SUBMISSION TO THE SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS (SCCP)

ZINC PYRITHIONE (ZPT)

SUPPLEMENTAL DOSSIER ADDRESSING USAGE LEVELS OF ZINC PYRITHIONE IN RINSE-OFF ANTI-DANDRUFF HAIR CARE PRODUCTS

Date: 3 September 2008

CONTENTS

1	INTRODUCTION	3
2	EXECUTIVE SUMMARY	4
3	CHEMICAL AND PHYSICAL SPECIFICATIONS	5
4	DETAILED REVIEW OF TOXICITY DATA DISCUSSED IN THIS SUBMISSION	6
5	EXPOSURE CALCULATIONS AND RISK ASSESSMENT	14

APPENDICES

I	REFERENCES	15
II	REVIEW OF STUDIES DISCUSSED IN THIS SUBMISSION	16

ANNEX 19

I FINAL REPORT: Physiologically-based pharmacokinetics model of zinc pyrithione in the rat (June 2006)

FINAL REPORT: Feasibility study for a physiologically-based pharmacokinetics model of zinc pyrithione (August 2004)

II FINAL REPORT: Disposition of zinc pyridinethione (ZPT) in female rats: repeated dose oral and dermal pharmacokinetic studies.

III FINAL REPORT: An evaluation of the deposition, absorption, and excretion of ^{14}C resulting from an anti-dandruff treatment regimen consisting of shampoo and a leave-on tonic containing ^{14}C-pyrithione zinc (Study Number CRB 0005-067 HC)

1. INTRODUCTION

This requests the 1% concentration of ZPT approved for use in rinse-off hair care products be increased to 2%. No changes are requested to the previously approved levels for leave-on or rinse-off preservative usage in hair care products.

The Scientific Committee on Cosmetic Products (SCCP) [formerly the Scientific Committee on Cosmetic Products and Non Food Products (SCCNFP)] previously reviewed a submission for the non-preservative use of zinc pyrithione (ZPT) in cosmetic rinse-off and leave-on hair care products and for the preservative use of ZPT in rinse-off hair care products[*]. Their opinion was issued in SCCNFP/0671/02 (December 2002) [Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers, 2002].

The requests to the SCCNFP detailed in SCCNFP/0671/02 were:

- *Can zinc pyrithione be safely used for non-preservative purposes in cosmetic rinse-off and leave-on products at a maximum concentration of 1.0 and 0.1%, respectively?*
- *Can zinc pyrithione be safely used for preservative purposes in cosmetic rinse-off hair care products at a maximum concentration of 1.0%?*
- *Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?*

Based on the data contained in the submission, the SCCNFP concluded the following in their opinion:

> *"The SCCNFP is of the opinion that zinc pyrithione does not pose a health risk when used:*
>
> - *for non-preservative purposes in cosmetic rinse-off and leave-on hair care products at a maximum concentration of 1.0% and 0.1% respectively;*
>
> *or,*
>
> - *for preservative purposes in cosmetic rinse-off hair care products at a maximum concentration of 1.0%.*
>
> *Zinc pyrithione should not be used in products for oral hygiene."*

In this supplemental dossier, a new human risk assessment for 2% ZPT in rinse-off hair care products has been prepared. This latest risk assessment is based on the existing analytical and toxicity data that were presented in the initial submission[*] together with newly generated "state-of-the-art" data. Specifically, a physiological-based pharmacokinetic (PBPK) model for ZPT was created and used to calculate the human dose of pyrithione equivalent to the no observable effect level (NOEL) obtained from rat studies. The predictive validity of the PBPK model was verified in repeat dose pharmacokinetic studies in rats. In addition, the "internal dose" of ^{14}C-PT (pyrithione or its metabolites) was measured in human subjects following repeated use of a rinse-off anti-dandruff shampoo containing 2% ^{14}C-ZPT + 0.25% ^{14}C-ZPT leave-in tonic-dandruff treatment. The human NOEL obtained using the ZPT PBPK model and the "internal dose" from the human clinical study were used in the calculation of the Margin of Exposure (MoE).

Collectively, these new data support the human safety of 2% ZPT used in rinse-off shampoo. A summary of these data and a new risk assessment are presented in the following sections. Based upon these new studies and the existing data, it is concluded that consumer use of a rinse off hair product containing 2% ZPT does not present a risk to human health.

[*] Application to the Scientific Committee for Cosmetics and Non Food Products. Proposal for Annex III listing of Zinc Pyrithione. Colipa, June 27, 2000.

2. EXECUTIVE SUMMARY

The toxicity profile of zinc pyrithione (ZPT), as a single ingredient or a component of cosmetic formulations, has been studied extensively in numerous preclinical studies with multiple species assessing acute/sub-chronic/chronic toxicity, genotoxicity, reproductive and developmental toxicity, skin and eye irritation, sensitisation and pharmacokinetics and in human clinical studies assessing irritation, sensitisation and pharmacokinetics. As an antidandruff active in cosmetic shampoo products, ZPT has been marketed safely for over 60 years at concentrations up to 2% in many regions of the world.

Additional support for the human safety of 2% ZPT in rinse-off shampoo products was obtained in three new studies. First, a "state-of-the-art" physiological-based pharmacokinetic (PBPK) model was developed simulating plasma, blood, and tissue kinetics of pyrithione (PT) and its two major metabolites, 2-(methylsulfonyl)-pyridine (MSP) and PT-glucuronide conjugates (SG). The model replicates experimentally-measured short-term elimination kinetics of PT and PT-derived carbon (PTC), i.e., ^{14}C from the administration of ^{14}C –ZPT, following single doses and longer-term temporal patterns of PTC and PT in blood/plasma during repeated dosing schedules.

The predictive validity of the ZPT PBPK model was verified in repeat dose pharmacokinetic studies conducted in female rats. Toxicologically-relevant doses of ^{14}C-ZPT were administered by gavage, in the diet, topically, or intravenously. Radiolabel (PTC from ^{14}C-ZPT) and parent (PT) concentrations were measured in blood and plasma, respectively, over the 14-day study (10-day dosing + 4-day recovery). These experimental observations confirmed the predictive validity of the ZPT PBPK model. Furthermore, these data provided additional support to estimates of the internal dose of ZPT in humans based on measures of cumulative urinary radioactivity following use of shampoos containing 2% ^{14}C-ZPT thereby linking PBPK estimates to the data obtained in human subjects.

