A Review on Toxicological Evaluation of Zinc Pyrithione; A Globally Recognized Antidandruff Active

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Abstract

Zinc pyrithione (ZPTO), also known as zinc pyridine-2-thiol-*N*-oxide or *bis*-[1-hydroxy-2(*H*) pyridinethionato]-zinc is a broad-spectrum antimicrobial agent and has been used as fungicide and bactericide in various personal care and pharmaceutical products, particularly in anti-dandruff shampoos. Many leading brands in anti-dandruff domain highlights advantages of ZPTO in eradicating dandruff as well as associated dandruff side effects. ZPTO is also being used as a biologically active agent, *i.e.*, antimicrobial in water-based paints, coatings, adhesives, as wet-state preservative, in hard surface cleaners, fabric care compositions, wood products, plastic products, for cutting oils and coolant systems, protecting cellulosic fibres from loss of tensile strength and/or in any other application where microorganism growth must be stopped or slowed.

As described above that ZPTO have proven wide range of applications but to control the growth of a wider range of organisms effectively and to attain higher bio-availability, higher concentrations of ZPTO is required. Whereas, the useful amount of pyrithione or its salts that can be added to a commercial product is limited to a lesser extent due to toxicological, environmental and economic considerations. Pyrithiones exhibit various biological effects, yet the active species and molecular mechanisms underlying their diverse biological activities remain enigmatic. Understanding the toxic effects of Zinc Pyrithione (ZPTO) is crucial for evaluating the risks associated with widespread pyrithione biocide release into the environment.

The present review article depicts the safety and toxicological aspects of Zinc Pyrithione, which is widely been used in anti-dandruff shampoos, soaps and various industrial formulations.

Keywords: Zinc Pyrithione, Antimicrobial, Fungicide, Bactericide, Anti-dandruff, Toxicology, Toxicokinetic and environmental effects.

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1. Introduction

Zinc Pyrithione (ZPTO) is authorized for use as a preservative in rinse-off (shampoo) and leave-on (hair cream) cosmetic products at concentrations 1-2% and up to 0.5%, respectively. It has been employed for over 60 years as an anti-dandruff agent in appropriate cosmetic products, providing significant practical

and epidemiological experience. Being a zinc based metal complex/chelate, the lipophilic character is very high in comparison to other organic anti-dandruff compounds.

The anti-microbial activity of ZPTO depends on the bio-availability of ZPTO molecules on the treated surface, in particular over the scalp to treat dandruff. Due to poor solubility in water (6-12 ppm), concentration of ZPTO molecules in aqueous media and thereafter on scalp and hair also remains low, leading to poor bio-availability. Also, when used in personal care formulations, it produces white suspension that precipitate in the packaging bottle. Therefore, to overcome these disadvantages of low water solubility, precipitations and poor bio-availability, ZPTO is generally used in excess (1-2% w/w) in rinse-off formulations as ready to add ***Fine Particle Suspension*** (with defined particle size and particle distribution), but higher concentration of ZPTO lacks ready consumer acceptability due to harshness and irritation. Numerous controlled toxicological and functional investigations in the scientific literature demonstrated moderate to low acute and subchronic toxicity of ZPTO, whether used alone, in market formulations, or after practical application to humans. These investigations were categorized as ingredient-based data and product-based data, where applicable to the mode of application. However, the knowledge about the toxic effect of ZPTO is crucial for the assessment of the risk implied by a high scale release of pyrithione biocides to the natural environment.

Early life stage tests exhibited significant teratogenic effects (morphologically visible wavy structures of the vertebral column) at very low concentrations of ZPTO in larvae of zebra fish and Japanese Medaka [1-3]. ZPTO exhibited EC_{50} values of 9 μg/L [28 nM] in zebra fish and 5 μg/L [16 nM] in Japanese Medaka. Kobayashi and Okamura [3] comparatively assessed the effects of tributyltin oxide and seven organotin antifoulant substitutes on sea urchin eggs and embryos. With No Observed Effect Concentrations (NOEC) of 0.03 femtoM, ZPTO was the most toxic antifoulant tested. $CuPT_2$ exhibited a NOEC value of 3 femtoM.

