Formulation and Artificial Sebum Effects on the Percutaneous Absorption of Zinc Pyrithione Through Excised Human Skin

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Supplementary Material

S1. Review of Zinc Pyrithione (ZnPT) skin penetration data

S1.1 In vivo percutaneous absorption of ZnPT in laboratory animals

Studies in multiple animal species show variability in the magnitude of percutaneous absorption for ZnPT (Fig. S1). In these studies, the amount of ZnPT systemically absorbed was determined by measuring a metabolite/degradation product of pyrithione excreted in the urine, the primary route of elimination [1-5]. The data generated by Howes and Black [6] showed that the percutaneous absorption rate of ZnPT deposited onto rat skin from shampoo remained relatively constant as the dose was increased from 12.8 to 230 μg/cm². Generally speaking, there is an inverse relationship between applied dose and percent absorption in these studies.

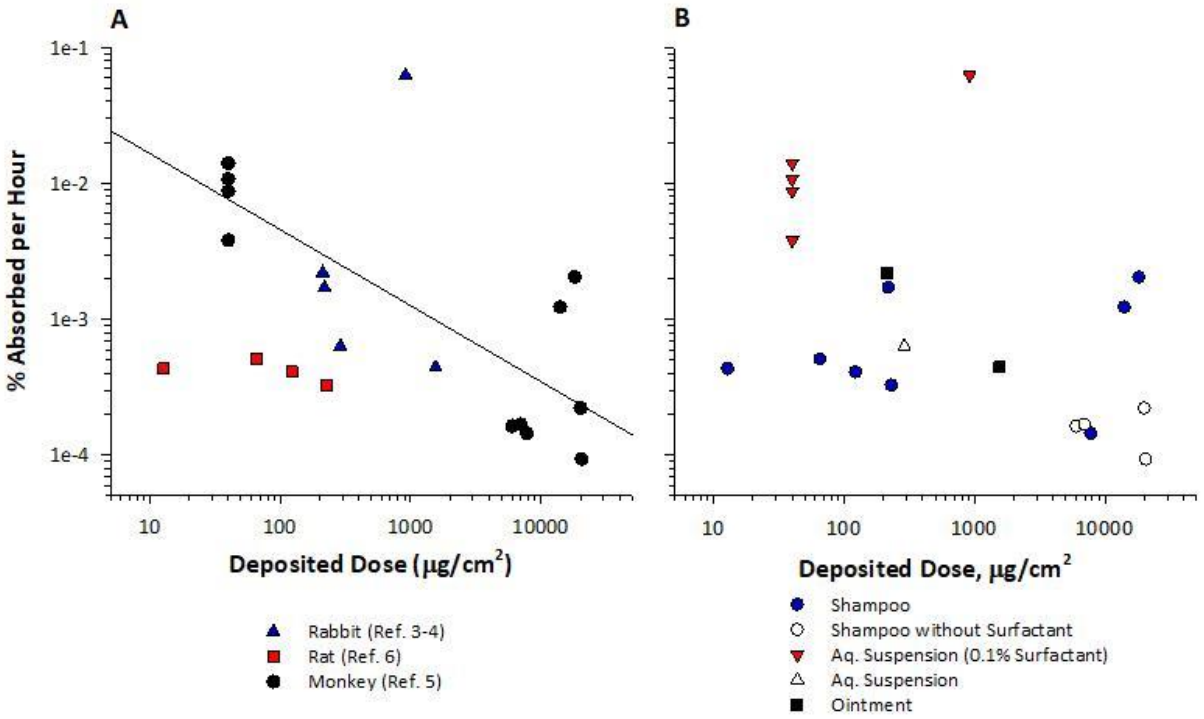

Figure S1. In vivo percutaneous absorption rates of ZnPT deposited (A) on the skin of different animal species and (B) from different formulations using the same data points as Panel A. The amount absorbed is considered to be the amount that was collected in the urine. Linear regression is plotted if the slopes in the dose response curves were significantly less than zero.