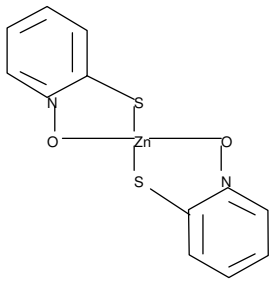
Finally, a human clinical study was conducted where the internal (systemic) dose of ZPT was determined after repeated use of a rinse-off shampoo containing 2% ^{14}C-ZPT + a 0.25% ^{14}C-ZPT leave-on hair tonic (3 applications). Measurement of steady-state cumulative 24-hr urinary excretion of ^{14}C-PTC was used to establish the internal dose which was a maximum of 4.66 ± 0.59 µg /kg/day.

Using the ZPT PBPK model and existing toxicology data, a human No Observed Effect Level (NOEL) was calculated and compared to the internal dose obtained in the human clinical study for the determination of the Margin of Exposure (MoE). Using this approach, the MoE was 465 [2170 µg/kg/day (human NOEL) ÷ 4.66 µg/ kg/day (internal human dose from use of 2% ZPT antidandruff shampoo)].

In summary, the PBPK model, verified by the rat pharmacokinetic studies, and human clinical study results provide new evidence supporting the human safety of 2% ZPT used in antidandruff shampoos. Additionally, these data generated from the PBPK model serve as an innovative approach for supporting the human safety of cosmetic ingredients in the future as the 7th Amendment to the Cosmetics Directive takes full effect. Most important, these new data, in combination with the existing data for all other toxicity endpoints support the safe non-preservative use of ZPT in rinse-off hair care products at levels up to 2%.

3 CHEMICAL AND PHYSICAL SPECIFICATIONS

A summary of the chemical and physical specifications of commercially available ZPT is presented in SCCNFP/671/02 [1]. Detailed below are the main chemical and physical properties of this material.

Chemical Name:	Bis[1-hydroxy-2(1H)-pyridine-thionato]zinc
INCI Name:	Zinc pyrithione
CAS No:	13463-41-7
EINECS No:	236-671-3
Synonyms:	Pyrithione zinc, Zinc pyridinethione, Zinc 2-pyridinethione-1-oxide, ZnPT
Empirical Formula:	$C_{10}H_8N_2O_2S_2Zn$
Chemical Structure:	
Physical Form:	White to slightly yellow crystals
Molecular Weight:	317.7
Melting Point:	240°C
Solubility:	Very low solubility in most solvents:
	Water: 0.0015 w/w% at 25°C
	Ethanol: 0.031 w/w% at 25°C
	Acetone: 0.07 w/w% at 25°C
	Chloroform: 0.34 w/w% at 25°C
	Mineral oil, light: 0.0001 w/w% at 25°C
Density:	1.782 at 25°C

4 REVIEW OF TOXICITY DATA DISCUSSED IN THIS SUBMISSION

4.1. PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL OF ZINC PYRITHIONE IN THE RAT (ANNEX I)

Background: There has been an increasing effort throughout the world to integrate physiological-based pharmacokinetic (PBPK) models into the risk assessment process (Bruckner, 2000; Krishnan *et al.*, 2002; Price *et al.*, 2003; Demchuk *et al.*, 2008). PBPK modelling integrates physiology, chemistry and biochemistry to estimate the "internal" dose resulting from exposure to a chemical. This tool has great potential in risk assessment of cosmetics particularly as conduct of animal toxicology studies for such ingredients become unavailable. For a compound like ZPT with such a vast toxicological and pharmacokinetic dataset, the development of a PBPK model serves as a test case to evaluate the utility and applicability of this innovative approach toward human risk assessment.

Overview: A rat PBPK model was developed for Zn-PT (a.k.a. ZPT) that includes compartments for plasma, liver, kidney, muscle, brain, and rapidly- and slowly-perfused tissue. Pyrithione (PT) metabolism to 2-(methylsulfonyl)-pyridine (MSP) and glucuronide conjugates (SG) was incorporated. The model replicated the observed short-term elimination kinetics of PT and PTC, i.e., ^{14}C derived from radiolabeled Zn-PT, following single doses and longer-term temporal patterns of blood concentrations during repeated dosing schedules. The model accounted for the production and rapid elimination of SG in urine, as well as production and slower elimination of 2-MSP, the major PTC species in blood within several hours following a dose of ZPT. This PBPK model was shown to have predictive validity and was used to calculate internal dose of PT for purposes of risk assessment.

Methods and Results: The general structure of the PBPK model is shown in Figure 1. The central distributing compartment is plasma. Seven tissue compartments are represented: kidney, liver, brain, skeletal muscle, other rapidly-perfused tissues (tissues other than liver and kidney that have a relatively high-blood flow/g tissue, e.g., heart, viscera), slowly-perfused tissue (e.g., adipose, skeletal muscle, skin), and red blood cells (RBC). Transfers of PT and metabolites between plasma and tissues other than RBC are believed to be sufficiently rapid to produce a steady state within the time of blood flow from the arterial to venous vasculature of each tissue (i.e., *flow-limited*). For plasma and RBCs, transfers are represented as first-order processes of unlimited capacity.

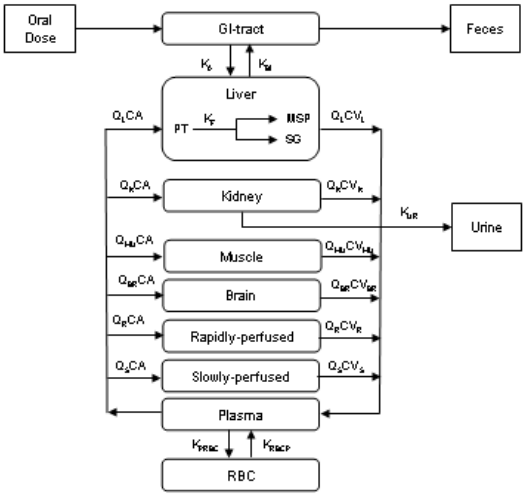


Figure 1. Conceptual diagram of rat PBPK model. The boxes represent compartments and arrows represent mass flows. A, amount (mg); B, bioavailable fraction; BI, bile; BR, brain; CA, arterial concentration (mg/L); CV, venous concentration (mg/L); GI, gastrointestinal; K, rate constant (hr-1); KI, kidney; L, liver; MSP, 2-(methylsulfonyl)pyridine; MU, muscle; PT, pyrithione; Q, plasma flow (L/hr); R, other rapidly-perfused tissue; RBC, red blood cell; S, other slowly-perfused tissue; SG, S-glucuronides; UR, urine; V, volume (L).

Figure 2 depicts the conceptual model of Zn-PT metabolism and disposition of metabolites that was used in the PBPK model based on published work (Min *et al.*, 1970; Kabacoff *et al.*, 1971; Ziller, 1977; Wedig *et al.*, 1978b; Wedig *et al.*, 1978a; Jeffcoat *et al.*, 1980). Conversion of Zn-PT to PT is believed to be

essentially instantaneous. PT is rapidly eliminated by S-glucuronide conjugation in liver, with secretion of the conjugates (SG) in urine and bile. A competing pathway in liver is S-methylation and oxidation to MSP, which undergoes secretion into bile. Rapid elimination of PT from plasma is attributed to the above two metabolic pathways. These are represented in the model as first-order processes of unlimited capacity.