Genotoxic effects induced by pyrithiones have been found in mammalian lymphoma cells with and without photo activation [4]. Pyrithiones are general inhibitors of membrane transport processes in fungi and have been found to distort membrane integrity in bacteria [5-8] and mammalian cultured cell lines [9]. Oxidative damage elicited by pyrithiones has been found in rabbit myocardium tissue [10]. Membrane depolarization induced by pyrithiones has been shown in mammalian cultured cells [11] and fungi [12]. Pyrithione biocides are capable of interaction with common biological molecules (phosphatidyl-ethanolamine, cysteine) [5] and have been shown to cause large decreases in ATP levels in bacteria [7] and fungi. As ionophores, pyrithiones affect homeostasis of different metal cations. This was found in different mammalian cultured cell lines [11, 13, 14] and rabbits (*in vivo*) [15] Further, pyrithiones affect amino acid metabolism in different mammalian cultured cell lines [14, 16, 17] as well as nucleoside metabolism in bacteria [18] different mammalian cultured cell lines [16-19] and the isolated salmon sperm DNA/bacterial RNA polymerase model [18] Pyrithiones affect cellular function regulating molecules (transcription factors NF-kB and AP-1) in different mammalian cultured cells [13].

2. Toxicological Study

Acute oral toxicity Studies of Zinc Pyrithione

Ingredient based data

LD50 values for zinc pyrithione have been determined in various species after oral administration. The values in the rat ranged from 92 to 266 mg/kg and in the mouse from 160 to 1000 mg/kg. 600 mg/kg was found to be the LD50 when administered orally to dogs [20-25]

Product based data

The oral LD_{50} values for shampoo formulations containing zinc pyrithione have been established in rats as 2.5 g/kg for a cream shampoo and 3.0 ml/kg for a lotion shampoo. An analysis of the acute data for rats from a number of studies provides the following mortality/dose levels:

Lethal dose	Cream shampoo (g/kg)	Lotion shampoo (ml/kg/*)
LD_1	1.4	4.5
LD_{25}	2.0	2.4
LD_{50}	2.5	3.0
LD_{75}	3.1	3.9
LD_{100}	4.5	6.0

* The density of the lotion shampoo form is approximately 1 and for practical purposes, gm and ml may be interchanged.

In addition to above, Snyder *et. al.* (1965) studied the acute oral toxicity of the cream shampoo product with higher levels of ZPTO and estimated the LD50. The results showed that increasing the level of ZPTO increases acute oral toxicity [26]

Emetic studies in dogs and pigeons showed that zinc pyrithione in this formulation is a potent emetic. A summary of the results follows:

Emetic dose	Cream shampoo (g/kg)	Lotion shampoo (ml/kg/*)
LD_0	0.007	0.04
LD_{50}	0.02	0.07
LD_{100}	0.07	0.25

For the cream shampoo form in pigeons, the ED100 was 0.1 g/kg, ED0 was 0.02 g/kg, and ED50 was approximately 0.05 g/kg.

In the emetic studies with dogs, the emesis typically occurred within 60 minutes of dosing, the average being 30 minutes and involved two to four episodes. Occasional bloody vomitus was seen which indicates the gastric irritation. The effect of the level of zinc pyrithione in the cream shampoo formulation upon the emetic potential in dogs is shown in the following table:

ZPTO (%)	Emetic Dose (g/kg)		
	ED_0	**ED_{50}**	**ED_{100}**
0	0.2	0.6	1.6
2	0.007	0.02	0.07
3	0.006	0.015	0.025
5	0.006	0.006	0.025

Overall, the ratios of ED_{50} to LD_{50} for both forms of the product are 1:125 for the cream shampoo and 1:42 for the lotion shampoo, therefore, it is unlikely that a human accidentally ingesting shampoo could retain a hazardous amount. This assumption is supported by the safe marketing history of zinc pyrithione containing cosmetic shampoos where no serious side effects have been reported [27-29].

2.1 Acute dermal toxicity Studies of Zinc Pyrithione

Ingredient based data

Dermal toxicity studies on albino rabbits showed that the LD_{50} values for rabbits varies from 2,000 mg/kg to 10,000 mg/kg. If the dose levels required to obtain dermal LD_{50} values are compared to values for other routes of administration, it is evident that ZPTO toxicity is much reduced by cutaneous application and therefore only a minimal risk exists from this type of exposure. This conclusion has been verified in several toxicokinetic studies and is supported by comparison of those data in which no-effect levels were determined during lifetime feeding studies in animals [30].

Product based data

A shampoo containing 2% ZPTO at levels of 2.5, 5.0, 10.0 and 20.0 g/kg was tested on rabbits. The shampoo was occluded with a rubber sleeve and left in place for 24 hours. There were no observable systemic effects in animals treated with 2.5, 5.0, or 10.0 g/kg. Two of the four animals dosed with 20 g/kg showed a slight temporary depression. There were no deaths at any level. These data are in line with a study on ZPTO alone indicating that its incorporation into a shampoo formulation does not significantly enhance penetration [26, 31].