S1.2 In vitro human studies

Although animal percutaneous absorption data can be used to estimate the upper limits of absorption in human skin, in vitro human skin studies provide assessments of permeability in the relevant species. A summary from one such study (Study 1 in Table S1) demonstrated this species difference by comparing the in vitro skin permeability of Zn[^{14}C]PT applied to rat and human skin from an aqueous 1% carboxymethylcellulose solution (CMC) (available in [7]). In the SCCS opinion [7], a 10-fold increase in total absorption of the radioactive dose was observed for rat skin compared to human skin, when total absorption was calculated as the sum of the amount of radioactivity (calculated as µg Zn[^{14}C]PT-equivalents/cm^2) collected in the receptor compartment, viable epidermis and dermis. This 10-fold increase is typical when comparing in vitro skin absorption data for these two species and has been suggested as a correction factor to adjust rat data for use in human dermal risk assessments [8].

The results of in vitro human skin penetration studies with Zn[^{14}C]PT in hair care formulations (i.e., shampoo or leave-on tonic) or a simple 0.1% surfactant solution are also shown in Table S1 (Study 2 [9] and Study 3 [10]). Taking all of the values from human skin and plotting percent absorption vs. deposited/applied dose (Table S1), an important finding emerges. As shown in Fig. S2 A and B, the skin penetration of radioactivity associated with Zn[^{14}C]PT was lower when dosed from formulations that did not contain surfactant compared to those that did contain surfactant ($p < 0.01$), which is consistent with present findings (see Table 2). This result may be attributed to the deposited ZnPT being more soluble in

vehicle containing surfactant and, perhaps, in more intimate contact with the skin surface and available to diffuse when dosed from surfactant suspensions.

Table S1. In vitro percutaneous absorption of Zn[^{14}C]PT following a 24 hour collection period. Results are expressed as % of radioactive dose or μg Zn[^{14}C]PT-equivalents/cm^2.

	Formulation	Dose	Cumulative Absorption		
			Receptor Fluid	Receptor Fluid + Skin	
		(μg/cm²)	(%)	(%)	(μg/cm²)
Study 1[a]					
Human	1% CMC	101	0.02	0.76[b], 1.3[c]	0.77[b], 1.3[c]
Rat		101	1.1	7.8[b], 9.3[c]	7.9[b], 9.3[c]
Study 2[d]					
Human	0.1% Surfactant	0.64	19.7	34.4[e]	0.22
		1.2	10.8	24.4[e]	0.29
		11.7	3.1	16.5[e]	1.9
		103	0.37	15.7[e]	16.2
	1% Shampoo	0.75	8.4	14.7[e]	0.11
	0.1 % Tonic	5.7	1.1	1.9[e]	0.11
	0.1% Tonic (No Silicone)	3.3	1.9	3.3[e]	0.11
Study 3[f]					
Human	Dilute Shampoo	0.32	13.6	34.7[e]	0.11
		0.69	9.9	25.2[e]	0.17
	Shampoo + Leave-on Tonic	0.87	8.9	23.3[e]	0.20
		1.7	7.0	18.6[e]	0.32
	Leave-on Tonic	5.0	0.83	2.5[e]	0.13
		17.7	0.36	1.2[e]	0.21
		20.1	0.39	1.2[e]	0.24
		30.5	0.43	1.2[e]	0.37
	Shampoo + Leave-on Tonic[g]	4.2	11.3	24.8[e]	1.0
		7.0	9.3	23.1[e]	1.6

[a] Data publically available in Ref **[11]**
[b] Receptor fluid + viable epidermis + dermis
[c] Receptor + viable epidermis + dermis + SC tape strips (6-20)
[d] Ref **[9]**
[e] Receptor + epidermis + dermis
[f] Ref **[10]**
[g] Repeat administration study (BID for 3 days)