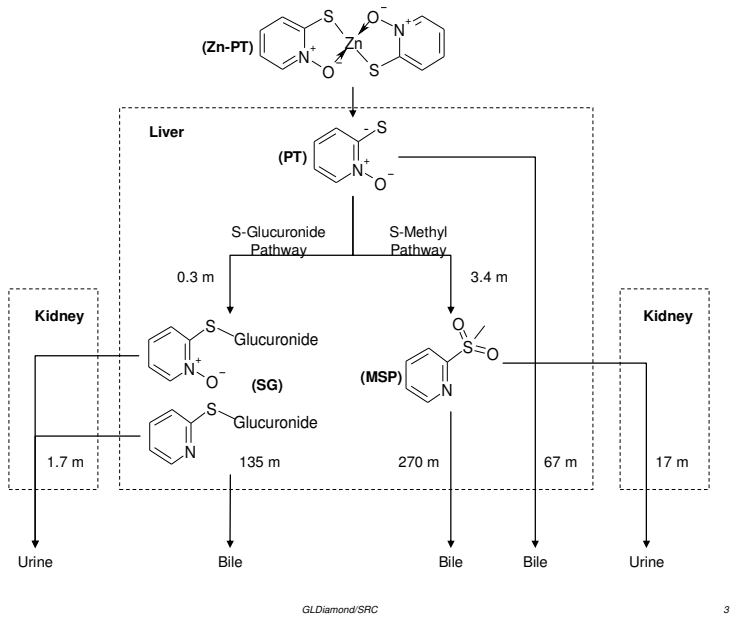


Figure 2. Conceptual model of Zn-PT metabolism and excretion of metabolites in the rat. Arrows depict metabolic and excretory pathways. Conversion of Zn-PT to 2-pryidinethiol-1-oxide (PT) is essentially instantaneous while all other metabolic and excretory pathways are first-order, without limitation in capacity. Numbers associated with pathways are approximate half-times (m= minutes) for the mass conversion or transfer from liver or kidney for a 0.2 kg rat. Metabolism of PT to SG and MSP is attributed to the liver. S-glucuronides are believed to be eliminated by rapid excretion into urine. Excretion of PT, SG, and MSP into bile is believed to occur more slowly.

The model was optimized based on pharmacokinetic data of PT in rats following single or repeated oral gavage doses of Zn-PT (see below and full report in ANNEX II). As illustrated in Figure 3, the PBPK model replicates the observed short-term elimination kinetics of PT following single doses and longer-term temporal patterns of blood concentrations of PT during repeated dosing schedules. As well, the model accounts for the production and rapid elimination of SG, the major metabolite of PT in urine, as well as production and slower elimination of the minor metabolite, MSP.

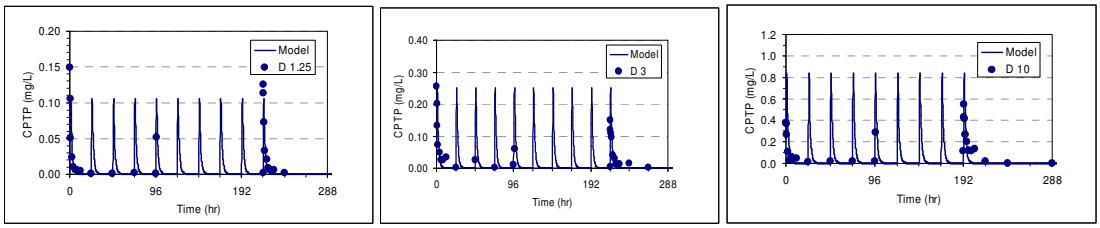


Figure 3. Observed and simulated plasma concentrations of PT (CPTC) in rats. Animals received 10 consecutive daily doses of 1.25 or 3 mg Zn-PT/kg/day or 9 consecutive daily doses of 10 mg Zn-PT /kg/day). Observations are mean values (closed circles). Solid lines represent model predictions.

An important feature of the PBPK model was the simulation of three species of PT, each having distinct elimination kinetics. As illustrated in Figure 4, the temporal profile for blood PTC (i.e., ^{14}C derived from radiolabeled Zn-PT) was a composite of the very rapid kinetics of PT ($t_{1/2}$≈1 hr), moderately fast kinetics of

SG ($t_{1/2}$≈9 hr), and slow kinetics of MSP ($t_{1/2}$≈70 hr). The relative contributions of each species to blood PTC levels changes over time and with exposure duration. Shortly after an oral dose, SG is the dominant contributor to blood PTC; however, after 12–15 hours following the dose, MSP dominates the PTC blood profile. With repeated daily exposures, periodicity occurs in the blood PT and SG concentrations, with near complete elimination between doses; however, the steady state PTC concentration is determined, largely, by MSP. This temporal profile is consistent with the observations available on the kinetics of blood SG and MSP in rats.

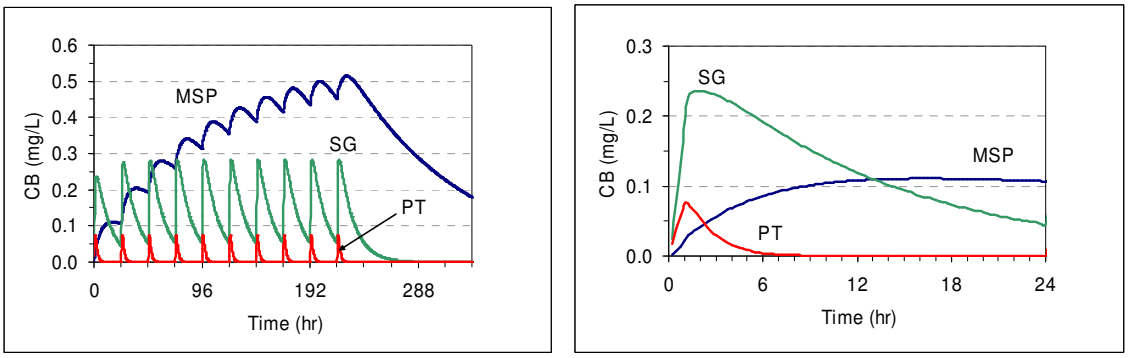


Figure 4. Simulated temporal profiles of parent compound and metabolites in blood. The panel on the left shows simulation of 10 consecutive daily doses of Zn-PT (1.25 mg/kg/day) and the right panel shows the same simulation for the first dose. Pyrithione (PT), 2-(methylsufonyl)pyridine (MSP), S-glucuronide conjugates of PT (SG).