In addition, intraperitoneal and intravenous data were also provided for comparison to the oral and dermal data. Intraperitoneal injection of ZPTO resulted in LD_{50} values of 36 mg/kg for rats and 500 mg/kg for mice. Generally, 25 mg/kg of ZPTO was fatal to both dogs and monkeys within 24 hours and produced cholinergic-like effects prior to death when injected intravenously. Doses of 15 and 20 mg/kg produced slight cholinergic stimulation in dogs but death did not result. One of two Yorkshire pigs died when injected intravenously with 20mg/kg and 10 mg/kg was a lethal dose for rabbits. Intravenous doses of 5 mg/kg or less produced only transient effects [32-34].

So far no data have been reported for acute inhalation toxicity and for repeated dose oral and dermal toxicity.

2.2 Sub-chronic toxicity Studies of Zinc Pyrithione

No significant effects have been noted other than the hind-limb weakness or paralysis which occurred in rats and rabbits within 8 to 14 days when ZPTO was administered in the diet at levels from 165 ppm to 330 ppm (8-16 mg/kg/day). Doses greater than 330 ppm required longer periods of administration before paralysis occurred. At levels of 1000 ppm or greater the animals usually died without developing a paralysis. The results of various Sub-chronic toxicological studies done by different groups of scientists [22, 25, 26, 35-46] are summarized below (**Table 1**):-

- ZPTO produces a reversible paralysis when fed to rats and rabbits at levels from 75 to 750 ppm (approximately 12-115 mg/kg/d) as part of the diet
- A concentration of 250 ppm (approximately 38 mg/kg/d) in diet seems to be the concentration at which the effects are most rapidly produced
- Monkeys do not become paralysed when fed ZPTO at levels of from 100 to 500 ppm (1.2 to 12.0 mg/kg/day) for up to 30 days but signs of neurologic deficit have been observed at a dose level of 5000 ppm (30 mg/kg/day)
- Neurological effects seen in some animal species exposed orally to ZPTO may not be demonstrated by the dermal route of exposure where ingestion was controlled
- Dermal application of 10% solutions of shampoo formulations containing up to 20 mg ZPTO/kg/d produced only mild to moderate irritation in rabbits.

Table 1: Summary of sub-chronic oral toxicity data for products containing ZPTO

Species	*Number of Animals*	*Route of administration*	*Dose [a] ZPT*	*Dosage (days)*	*Observation*
Rat	4	Stomach tube	10 mg/kg/day	15	No effects
Rat	13	Diet	1 mg/20 g diet [b]	63	Muscle relaxation
Rat	13	Diet	5 mg/20 g diet [c]	7	Muscle relaxation
Rabbit	2	Diet	3.5 mg/kg/day	14	No effects
Rabbit	2	Diet	17.5 mg/kg/day	14	Muscle relaxation
Rabbit	2	Diet	70 mg/kg/day	14	Muscle relaxation
Rabbit	6	Stomach tube	20 mg/kg/day	15	No symptoms
Rabbit	6	Stomach tube	40 mg/kg/day	15	No symptoms
Rabbit	6	Stomach tube	80 mg/kg/day	15	No symptoms
Rabbit	4	By mouth [d]	15 mg/kg/day	20	No paralytic symptoms
Dog	2	Oral	25 mg/kg/day [e]	14	Emesis, pupil dilation, blindness
Dog	4	Stomach tube	1 mg/kg/day	1	Emesis
Dog	1	Stomach tube	20 mg/kg	3 d/week for 8 w	Emesis
			100 mg/kg	3 d/week in week 9	emesis
Monkey	1	Stomach tube	10 mg/kg/day	80	No effects
Monkey	2	Stomach tube	25 mg/kg/day	8	Emesis and diarrhoea
Monkey	3	Diet [f]	1.2-1.8 mg/kg/day	30	No effects
Monkey	3	Diet [f]	3.6-5.4 mg/kg/day	30	No effects
Monkey	3	Diet [f]	6.0-9.0 mg/kg/day	30	No effects

[a]Dosed as shampoo base containing 10% ZPTO, except where indicated
[b]20mg/kg/day for 50 g weanlings; 5 mg/kg/day for 9-week weight of approx. 200 g
[c]100 mg/kg/day for 50 g weanlings; 20 mg/kg/day for 7-week weight of approx. 250 g
[d]Ten divided daily doses
[e]Dosed as w/o emulsion
[f]ZPTO palletised with food