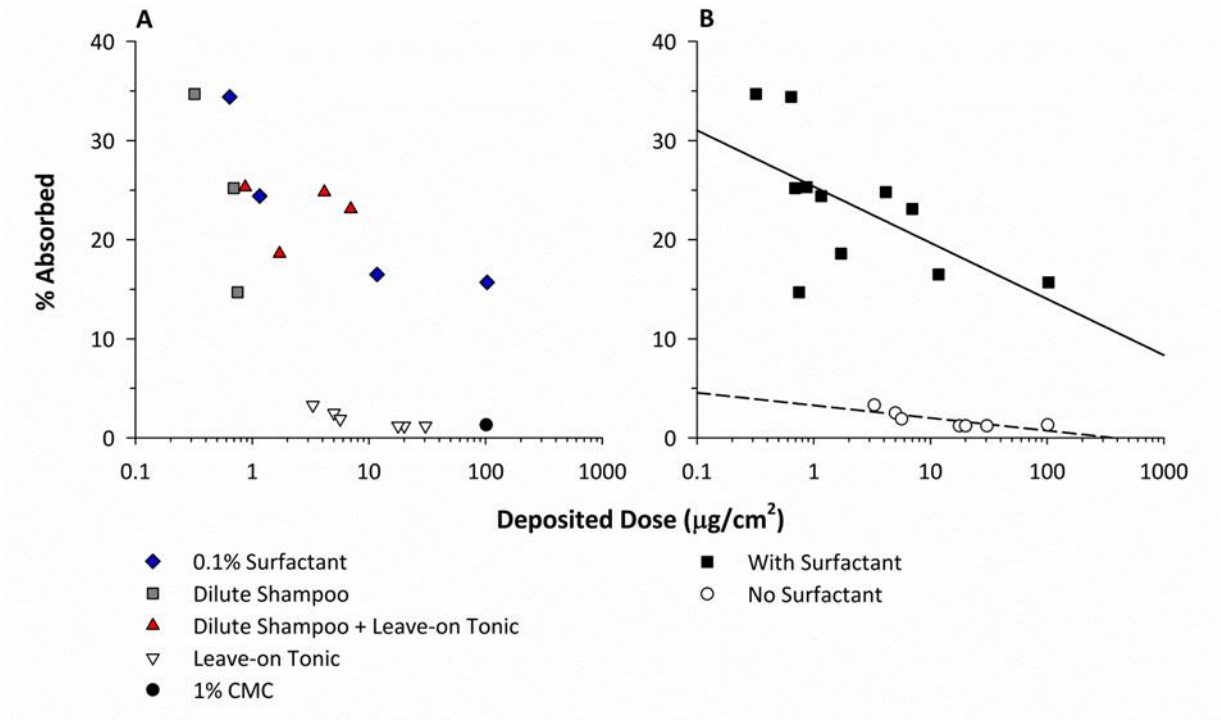

Figure S2. Summary of the Zn[^{14}C]PT *in vitro skin penetration data presented in Table S1 for (A) variable formulations (B) with and without surfactant. Non-linear regression is plotted if the slopes in the dose response curves were significantly less than zero.*

S1.3. Knowledge gaps in ZnPT skin penetration data

The available data from numerous ZnPT skin penetration studies are difficult to distill into a consise, singular conclusion given the wide range of exposure conditions and lack of replication. Skin dispositions vary between the studies due to intrinsic inter- and intra-species variations in the biophysical characteristics of skin [12] as well as differences in experimental protocols (e.g. vehicles/formulation, dose level, study duration) and subsequent analysis of the data [13-15]. For most compounds, percent absorption is inversely related to mass coverage over a certain dose range and is a relative term that should be interpreted cautiously [16]. Recent analyses have put these relationships on a more quantitative basis [17, 18]. A majority of the ZnPT doses used in the in vivo animal studies and in the cited in vitro human study far exceeded clinically relevant deposition amounts. Considering the low solubility of ZnPT and the fact that only solubilized ZnPT will be absorbed, a majority of these doses remain on the skin surface in crystalline form. Therefore absorption was likely limited by the dissolution rate of ZnPT in the dried product film and/or on the skin surface. In regards to the latter, the process is expected to be slow based on the model-derived [19, 20] value for the amount of ZnPT required to saturate the upper layers of the stratum corneum (SC) barrier (M_{sat} = 0.011 µg/cm²). Calculation of this concentration was based on the surface area of the evaporated dose (cm²) and the following set of equations:

$$M_{sat} = C_{sat} \times h_{dep} \quad \text{(S1)}$$

$$C_{sat} = S_w \times K_{sc/w} \quad \text{(S2)}$$

$$K_{sc/w} = 0.04 \times K_{o/w}^{0.81} + 0.0359 + 4.057 \times K_{o/w}^{0.27} \quad \text{(S3)}$$