One noteworthy application of PBPK model is the calculation of rat and human doses required to produce the area under the curve (0-72 hr) for plasma PT equivalent to the NOEL (Table 1). The rat NOEL for ZPT is 500 µg/kg/day based on hindlimb weakness observed in a 2-year study in rats (Larson, 1958), which was presented in the original submission (Application to the Scientific Committee for Cosmetics and Non Food Products. Proposal for Annex III listing of Zinc Pyrithione. Colipa, June 27, 2000). Using the PBPK model, the human dose equivalent to the rat NOEL producing the PT $AUC_{0\text{-}72\ hr}$ 260 ng/ml ∗ h was determined to be 2170 µg /kg/day.

Table 1. Calculation of the area under the curve (0-72 hr) for plasma PT in rat and human. The rat NOEL for ZPT is 500 µg/kg/day based on hindlimb weakness observed in a 2-year study in rats Larson, 1958. In the repeat dose pharmacokinetic study, the plasma concentration of PT where there was no effect in rats was 260 ng/ml ∗ h. Using the PBPK model, the doses of ZPT needed to produce the AUCs of 260 ng/ml ∗ h were determined.

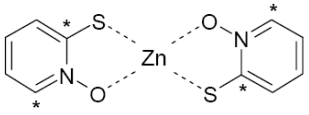
NOEL: Rat	AUC $PT_{0\text{-}72\ hour}$	Human Equivalent
µg/kg/day	ng/ml ∗ h	µg/kg/day
500	260	2170

Conclusions: A PBPK model for Zn-PT in the rat was developed for simulating plasma, blood, and tissue kinetics of PT and its two major metabolites, SG(s) and MSP. The model replicates experimentally-observed short-term elimination kinetics of PT and pyrithione-derived carbon (PTC) i.e., ^{14}C derived from the administration of ^{14}C –ZPT, following single doses and longer-term temporal pattern blood concentrations of PTC during repeated dosing schedules. The model also accounts for the production and rapid elimination of SG, the major metabolite of PT in urine, as well as production and slower elimination of the minor metabolite, MSP. The PBPK model was used to determine the human dose, 2170 µg/kg/day (Table 1), needed to achieve plasma AUC for PT (i.e., reverse dosimetry) equivalent to the NOEL in the rat (i.e., 500 µg/kg/day).

4.2 DISPOSITION OF ZINC PYRIDINETHIONE (ZPT) IN FEMALE RATS: REPEATED DOSE PHARMACOKINETIC STUDIES (ANNEX II).

Background: The pyrithione (PT) moiety of ZPT is responsible for the toxicological effects. The evidence for this is based on the comparable toxicological responses for PT salts of sodium, copper, magnesium and zinc which differ mainly on their solubility (Howes *et al.*, 1975; Mitoma *et al.*, 1983; Gibson *et al.*, 1985). To date, pharmacokinetic studies with ZPT have relied on measures of ^{14}C (PTC) which includes parent, PT, and any metabolites. The studies presented herein are the first to directly measure PT in rats at select time points after 10-day administration of ^{14}C-ZPT by gavage, diet, and dermal routes of exposure. Hindlimb muscle weakness and tone were measured in the same group of rats to determine the relationship between PT plasma concentrations and toxicity. Tissue disposition of radiolabel was measured to determine if there was any evidence of accumulation from different routes of administration. Data in these studies were used to verify/validate the PBPK model and human measures of 24-hr urinary output

Methods: Female, Sprague Dawley rats, 11-12 weeks of age weighing 182–235 g at study initiation were used in all studies. ^{14}C-labeled Zinc Pyrithione (CAS No. 13463-41-7, * indicates location of ^{14}C-label, 2 and 6 positions of pyridine rings) was used.



Rats were prepared with an indwelling jugular cannula for collection of serial blood samples. Blood was sampled through indwelling jugular cannulae from each animal, pre-dose (time 0) and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h following day 1 and day 10 of gavage, dietary, and dermal administration. Approximately 250 µL of blood was withdrawn using heparinised syringes. Aliquots of blood were taken, centrifuged to obtain plasma and frozen. Blood urine, faeces, and tissue samples were assayed for total radioactivity by liquid scintillation spectrometry (LSS). Plasma was analyzed by for pyrithione (PT) by mass spectrometry (LC/MS/MS).

Animals were weighed prior to each dose administration. Oral gavage and dermal routes were administered a dose of ZPT on a mg/kg body weight. Animals in the dietary groups received dose as concentration (ppm) in the feed.

- Dietary**:** Rats were dosed with ZPT in the feed for up to 10 days at a concentration of 250 ppm ZPT. The feed remained with the animal for 8 h during the light part of the light/dark cycle. Consumption was calculated as the difference in the weight of the jar at times zero and 8 h.
- Oral Gavage: Rats received an oral dose of 1.25, 3.0 or 10.0 mg/kg ZPT directly into the stomach via a syringe equipped with a 16-gauge ball-tipped stainless steel feeding needle with a 3-mm ball tip.
- Dermal Exposure: Applied dermal doses were 10, 30 and 100 mg/kg ZPT. On the day before the first dose and as needed thereafter, the fur within the dose site was removed with clippers so that the test material was applied to bare skin. The dose site area, 12 cm^2 (3 x 4 cm), was outlined on the back of each rat and a protective foam appliance was secured to the shaved area. After each dose was applied, a non-occlusive cover was placed over the appliance. Six hours after application, the appliance was removed. The dose site was gently scrubbed with gauzes, rinsed and wiped with dry. A new appliance was secured.
- Intravenous administration: In the pilot study, the intravenous dose was a single bolus administration of 0.5 mg/kg. Rats received an iv bolus dose by tail vein injection using a 1 mL disposable syringe equipped with a 27 gauge x ½" needle.

Pharmacokinetic calculations using plasma concentration-time data from individual animals were analyzed, as appropriate, by non-compartmental (model-independent) methods using the least-squares fitting program WinNonlin (Statistical Consulting Inc., Cary, NC). Pharmacokinetic estimates were made after fitting a model to the plasma concentration-time data.

After day 1 and 10 of dosing, muscle strength was determined. For *muscle tone*, the rat was supported by grasping the thorax gently from behind. With the free hand, the tester gently pressed the tips of two fingers (or one finger and thumb) into the middle of the plantar surface (i.e., footpads) of each hind limb (one digit into each footpad). As the rat extended the hind limbs, the presence/strength of the extensor response was

evaluated subjectively and extensor response was recorded as: (a.) None, (b.) Low, (c.) Moderate or (d.) High. For *muscle mass,* the tester "rolled" the extensor muscles of the tarsus between the tester's thumb and forefinger and evaluated the muscle mass subjectively via digital palpation. The response is recorded as follows: (a.) Normal muscle mass, (b.) Slightly reduced muscle mass, (c.) Greatly reduced muscle mass.