2.3 Chronic toxicity Studies of Zinc Pyrithione

Ingredient based data

A two-year feeding study was conducted by Larson [97]. Young Wistar rats in groups of ten males and ten females were fed diets containing ZPTO at levels of 0, 2, 5, 10, 25 and 50 ppm. These levels correspond to approximately 0, 0.1, 0.25, 0.5, 1.25 and 2.5 mg/kg/day for adult animals. At the start of the study the corresponding levels were 0, 0.2, 0.5, 1.0, 2.5 and 5.0 mg/kg/day for the young rats. Survival in males was not adversely affected by ingestion of the compound but the highest level caused hind-limb paralysis in some animals. None of the females on the 50 ppm diet lived beyond 80 weeks and death was commonly preceded by paralysis. Mortality was also increased at 25 ppm and paralysis occurred in some animals prior to death. In females, growth depression was marked at 50 ppm. Dietary concentrations of 2, 5 and 10 ppm appeared to have an accelerating effect on weight gain in both sexes and males showed a comparable stimulation at 25 ppm. The no-effect level for males and females was 10 ppm (0.5 mg/kg/day).

The only unusual finding upon termination of the study was an increase in neutrophil versus lymphocyte counts in males on the 50 ppm diet. Ratios of organ weights to body weights did not differ significantly among the surviving groups at termination. Histopathologic examinations did not reveal any lesions that appeared to be attributable to the administration of ZPTO. These observations included careful attention to retina, optic nerve, cerebral cortex and other parts of the central and peripheral nervous systems. There were no significant differences in the rate of frequency of neoplasms between any of the groups.

The chronic studies summarised above provide two essential pieces of information relevant to the safety evaluation of ZPTO:

- 0.5 mg/kg/day (500 µg/kg/d) given orally to rats is a no-effect level
- No evidence of a carcinogenic response was seen when ZPTO was applied topically (up to 100 mg/kg/d) or given orally (up to 5 mg/kg/d) in lifetime studies using mice and rats.

2.4 Irritation and Corrosivity Studies of Zinc Pyrithione

2.4.1. Irritation (skin)

Irritation studies have been conducted with marketed shampoos containing ZPTO [48]. In a study (based on human skin pigmentation at sub irritating levels) product was applied daily at 0.2, 0.4, and 2.0% under non-occluded dressings to each of eight Caucasians and eight black males for 64 consecutive days. Under the experimental conditions, the cream and lotion shampoos did not produce any skin irritation nor did they change the skin pigmentation level in Caucasian or black skin.

However, a case report described a reaction by a patient to a shampoo containing 2% ZPTO. The patient had had a similar reaction after using a hair cream with a lower level of ZPTO. Another report described a case of eczema of the scalp and face after using a shampoo containing 2% ZPTO for a short period [49, 50].

These studies indicate that the presence of ZPTO in cosmetic formulations did not impact upon the low irritation potential of the formulations.

2.4.2. Irritation (mucous membranes)

The eye irritation potential of ZPTO has been evaluated in a number of product types:

- Instillation of a soap solution containing 0.25% ZPTO to rabbit eyes [26] produced slight transient irritation with the peak effect occurring during the first 4 hours and having disappeared completely in 2-4 days
- In another study, undiluted and diluted solutions of shampoo with or without ZPTO (2%) were tested. Undiluted solutions produced extensive damage to the eyes of rabbits which was characterized by opalescence of the entire cornea, severe iritis and marked conjunctivitis. In all cases, rinsing was particularly effective in alleviating the condition with very slight to moderate conjunctivitis being observed. In all rinsed cases, damage had cleared by the third day whereas in unrinsed eyes the condition had not cleared by day 42. Dilution of these test solutions to 10% also reduced the ocular irritation and in all cases the condition was cleared by day 7. Again, rinsing was effective in alleviating the condition. No significant differences were observed between the control and the test animals in this study
- Repetition of the above study in monkeys with no rinsing produced superficial damage to the corneal epithelium and/or slight conjunctival irritation when the 2% ZPTO shampoo was instilled undiluted. Instillation of the shampoo formulation diluted to 10% (0.2% ZPTO) resulted in no ocular irritation.