In Eqs. S1-S3, C_{sat} is the permeant solubility in the SC (g/cm^3), h_{dep} is the initial deposition depth of the permeant (assumed to be 10% of total SC thickness [21] ~ 1.34 µm for partially hydrated SC), S_w is the water solubility of the permeant at skin temperature (32°C), and $K_{sc/w}$ is its SC/water partition coefficient [21, 22]. The concept of a deposition depth is associated with the desquamating layers of the SC and has been useful for describing the skin disposition of small doses of other topically-applied compounds [21, 23, 24].

S2. Results for formulation and sebum effects on the in vitro skin penetration of ZnPT

S2.1. $^3H_20$ skin permeation and Zn[^{14}C]PT dose deposition amounts

$^3H_20$ penetration and Zn[^{14}C]PT deposition amounts are summarized in Figure S3. Significant differences between donors ($p < 0.001$) were observed in the ^3H_2O flux (Panel A of Fig. S3). Significant differences were also observed in the amount of Zn[^{14}C]PT deposited from the three ZnPT formulations (Panel B of Fig. S3).

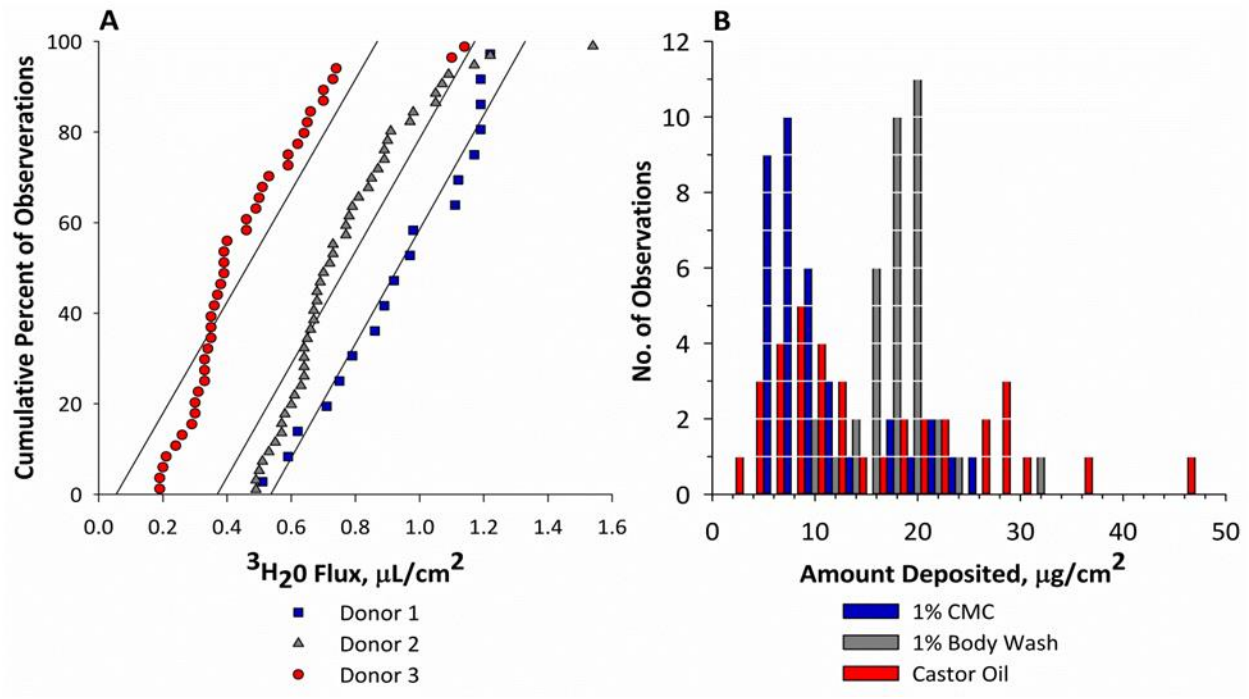

Figure S3. (A) Cumulative distribution of ^3H_2O permeation for skin samples accepted into each study. (B) Frequency distribution of Zn[^{14}C]PT dose for the three test formulations.