Results: [^{14}C]-ZPT administration to female rats by intravenous injection, dermal application, oral gavage or in diet was excreted primarily in the urine, consistent with earlier studies (Wedig, et al., 1978b; Mitoma et al., 1983). As such, urinary excretion can be used as a surrogate measure of absorption into the systemic circulation. Table 2 presents the percent cumulative radiolabel measured in urine and feces at 72 hr. The total percent dose recovered represents all possible sources of radioactivity including urine, feces, carcass, tissues, etc.

Table 2. Cumulative Excretion of Radioactivity

Route	Dose	Cumulative % of dose recovered in:		Total % dose recovered
		Urine	Feces	
i.v.	0.5 mg/kg	83	1.8	92
Gavage	0.5 mg/kg	82	3.6	92
	5.0 mg/kg	76	7.0	88
Diet	10 ppm	68	12.6	90
	50 ppm	72	7.5	90
	250 ppm	67	2.7	87
Dermal	0.3 mg/kg	4.6	0.6	16/76*
	10 mg/kg	2.0	0.2	5/86*
	100 mg/kg	2.8	0.2	6/77*

*Percent of applied dose delivered/percent of applied dose unabsorbed

The concentration of total radiolabel in blood was higher and slightly more persistent in blood compared to parent, i.e., PT, concentration in plasma. This is consistent with previous studies that identified PT metabolites as the main constituents in serum with longer half-life (Wedig, et al., 1978a; Gibson *et al.*, 1982).

A partial summary of the results from the repeat dose PK studies is presented in Table 3.

TABLE 3. Summary of Non-compartmental Pharmacokinetic Estimates for ZPT Administered Orally[1,2]

dose	Study		t_{max}	C_{max}	k_{el}	AUC 0-∞	V_d/F	CL/F	AUC ratio
mg/kg/day	Day	N	h	ng/ml	h^{-1}	h x ng/mL			0-24 h
1.25	1	6	0.4 ± 0.3[3]	151 ± 65	0.29 ± 0.13	268 ± 100	22.2 ± 14.1	5.0 ± 1.4	
1.25	10	6	0.5 ± 0.1	129 ± 62	0.28 ± 0.06	291 ± 37	16.1 ± 4.9	4.3 ± 0.5	1.3 ± 0.2
3	1	7	0.4 ± 0.3	268 ± 94	0.16 ± 0.03	884 ± 191	21.7 ± 4.1	3.5± 0.6	
3	10	4	0.9 ± 0.8	156 ± 53	0.15 ± 0.12	826 ± 181	34.2 ± 15.9	3.7± 0.7	1.0 ± 0.5
10	1	7	0.4 ± 0.3	415 ± 45	0.12 ± 0.02	1495 ± 400	59.9 ± 11.3	7.1± 1.9	
10	9	6	0.6 ± 0.3	608 ± 172	0.11 ± 0.05	3422 ± 1677	31.7 ± 12.7	3.5 ± 1.4	2.8 ± 1.8

1: Parameters are: t_{max},: time at which C_{max} was observed; C_{max}: observed maximum concentration in plasma; k_{el}: terminal elimination rate constant, AUC: area under the plasma concentration vs. time curve; V_d/F: volume of distribution, CL/F: systemic clearance, C_{max} ratio: ratio of C_{max} day 10 (or 9) to that for day 1, AUC ratio$_{0\text{-}24\ h}$: ratio of AUC for day 10 (or 9) to that for day 1.
2: Any concentrations < limit of quantitation were set to zero for calculation of pharmacokinetic parameters
3: All values are mean ± standard deviation

In gavage-treated rats, concentrations of PT in plasma reached maximal levels within 0.4–0.9 h, then declined with mean half-lives that ranged from 2.6 to 6.9 h with no difference between first and last dose. Ratios of day 10 (9 for the high dose) to day 1 for both C_{max} and AUC were less than 3 at all doses, suggesting that accumulation of PT was minimal.

There was a strong relationship, $r^2 = 0.89$, between muscle weakness (tone + mass) on day 10 and the AUC plasma concentration of pyrithione obtained after the last dose (Figure 5). Limit of detection for PT was used to calculate the AUC$_{0\text{-}72\ h}$ equivalent to 0 muscle weakness. The 7 treatment groups, gavage 1.25, 3.0

and 10.0 mg/kg, dermal, 10, 30 and 100 mg/kg and dietary, 250 ppm, are represented in the figure. The NOEL (no observed effect level) was 0.5 mg/kg/day and equivalent to "normal" muscle mass and tone.

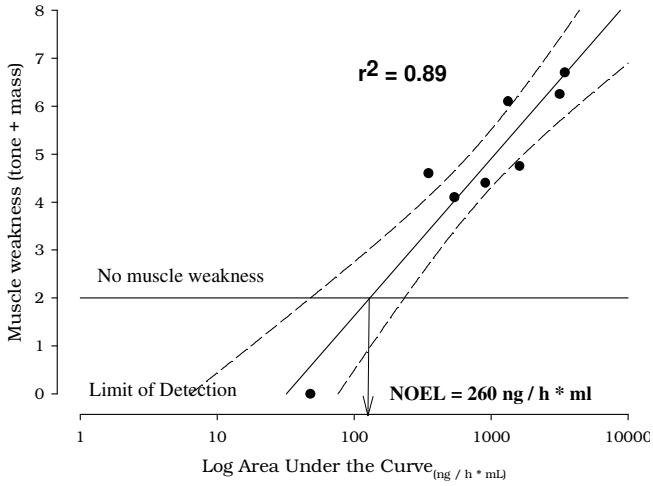


Figure 5: Relationship between muscle weakness on last day of dosing (Day 10) and AUC for plasma pyrithione. Each point represents the mean of 4-7 rats for the 7 different treatments. The limit of detection for plasma PT was assumed to be equivalent to 0. The NOEL, 0.5 mg/kg/day, was 260 ng/h*ml.

Conclusions: Urine excretion was the primary route of elimination for ZPT from all routes of administration, supporting the use of 24-hr cumulative urinary as a measure of bioavailability from human studies. The concentration of total radioactivity in blood was higher and more persistent than plasma PT, consistent with previous studies in which PT metabolites, including 2-methylsulfonylpyidine (MSP), were found as the main constituents in the blood. There was no apparent bioaccumulation of radiolabeled PT in any of the tissues evaluated in this study. The highest amount of radioactivity was in the liver, kidney and blood for all routes of administration.