2.4.3. Sensitization Studies

Ingredient based data

The work by the Danish Contact Dermatitis Group [49] was described in which ZPTO (1%) was added to the European Standard Patch Test Series. 1652 consecutive dermatitis patients were tested and only in three cases positive reactions were found. The authors stated that in only one of these was the ZPTO reaction interpreted with certainty as being of present relevance. They also point out the wide use of ZPTO in shampoos, hair creams and cosmetics. Bearing this in mind and recalling that all the subjects tested had known skin problems, this is a remarkably low incidence of reactions and underline the very low risk from ZPTO in the sensitization area.

A multi-centre investigation was conducted in France in order to evaluate the risk of sensitization by a number of preservatives. 465 subjects were tested and only two patients (0.4%) gave positive patch tests to ZPTO [79].

In another study by the scientists of P&G, ZPTO was evaluated for its potential to induce contact hypersensitivity to guinea pigs. Using the procedure of Buehler [51] to detect contact hypersensitivity, 40 animals were exposed to a 50% aqueous slurry of ZPTO. No reactions indicative of contact hypersensitivity were seen in any of the animals at challenge.

Product based data

Many sensitization studies have been conducted with marketed shampoos containing ZPTO using both animals and humans. Several human repeat insult patch tests (HRIPT) have been conducted employing over 1000 human volunteers. The irritation potential was also investigated in all HRIPT's.

A 0.1% solution of the ZPTO (1% ZPTO) soap was injected intracutaneously into depilated guinea pig skin at an initial dose of 0.05 ml and nine subsequent doses of 0.1 ml on alternate weekdays. A single challenge dose of 0.05 ml was injected two weeks later. There was no evidence of sensitization [26].

Two separate closed-patch test studies on human volunteers were conducted. A 1% aqueous solution of shampoo containing 2% ZPTO was used. The test solution was placed on the upper arm of the subjects and occluded. Nine serial applications were made on alternate weekdays for three weeks, followed by challenge two weeks later. Challenge patches with the same concentration of test material were placed on both the original site of insult and on an alternate site on the opposite arm to distinguish between skin fatigue and sensitization.

Reactions were scored at both 48 and 96 hours. One subject gave papular reaction at 48 hours, which was scored negative at 96 hours. Unfortunately, no follow-up was done with individual ingredients, so it is impossible to determine whether indeed the subject was sensitized and if so, what the offending material was?? The remaining subjects gave only a transient erythematous response indicative of irritation [26].

Cream and lotion shampoo products were tested in two separate HRIPT's. Both studies were conducted according to the modified Draize procedure described above, in which 0.25% shampoo was patched. No sensitization was detected in the 82 subjects exposed to the cream nor in the 78 subjects exposed to the lotion. The only responses noted were transient primary irritation in some subjects [52, 53]

A hair dressing cream containing 0.5% ZPTO was used to patch test over more than 100 women for five months. A minimum of 80% of the subjects were patched weekly for 20 consecutive weeks. Patches were left in place for 48 hours and sites graded 72 hours after removal. Throughout the entire test program, no instances of any skin reactions were observed. Thus, it was concluded that the hairdressing product possessed an extremely low index of sensitization in humans [40, 41]

Marketing experience with a commercially available formulation has conclusively demonstrated that ZPTO at worst is a very weak sensitizer. Although, few reports of sensitization have appeared in the literature [49, 50, 54, 55].

In general, whether injected intradermally or applied topically and regardless of the species, ZPTO has been repeatedly demonstrated to be rare or even a non-allergenic. When tested alone, ZPTO has a low potential to induce contact hypersensitivity and when tested as part of a cosmetic formulation, ZPTO has a low potential to induce contact hypersensitivity [56-60].

2.5 Reproductive Studies of Zinc Pyrithione

Several teratology/reproduction studies [31, 61-64] have been conducted using rats and rabbits, in which ZPTO was either applied topically or given orally. Topical application (with ingestion during grooming) of levels up to 15 mg/kg/day did not adversely affect reproduction in rats. When pregnant rats were gavaged with 15 mg/kg/day of ZPTO, there was an increase in the incidence of forked and fused ribs in the neonates. A dose level of 2.5 mg/kg/day given orally is a no-effect level for teratogenicity/embryotoxicity. No material toxicity was observed in these studies. Teratology data are summarized in the **table 2.**