S2.2. Localization of Zn[^{14}C]PT in the excised skin samples

Recovery of radioactivity, expressed as Zn[^{14}C]PT-equivalents, in the various samples is shown in Figure S4. Most of the radioactivity remained on the skin surface and was recovered in the wash samples following a 72-hour exposure period to all six of the topical treatments. In the absence of an artificial sebum layer, less than 3% of the dosed radioactivity penetrated the skin when Zn[^{14}C]PT was applied from the 1% CMC suspension. Under these conditions, the percent of dose penetrated was the highest for the castor oil formulation. Significant differences between formulations ($p < 0.001$) were observed for the wash (i.e. skin surface), epidermis and receptor fluid samples. The presence of the artificial sebum layer did not affect the skin disposition of radioactivity when dosed from the castor oil formulation. However,

significant reductions in the wash amounts were observed for the aqueous suspensions on sebum-supplemented skin compared to untreated skin ($p < 0.001$). Following the deposition of these treatments, more of the compound penetrated into the viable epidermis ($p < 0.001$ for 1% CMC, $p < 0.01$ for 1% body wash) or permeated into the receptor fluid ($p < 0.001$).

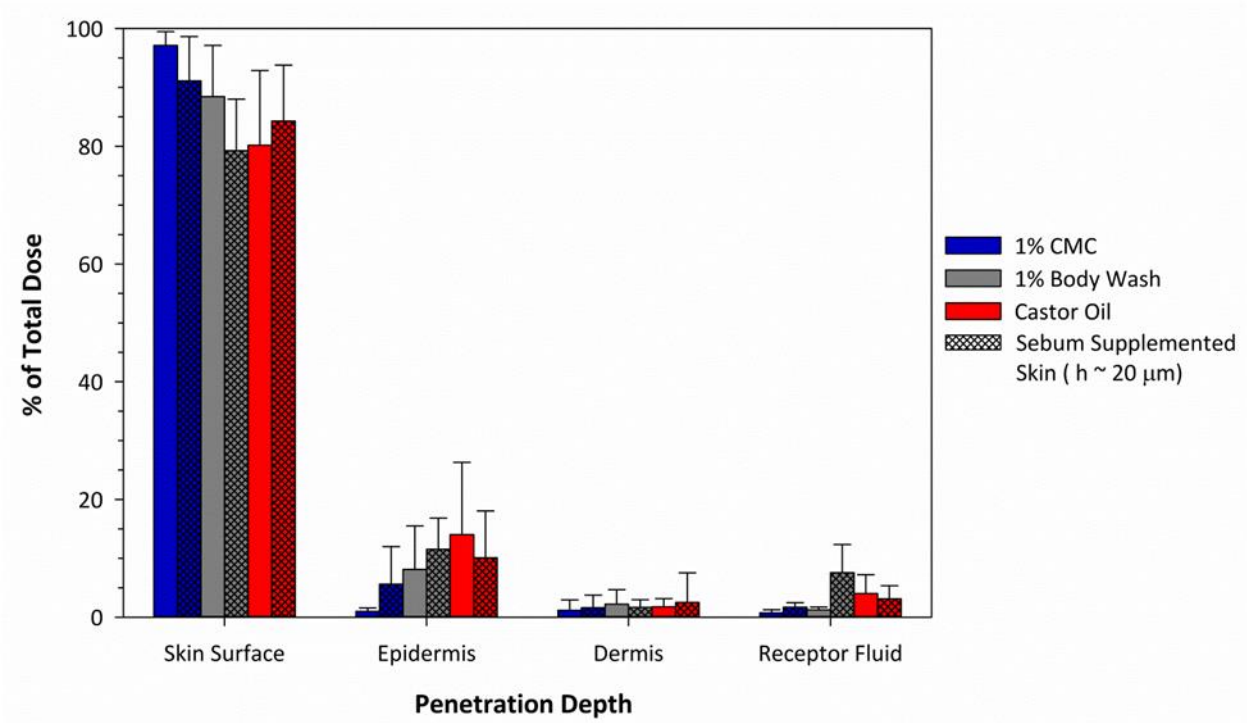

Figure S4. Skin disposition of radioactivity associated with Zn[^{14}C]PT for the three test formulations in the presence (cross-bar) and absence of an artificial sebum film (h ~ 20 µm).