Approximately 80-90% of the gavage or dietary ^{14}C-ZPT was bioavailable compared to less than 10% for dermal application. There was an 8-12 hr lag time for ^{14}C-PT or PT to appear in the blood/plasma following dermal exposure. There was a strong positive relationship between area-under-the-curve plasma PT concentration and muscle weakness independent of route of administration.

From this study, it is concluded that urinary excretion is the principle route of elimination of ZPT, systemic bioavailability of ZPT following topical administration is limited and concentration of blood ^{14}C-PT over predicts plasma PT levels. As well, plasma PT appears to be positively-related to muscle weakness, the critical toxicological effect attributed to ZPT administration.

Data obtained in the oral gavage repeat dose pharmacokinetic studies were used to verify and optimize the PBPK model.

4.3 AN EVALUATION OF THE DEPOSITION, ABSORPTION, AND EXCRETION OF ^{14}C RESULTING FROM AN ANTI-DANDRUFF TREATMENT REGIMEN CONSISTING OF SHAMPOO AND A LEAVE-ON TONIC CONTAINING ^{14}C-PYRITHIONE ZINC (ANNEX III)

Background: In the previously reviewed submission for the non-preservative use of ZPT in cosmetic rinse-off and leave-on hair care products and for the preservative use of ZPT in rinse-off hair care products Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers, 2002[†], a clinical study measuring the bioavailability of 1% ZPT from an antidandruff shampoo was presented. This study was essential to the calculation of the MoE.

In this regard, the new study presented herein was designed to measure the deposition, systemic absorption and excretion of ^{14}C-radiolabeled pyrithione zinc (ZPT) resulting from the repeated application of treatment regimens including 2% ZPT containing antidandruff shampoo. These data were instrumental in our determination of the MoE for 2% ZPT.

Methods: This was a 7-day randomized, parallel group comparison study. It consisted of a 4-day treatment period followed by a 3-day wash-out period. Subjects who agreed to participate in this study were sequestered at the testing facility for the entire study period. A total of thirty (30) male and female subjects between the ages of 18 to 65 were enrolled. To qualify for enrollment, the subjects had to have mild to severe dandruff or seborrheic dermatitis of the scalp with a total adherent scalp flaking score (ASFS) of $\geq$12.

During the 4-day treatment, subjects washed their own hair with 10 g of the shampoo containing ^{14}C-ZPT. The shampoo was applied only one time each day and was rinsed from the hair. All of the water used for rinsing the shampoo lather from the subjects' hair and hands was collected in a single container for analysis of ^{14}C-radioactivity. The treatment regimens were:

Treatment Regimen	Test Products
A	Shampoo with 1% ^{14}C-ZPT + Leave-on Tonic with 0.1% ^{14}C-ZPT (2 applications)
B	Shampoo with 2% ^{14}C-ZPT + Leave-on Tonic with 0.1% ^{14}C-ZPT (2 applications) + Leave-on Tonic with 0.25% ^{14}C-ZPT (1 application)
C	Shampoo with 2% ^{14}C-ZPT + Leave-on Tonic with 0.25% ^{14}C-ZPT (3 application)

Immediately following rinsing of the shampoo, subjects applied the 1st – 4g dose of the ^{14}C-ZPT containing leave-on tonic to their wet hair. Following application, the subjects' hair was blown dry. Subjects assigned to Treatment Regimen A, applied 2 doses of the leave-on tonic. The 1st dose was applied immediately after the shampoo application and the 2nd dose was applied 8 hours later. Subjects assigned to treatment regimens B and C, applied 3 doses of the tonic. Their 1st dose was also applied immediately shampoo application. The 2nd and 3rd doses were applied at 4 to 6 hour intervals after the 1st dose. These doses were applied to dry hair.

To measure the deposition of the ^{14}C-ZPT on the scalp and hands, tape-stripping of the scalp and/or fingertips was done on days 1, 2, 3 and 4 after the daily treatment products were applied; on day 5 (24 hours after the 4th application of the treatment regimen) and, on day 7 of the wash-out period (after the 3rd hair wash with a regular shampoo). In addition to tape stripping, hair specimens were clipped from the subjects' scalps at the same time that the tape-stripping was done to measure the deposition of ^{14}C-ZPT on the hair. To measure the systemic absorption and excretion of ^{14}C-ZPT, 24 hour urine specimens were collected during the entire study period. Subjects were dismissed on day 8 at the completion of the 24 hour urine collection at day 7.

Results: The measured scalp deposition did not increase with repeated. As expected, the scalp deposition rate was generally highest in Treatment Regimen C (max. 1.92 µg/cm^2), followed by B (max. 1.39 µg/cm^2), then A (max. 0.51 µg/cm^2). The total mass of ZPT deposited on the hands was typically about 25% - 50%

† * Application to the Scientific Committee for Cosmetics and Non Food Products. Proposal for Annex III listing of Zinc Pyrithione. Colipa, June 27, 2000.

of that on the scalp. Scalp + Hands deposition as a percentage of the applied quantity was less than 1% in all treatment groups (max. 0.52% for A, 0.63% for B and 0.88% for C).

The quantity of ZPT deposited on the hair was typically about 5x-10x higher than the amount deposited on the scalp, demonstrating that most of the ZPT deposited is not on the skin. Hair deposition ranged from approximately 2%-4% in Treatment Regimens A and B and about 4%-6% in Treatment Regimen C.

Urinary ZPT excretion reached an apparent steady state for all three treatment groups during the 4-day treatment period. This was demonstrated by the lack of significance difference between the mass excreted on day 4 vs. day 3 in all treatment groups. Therefore, the mean values calculated on day 4 were used to approximate daily ZPT absorption and excretion during longer term consumer use. The 24 hour excretion measurements were normalized to body weight for each subject to provide estimates of daily internal exposure (systemic load). Systemic loads were significantly higher for Treatment Regimens C (4.66 µg/kg/day) and B (4.38 µg/kg/day) compared to A (2.82 µg/kg/day).

There was no significant difference by gender in scalp deposition, absorption, mass excreted or systemic load of ZPT during the 4-day treatment period.

Conclusions: Data for urinary excretion of ZPT provides a reasonable surrogate for internal exposure that can be used for safety assessments (i.e., MoE calculations). There are several studies that have shown that ZPT is almost exclusively excreted in the urine and that ZPT disposition is similar among several species (Wedig, et al., 1978b; Jeffcoat, et al., 1980). Deposition of ZPT on skin appears to be a saturable phenomenon. The systemic load (internal dose) of PTC following use of a shampoo containing 2% ZPT was 4.66 ug/kg/day which was used in the calculation of Margin of Exposure.