Table 2: Summary of teratogenicity studies

Species	*Route of administration*	*Dose levels (mg/kg)*	*Teratology findings*	*Ref.*
Rat	Oral	7.5 15.0	7.5 – none 15.0 – increased incidence of fused or forked ribs	24
Rat	Oral	7.5 15.0	7.5 – none 15.0 – increased incidence of fused or forked ribs	43
Rat	Topical with ingestion of applied material	2.5 7.5 15.0	2.5 – none 7.5 – none 15.0 - none	43
Rat	Topical	2.5 7.5 15.0	2.5 – none 7.5 – none 15.0 - none	43
Rabbit	Oral	5.0 10.0 20.0	5.0 - fatal to 6/15 dams, no teratogenic effects 10.0 - fatal to 10/15 dams, no teratogenic effects 20.0 - fatal to 15/15 dams	43
Rabbit	Oral	1.0 2.5 5.0	1.0 – none 2.5 – none 5.0 - none	43
Rabbit	topical	25.0 50.0 100.0	25.0 – none 50.0 – none 100.0 - none	43

In summary:

- 2.5 mg/kg/d administered orally to rats is a no effect level for teratological effects
- No reproductive effects have been observed when ZPTO was applied topically to rats and rabbits at levels up to 15 and 100 mg ZPTO/kg/d respectively (highest doses tested) and ingestion of the test material was controlled.

- No reproductive or teratogenic effects have been observed in rabbits and pigs following topical application of shampoo formulations containing 50 and 400 mg ZPTO/kg/d, respectively.

3. Toxicokinetics Studies of Zinc Pyrithione (incl. Percutaneous Absorption)

Deposition and absorption

Ingredient based data

The effect of topical application of the anti-dandruff agent ZPTO on epidermal DNA synthesis in normal and hexadecane stimulated rat skin was investigated [65]. Autoradiography was used to determine the percentage of epidermal cells labelled with [^{3}H] thymidine (labeling index). ZPTO at 1% in shampoo base caused a slight increase in the labeling index in normal skin, similar to the effect of the shampoo base alone. No effect of 1% ZPTO as an aqueous dispersion was observed. ZPTO at 1% in shampoo base did not reduce the large increase in the labeling index produced by hexadecane, nor did shampoo base alone or 1% ZPTO in water. The shampoo base with or without 1% ZPTO had only a very slight effect on the histopathology of normal and hexadecane treated skin and 1% ZPTO in water had no effect. It is concluded that *in vitro* potential of ZPTO to cause growth inhibition is not achieved *in vivo*, presumably because of low percutaneous absorption. Thus evidence does not support a cytostatic mode of action in clearing dandruff.

Parran [66] showed that particles of ZPTO were deposited on the scalp from shampoos. He stated that the particles could not be removed by vigorous and prolonged rinsing with just water but gradually decreased in number with some still detectable factors within two to three days after shampooing.

Okumura *et. al.* [67] found the adsorption of ^{35}S-ZPTO from a 1% ZPTO shampoo to be 0.1 µg/cm^2 for mice and about 10.1 µg/cm^2 for rats. The data for mice were derived from counting skin samples, whereas the rat data were estimated from counts of successive tape strippings. The difference in the amounts adsorbed between two similar animals is much larger than would be expected but could be partially accounted for by differences in experimental procedures.

Black *et. al.* [68] determined the deposition of ^{35}S-ZPTO from shampoo on rat skin as a function of concentration in shampoo, duration of contact with the skin, pH of shampoo and nature of the detergent in the shampoo formulation. In experiments with 0.1% to 2.0% ZPTO in shampoos, the deposition increased from 0.03 to 6.44 p.g/cm^2. The duration of contact and pH of the shampoo did not affect deposition but different detergent compositions did. These results agree with those reported previously by Snyder *et. al.* [26] and Parran [66].

Okamoto *et. al.* [69] determined the substantivity of ZPTO to the skin of rats and rabbits using shampoos with ^{35}S-ZPTO for rats and ^{65}Zn-ZPTO for rabbits. Substantivity to clipped rat skin following shampoos of one and five minutes with a 1.82% ZPTO shampoo followed by rinsing was 9.2 and 11.6 µg/cm^2, respectively. These data agree with those reported by Snyder *et. al.* [26], Okumura *et. al.* [67] and Black *et. al.* [68].

Rutherford and Black [70] used autoradiography to study the localization of ZPTO in guinea pig skin. They found clear evidence that ZPTO particles are adsorbed on skin and hair and that solubilized ZPTO enters hair follicles. A quantitative estimate of the amount of ^{35}S-ZPTO adsorbed on skin was made by counting 25-µm serial sections of guinea pig skin cut parallel to the surface. The adsorbed ZPT was found to be 1.4 to 3.6 µg/cm^2.

The data from the various studies summarized above suggest that deposition on animal skin is equal to approximately 1% of the applied ZPTO and is therefore concentration dependent but independent of contact times from 1 to 32 minutes.