S3. Model-derived diffusivities of hypothetical pyrithione moieties

Molecular transformations of ZnPT would modify the physicochemical properties, solubility, and successive mass transport rates of the carbon-14 labeled pyrithione species (Table S2). It is likely that these transformations explain the treatment effects on percutaneous absorption that were measured in this study. While no experimental data is available regarding the speciation of ZnPT, solubility data using LCMS and ICP to detect pyrithione or zinc concentrations, respectively, can be compared among the formulations used in the present study. These analyses revealed reductions in the pyrithione to zinc ratio when ZnPT was solubilized in castor oil (1.6:1) or the artificial sebum composition (0.43-0.9:1) compared to water (2.2:1). These ratios indicate an increase in the concentration of solubilized pyrithione species containing zinc. The predominant pyrithione species that are present within the stable pH range in water are the 1:1 $ZnPT^+$ monomer and ionized pyrithione, each accounting for approximately 40-50% of the total pyrithione concentration[1]. We surmise that coordinate covalent bonding between the 1:1 $ZnPT^+$ monomer and anions in the formulation or on the skin surface is probable. Theoretical structures were proposed in [25] for the complexation of the 1:1 $ZnPT^+$ monomer species to the anionic sodium lauryl sulfate surfactant (ZnPT-SLS) present in the body wash or the deprotonated oleic acid (ZnPT-OA) in the artificial sebum composition. Compared to the intact 1:2 ZnPT monomer, these ligand exchange products

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would enhance the solubility of the zinc chelate and help to explain the decreases in the pyrithione to zinc ratios. When oleic acid was removed from artificial sebum composition, the pyrithione to zinc ratio was increased to 1.5:1 (E.D. Smith and J.M. Heinrich, personal communication). Furthermore, a significant ($p < 0.001$) increase was measured for the solubility of Zn[^{14}C]PT in the artificial sebum comprised of oleic acid and olive oil (95 ± 18 PPM) compared to olive oil only (27 ± 1 PPM). This fluctuation in the pyrithione to zinc ratio along with increased total solubility suggests that the oleic acid is involved in the dissolution of the zinc complex, potentially through a ligand exchange mechanism.

Simulations using the Wang et al. model [19, 20] illustrate the variability in the predicted absorption of the various pyrithione species over time (Fig. S5). The calculated curves that are presented in Fig. S5 are for doses that are stoichiometrically equivalent to 13.8 µg/cm² ZnPT, the average amount deposited in the in vitro skin penetration study results presented in the main text. According to the model-derived values [19, 20], only the free pyrithione, dipyrithione, and $ZnPT^+$ complex ion species have M_{sat} values above the doses deposited in this study to enable diffusion-limited permeation. Conversely, the lower permeation and higher lag times of the other pyrithione species is likely attributable to dissolution rate-limited permeation. Although the high lipophilicity of the ligand exchange products, i.e., ZnPT-SLS, ZnPT-OA, could potentially enhance partitioning into the SC barrier, the sizes of these complexes are larger than SC lipid molecules (MW ~ 400 Da); therefore permeation kinetics would likely be limited by the lateral diffusivity of the lipids around which the solutes are intercalated [43]. Comparison of the relatively large ZnPT ligand exchange products demonstrates the dependence of the aliphatic chain size and configuration on permeation. The linear, C12 chain ZnPT complex with sodium lauryl sulfate (ZnPT-SLS) was predicted to penetrate faster and to a greater extent than ZnPT2 and the branched, C18 chain ZnPT complex with oleic acid (ZnPT-OA).

Table S2. Physicochemical properties and model-derived [37,38] transverse diffusivities of the probable diffusing pyrithione species.