5 EXPOSURE CALCULATIONS AND RISK ASSESSMENT

The exposure assessment is an important calculation for consideration when assessing the risk to human health associated with exposure to ZPT through consumer use of rinse-off hair care products.

The exposure assessment is based on data generated in the human pharmacokinetic study with 2% ZPT (ANNEX III) and the PBPK model (ANNEX I). The following values were used:

- Systemic (internal) dose of ZPT = 4.66 µg/kg/day or 4.66 x 10^{-3} mg/kg/day (ANNEX III)
- Rat reference NOEL = 0.500 mg/kg/day based on rat oral administration study (Larson, 1958).*

There are several studies including the new ones presented in ANNEX II that have shown ZPT is excreted predominantly in the urine and that ZPT disposition is similar among several species (Wedig, et al., 1978a; Wedig et al., 1978b; Jeffcoat et al., 1980). In these studies and current ones (ANNEX II), ZPT was shown to be well absorbed from the GI tract and, therefore, the oral NOEL does not require an absorption adjustment for the purposes of comparison with human systemic exposure estimates as determined in the clinical pharmacokinetics study.

Standard calculations of Margin of Exposure (MoE):

MoE – human exposure versus rat NOEL = NOEL/Systemic exposure
= 0.5 mg/kg/day ÷ 4.66 x 10^{-3} mg/kg/day
= 107

Calculations of MoE using PBPK model

MoE – human exposure verse rat NOEL = Human dose equivalent to rat NOEL[A]/Systemic exposure
= 2.17 mg/kg/day ÷ 4.66 x 10^{-3} mg/kg/day
= 465

[A]The human equivalent to the NOEL in the rat was calculated to be 2170 µg/kg/day, using the ZPT PBPK model.

Based upon these calculations, there is an approximate 465-fold difference (2170 µg/kg/day ÷ 4.66 µg/kg/day) between the internal human dose from the exaggerated use of a 2% ZPT shampoo and the external human dose equivalent to the NOEL in rat

It is concluded that consumer use of a rinse off hair product containing 2% ZPT dose not present a risk to human health.

* Application to the Scientific Committee for Cosmetics and Non Food Products. Proposal for Annex III listing of Zinc Pyrithione. Colipa, June 27, 2000.

APPENDIX I REFERENCES

1. Bruckner, J. V. (2000). Differences in sensitivity of children and adults to chemical toxicity: The NAS panel report. Regulatory Toxicology and Pharmacology **31**, 280-285.

2. Demchuk, E., Ruiz, P., Wilson, J. D., Scinicariello, F., Pohl, H. R., Fay, M., Mumtaz, M. M., Hansen, H., and De Rosa, C. T. (2008). Computational toxicology methods in public health practice. Toxicology Mechanisms and Methods **18**, 119-135.

3. Gibson, W. B., Jeffcoat, A. R., and Turan, T. S. (1982). Zinc pyridinethione: Serum metabolites of zinc pyridinethione in rabbits, rats, monkeys, and dogs after oral dosing. Toxicology and Applied Pharmacology **62**, 237-250.

4. Gibson, W. T., Chamberlain, M., Parsons, J. F., Brunskill, J. E., Leftwich, D., Lock, S., and Safford, R. J. (1985). The effect and mode of action of zinc pyrithione on cell growth. I. In vitro studies. Food Chem Toxicol **23**, 93-102.

5. Howes, D. and Black, J. G. Comparative percutaneous absorption of pyrithiones. Toxicology 5, 209-220. 1975.

6. Jeffcoat, A. R., Gibson, W. B., Rodriguez, P. A., Turan, T. S., Hughes, P. F., and Twine, M. E. (1980). Zinc pyridinethione: urinary metabolites of zinc pyridinethione in rabbits, rats, monkeys, and dogs after oral dosing. Toxicol Appl.Pharmacol **56**, 141-154.

7. Kabacoff, B. L., Fairchild, C. M., and Burnett, C. (1971). Pyridinethione glucuronide as a metabolite of sodium pyridinethione. Food Cosmet.Toxicol **9**, 519-526.

8. Krishnan, K., Haddad, S., liveau, M., and Tardif, R. (2002). Physiological modeling and extrapolation of pharmacokinetic interactions from binary to more complex chemical mixtures. Environmental Health Perspectives **110**, 989-994.

9. Larson, Paul S. Toxicologic Observations on the Effect of adding Zn-Pyridinethione to the diet of rats for a period of two years. 1-15. 1958.

10. Min, B. H., Parekh, C., Golberg, L., and McChesney, E. W. (1970). Experimental studies of sodium pyridinethione. II. Urinary excretion following topical application to rats and monkeys. Food Cosmet.Toxicol **8**, 161-166.

11. Mitoma, C., Steeger, T., Rogers, J., Thomas, D., and Wedig, J. H. (1983). Metabolic disposition of pyrithiones. Fundam.Appl.Toxicol **3**, 256-263.

12. Price, P. S., Conolly, R. B., Chaisson, C. F., Gross, E. A., Young, J. S., Mathis, E. T., and Tedder, D. R. (2003). Modeling Interindividual Variation in Physiological Factors Used in PBPK Models of Humans. Critical Reviews in Toxicology **33**, 469-503.

13. Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Opinion Concerning Zinc Pyrithione, Colipa No. P81. SCCNFP/671/02. 12-17-2002. 12-17-2002.

14. Wedig, J. H., Mitoma, C., Howd, R. A., and Thomas, D. W. (1978a). Identification of metabolites from salts of pyridine-2-thiol-1-oxide following intravenous and dermal adminstration to swine. Toxicol Appl.Pharmacol **43**, 373-379.

15. Wedig, J. H., Wentworth, R. A., and Gallo, M. A. (1978b). Disposition of zinc pyrithione in the rat. Food and Cosmetics Toxicology **16**, 553-561.

16. Ziller, S. A. (1977). Absorption, excretion and tissue distribution of 2-pyridinethiol-1-oxide. Food Cosmet.Toxicol **15**, 49-54.