Gibson and Calvin [71] used rhesus monkeys to study absorption from a three-hour application of 2% ^{35}S-ZPTO to a 10 cm^2 area of the abdomen. They found that 0.03-0.04% of the administered dose was absorbed. The absorption was increased approximately 10 times using abraded or stripped skin. In separate monkey studies using scalp exposure to ^{14}C-ZPTO, about 3.4% of the ZPTO was absorbed whether a 3- or 72-hour exposure was used, confirming the data of Howes and Black. Blood levels, however, were below the limit of detectability (1 ppb). These differences in percentage absorption may be explained by factors such as vehicle differences, differences in skin site and therefore the presence/absence of hair follicles.

Spiker and Ciuchta [72] have conducted a rabbit study in which they made dermal application of a shampoo containing 0.75% ZPTO twice daily for four days. They found that surfactant irritation had occurred but no ZPTO penetration was observed as measured by whole-blood zinc levels. They did report, however, that 3.75% ZPTO in 28% ammonium lauryl sulfate produced large increases in whole blood zinc levels with reductions in plasma zinc 6 hours after a 24-hour exposure. They attributed these changes to the chelating nature of the pyrithione molecule.

Summary of Percutaneous Absorption Studies

- Percutaneous absorption of ZPTO varies from approximately 0.03 to 3.4%
- The distribution of radioactivity in tissues after oral administration of labeled ZPTO showed that the radioactivity rapidly disappeared from the blood and the primary route of excretion was *via* the urine. The residual radioactivity was low (4.5% of dose) indicating ZPTO was distributed throughout the body and was not concentrated in any particular tissue.
- All animal species investigated (rat, rabbit, dog, and monkey) biotransformed ZPTO in qualitatively similar ways. This similarity with regard to ZPTO metabolism suggests that human metabolism is likely to be similar. This has been confirmed by Wedig *et. al.* [73]

Product Based Data

A clinical pharmacokinetic study has investigated deposition, absorption and excretion of ^{14}C radio labelled ZPTO resulting from the use of a ZPTO containing shampoo alone (1% ZPT) and in combination with a ZPTO containing leave on hair tonic (0.1% ZPTO). Measurements of ZPTO deposition and excretion were made by analysis of clipped hair, tape stripping areas of the scalp and hands and urinalysis, respectively. Previously, preclinical studies have demonstrated that ≥ 90% of absorbed ZPTO is excreted in the urine within 24 hours, therefore, for the purposes of this study the level of ZPTO excreted was taken to represent the level of ZPTO absorbed

This study demonstrated that systemic loading of ZPTO was increased significantly in those subjects using the shampoo/tonic combination compared with those using the shampoo alone. Additionally, absorption of ZPTO in patients with compromised scalps was not found to be statistically different to normal scalps patients.

Main results are shown in the following tables:

Table 3: Mean ^{14}C-ZPTO Skin Deposition Measurements (Scalp and Hands)

Day[#]	1% ^{14}C-ZPT Shampoo		1 % ^{14}C-ZPT Shampoo + 0.1 % ^{14}C -ZPT Tonic	
	LSM amount of ZPT deposited (µg /cm^2)	Estimated total ZPT deposited (µg)	LSM amount of ZPT deposited (µg/cm^2)	Estimated total ZPT deposited (µg)
1	0.39	288.00	0.88	644.93
2	0.43	317.60	0.96	693.19
4	0.50	363.30	1.33	967.60
5	0.20	146.80	0.40	296.31

[#] Day 3 deposition measurements not recorded as a practical consideration to study participants to avoid excess hair removal

LSM = Least Squares Mean

Table 4: Mean ^{14}C-ZPTO Systemic Load

Day	1% ^{14}C-ZPT Shampoo		1 % ^{14}C-ZPT Shampoo + 0.1 % ^{14}C -ZPT Tonic	
	LSM systemic load (µg /kg/d)[#]	SEM	LSM systemic load (µg /kg/d)[#]	SEM
1	1.02	0.14	1.39	0.14
2	2.54	0.33	3.31	0.33
3	2.73	0.32	3.32	0.32
4	2.76	0.35	3.43	0.35
5	1.96	0.32	2.29	0.32

LSM = Least Squares Mean; SEM = Standard Error of the Mean

[#]Average body weight per group used for calculation of µg/kg/d values

Table 5: Comparison of Mean ^{14}C-ZPTO Absorption in Normal and Compromised Scalp Subjects