Molecular Species	MW	a_s [b], Å	Log $K_{o/w}$ [a]	mp [g], °C	M_{sat} [c], µg/cm²	$D_\perp$ (calc)[d] x 10^{12}, cm^2s^{-1}
$ZnPT_2$	317.7	5.1	0.97[f]	240	0.0011	1.1
$ZnPT^+$	192.6	2.7	-1.33[g]	78	130	0.56
Pyrithione (HPT)[e]	127.2	2.7	-0.30[g]	25	100	5.1
Dipyrithione (PT_2)	252.3	4.0	0.60[h]	191	12	1.6
ZnPT-SLS	456.9	5.9	7.23[g]	262	0.0041	5.7
ZnPT-OA	473.0	6.4	8.43[g]	260	0.0011	3.6

[a] Octanol/water partition coefficient
[b] Estimated hydrodynamic radius
[c] Equation 4.1
[d] Ref **[19, 20]**
[e] Predominant pyrithione tautomer **[26]**
[f] Ref **[11]**
[g] Ref **[27]**
[h] Ref **[28]**

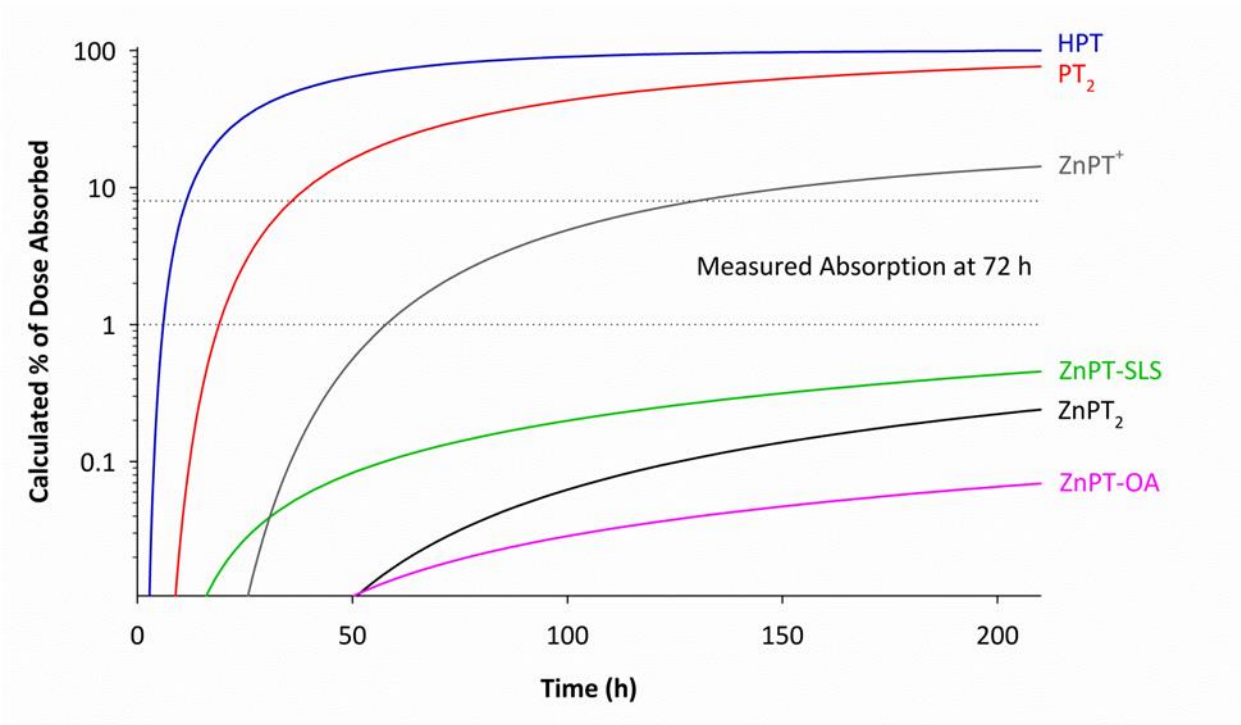

Figure S5. Simulated percutaneous absorption of the various pyrithione species as a function of time for a 13.8 µg/cm² ZnPT dose. Absorption values between 1-8% are bracketed to display the percent absorption measured in this study after 72 hours of dermal exposures to the various treatments.

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