APPENDIX II BREAKDOWN OF NEW REFERENCE STUDIES

PHYSIOLOGICAL-BASED PHARMACOKINETIC MODEL

Reference: Diamond (2004) and Diamond (2006)

Results: A physiologically-based pharmacokinetic (PBPK) model of ZPT was developed to further understand relationships between toxicity and internal dosimetry of ZPT in the rat. The model simulates the kinetics of absorption, distribution, and excretion of pyrithione (PT) and its major metabolites 2-(methylsufonyl)pyridine (2-MSP) and the S-glucuronide (SG) conjugates of 2-pyridinethiol and 2-pyridinethiol-1-oxide following single or repeated oral administration of [^{14}C]ZPT to female rats. Tissue compartments represented are: kidney, liver, brain, skeletal muscle, other rapidly-perfused tissues (tissues other than liver and kidney that have a relatively high-blood flow/g tissue; e.g., heart, viscera), slowly-perfused tissue (e.g., adipose, skeletal muscle, skin), and red blood cells (RBC). Transfers of PT and metabolites between plasma and tissues other than RBC are believed to be sufficiently rapid to produce a steady state within the time of blood flow from the arterial to venous vasculature of each tissue (i.e., flow-limited). For plasma and RBCs, transfers are represented as first-order processes of unlimited capacity. Metabolism is attributed to the liver compartment and is represented as distinct first-order (unlimited capacity) processes for conversion of PT to MSP and SG. The model was used to calculate human doses needed to achieve plasma AUC for PT (i.e., reverse dosimetry) in the rat. The dose of ZPT in the human equivalent to the NOEL in the rat (i.e., 500 μg/kg/day) was 2170 μg/kg/day. The PBPK model is supportive evidence of the human safety of ZPT used in antidandruff shampoos. These new data more accurately define the difference between plasma concentrations of PT expected from use of a shampoo and the rat NOEL.

Publications: Nash, J.F., Diamond, G.: A physiologically-based pharmacokinetic (PBPK) model of zinc pyrithione in the rat. *Society of Toxicology,* 2006.

Diamond, G.L., Nash, J.F. Use of a Physiologically-based Pharmacokinetics (PBPK) Model to Predict Internal Dosimetry of Zinc Pyrithione (ZPT) in Rats and Humans. *Society of Toxicology,* 2008.

Diamond, G, Skoulis, N.P., Jeffcoat, A.R., Nash, J.F. A physiological-based pharmacokinetic model for the antimicrobial zinc pyrithione. *Toxicological Sciences*, submitted, 2008.

PHARMACOKINETICS (RAT)

Reference:	Jeffcoat *et al.* (2007)
Substance/Purity:	Zinc Pyridinethione (CAS No. 13463-41-7) The radiochemical purity of Zn-^{14}C-PT in 0.1% triethanolamine lauryl sulfate was 95.8%.
Species/Sex:	Female, Sprague Dawley rats, 11-12 weeks of age weighing 182−235 g at initiation of dose administration were used
Number of animals:	N=162 (5-7/treatment group); 2/treatment in pilot studies
Route of exposure:	gavage, diet, intravenous, and dermal
Vehicle:	oral and dermal: 0.1% triethanolamine lauryl sulfate
Dosing:	gavage: 1.25, 3.0 and 10.0 mg/kg/day; dermal: 10, 30 and 100 mg/kg/day; diet: 250 ppm; intravenous: 0.5 mg/kg in
Duration:	9-10 days + 4 day recovery
Results:	Urine excretion was the primary route of elimination for all routes of administration. The concentration of total radioactivity in blood was higher and more persistent than plasma PT. This is consistent with previous studies in which PT metabolites, including 2-methylsulfonylpyidine (MSP), were found as the main constituents in the blood. There was no apparent bioaccumulation of radiolabeled PT in any of the tissues evaluated in this study. The highest amount of radioactivity was in the liver, kidney and blood for all routes of administration. Approximately 70-80% of the gavage or dietary 14C-ZPT was bioavailable compared to 4% for dermal application. There was an 8-12 hr lag time for ^{14}C-PT or PT to appear in the blood/plasma following dermal exposure. From this study, it is concluded that urinary excretion is the principle route of elimination of ZPT, systemic bioavailability of ZPT following topical administration is limited and concentration of blood ^{14}C-PT over predicts plasma PT levels.
Publications:	Nash, J.F., Jeffcoat, A.R., DP Coleman, D.P., Parham, A.J., Garner C.E., Skoulis, N.P. Disposition of Pyrithione and Muscle Weakness in the Rat after Repeat Dose Administration of Zinc Pyrithione. *Society of Toxicology,* 2007. Skoulis, N.P., Jeffcoat, A.R., DP Coleman, Valentine, J.L., Nash, J.F., Disposition of Pyrithione in the Rat After Oral, Dermal and Intravenous Routes of Administration. *Society of Toxicology,* 2007.

PHARMACOKINETICS (HUMAN)

Reference: Vater (2000)

Substance: ^{14}C-zinc pyrithione

Species/Sex: Human/male and female

Number of subjects: N=30 (10/treatment group)

Route of exposure: Topical (dermal/scalp) application of shampoo + tonic

Vehicle: shampoo

Dosing: 2% ZPT Shampoo (10 g); Hair tonic (4 g)

Duration: 4 days + 3 day wash-out

% Dose Recovered: 61-86%

Results: The measured scalp deposition did not increase with repeated exposure except in treatment C where deposition did show a directional increase. The quantity of ZPT deposited on the hair was typically about 5x-10x higher than the amount deposited on the scalp, demonstrating that most of the ZPT deposited is not on the skin. Urinary ZPT excretion reached an apparent steady state for all three treatment groups during the 4-day treatment period. This was demonstrated by the lack of significance difference between the mass excreted on day 4 vs. day 3 in all treatment groups. Therefore, the mean values calculated on day 4 were used to approximate daily ZPT absorption and excretion during longer term consumer use. The 24 hour excretion measurements were normalized to body weight for each subject to provide estimates of daily internal exposure (systemic load). Systemic loads were significantly higher for Treatment Regimens C (4.66 µg/day) and B (4.38 µg/day) compared to A (2.82 µg/day). Excretion data were also used to calculate dermal absorption of ZPT as a percentage of the total quantity deposited on the scalp (scalp deposition alone was judged to be the appropriate denominator because most of the ZPT on the hands was shown to be removed by hand washing). The corresponding mean percentages absorbed (as a function of scalp deposition; excluding deposition on hands) were 71.6%, 33.6% and 26.5%, respectively, for Treatment Regimens A, B and C. Thus, it appeared that the percentage absorbed decreased with increased deposition rate on the scalp. There was no significant difference by gender in scalp deposition, absorption, mass excreted or systemic load of ZPT during the 4-day treatment period.

ANNEX

I FINAL REPORT: Physiologically-based pharmacokinetics model of zinc pyrithione in the rat (June 2006)

FINAL REPORT: Feasibility study for a physiologically-based pharmacokinetics model of zinc pyrithione (August 2004)

II FINAL REPORT: Disposition of zinc pyridinethione (ZPT) in female rats: repeated dose oral and dermal pharmacokinetic studies (2007).

III CLINICAL STUDY FINAL REPORT: An evaluation of the deposition, absorption, and excretion of ^{14}C resulting from an anti-dandruff treatment regimen consisting of shampoo and a leave-on tonic containing ^{14}C-pyrithione zinc (Study Number CRB 0005-067 HC)