Day[#]	LSM amount of ZPT absorbed (% of dose deposited)				Statistical analysis – p-values	
	1% ^{14}C-ZPT Shampoo		1 % ^{14}C-ZPT Shampoo + 0.1 % ^{14}C -ZPT Tonic			
	Normal scalp	Compromised scalp	Normal scalp	Compromised scalp	ASF	ASF*TRT
1	25.39	46.48	20.58	20.84	0.24	0.25
2	68.08	57.26	49.75	47.57	0.58	0.71
4	57.51	52.67	43.85	27.39	0.39	0.64

[#] Day 3 deposition measurements not recorded as a practical consideration to study participants to avoid excess hair removal

LSM = Least Squares Mean; ASF = Adherent Scalp Flaking (normal versus compromised scalp); TRT = Treatment group

Analyses of the dermal deposition data indicated:

- ZPTO deposition on the hands between the treatment groups* A and B was not significantly difference throughout the study except on day 2
- ZPTO deposition on the scalp between the treatment groups A and B was statistically
- Different with deposition in Group B being significantly higher than group A
- ZPTO deposition on the hands was determined to be approximately half the level deposited on the scalp
- ZPTO deposition on the hair between the treatment groups A and B was statistically different with deposition on hair in Group A being half the level of Group B

* [Patients using ZPTO containing shampoo alone (Group A) and patients using the ZPTO containing shampoo and ZPTO containing tonic combination (Group B)].

Analysis of individual subject absorption data indicated that individuals with compromised scalps demonstrated no greater absorption than individuals with normal scalps. Analysis of the urinary excretion curves indicates that steady state conditions were reached within the 4 day treatment period of this study. Statistical analysis indicated that the amount of ZPTO excreted in the urine (indicative of systemic exposure) was significantly higher in the shampoo + tonic group (**B**) compared with the shampoo only group (**A**) throughout the study. However, the increase was less than what would have been expected from the increase in skin deposition. This suggests a rate limiting mechanism exists for the absorption of ZPTO across the skin.

4. Mutagenicity/Genotoxicity Studies of Zinc Pyrithione

In vitro and *in vivo* studies carried out by Skare and Wong [74] and Skoulis *et. al.* [75] have indicated that ZPTO did not show any mutagenic effect.

Till date no data are available for the carcinogenic effect of ZPTO.

5. Conclusions

It could be demonstrated that the acute as well as the subchronic toxicity of Zinc Pyrithione is moderate to low for both ZPTO alone or incorporated in market formulations and in animal experiments or after practical application to humans. Thus, the scientific evaluation of the submission, the results of these investigations on ZPTO have been separated as to (i) ingredient based data (ii) product based data and where applicable to the mode of application.

As to probable neurotoxicological properties of ZPTO the results of investigations in several species can be summarized as follows:

In chronic toxicity experiments it was shown that an oral application of 500 µg/kg/d applied over (~18 m) can be regarded as NOAEL. No evidence of a carcinogenic response was seen when ZPTO was applied topically up to 100 mg/kg/d or given orally up to 5 mg/kg/d in lifetime studies.

The presence of ZPTO did not impact upon the low irritation potential of the cosmetic formulations tested. The same observation was valid for a shampoo formulation investigated in a mucous membrane test. The appropriate experiments showed that ZPTO alone as well as part of cosmetic formulations had a low potential to induce contact hypersensitivity.

In teratological studies, 2.5 mg/kg/day applied orally to rats was the NOAEL maternally, no reproductive effects were observed in rabbits and rats when ZPTO was topically administered up to 400 mg/kg/day.

Toxicokinetic investigations revealed:

- Percutaneous absorption of ZPTO varies from approximately 0.03 to 3.4%
- The distribution of radioactivity in tissues after oral administration of labeled ZPTO showed that the radioactivity rapidly disappeared from the blood, and the primary route of excretion was via the urine. The residual radioactivity was low (4.5% of dose), ZPTO was distributed throughout the body and was not concentrated in any tissue.
- All animal species investigated (rat, rabbit, dog, and monkey) bio transformed ZPTO in qualitatively similar ways. This similarity with regard to ZPTO metabolism suggests that human metabolism is likely to be similar. This has been confirmed by Wedig *et. al.* [73].

In mutagenicity/genotoxicity studies ZPTO has shown no effects in any of the *in vitro* and/or *in vivo* studies conducted.

Therefore, in-line with the overall exposure studies reported herein and taking into account the scientific data provided, the Zinc Pyrithione (ZPTO) can be considered as safe when used as an anti-dandruff in rinse-off hair products up to a maximum concentration of 1% and upto 0.5% when used in leave-on products.

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