Scientific Committee on Consumer Safety

SCCS

OPINION ON

Zinc pyrithione

COLIPA n° P81

- Text from previous opinion has not been edited -

The SCCS adopted this opinion at its 2nd plenary meeting of 18 June 2013

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS
The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

Zinc pyrithione (ZPT) (CAS 13463-41-7; EU 236-671-3) with the chemical name: Bis[(2-pyridyl-1-oxo)-thio]zinc was introduced into the Cosmetics Directive as a preservative by Directive 82/368/EEC. It was authorised as a preservative at the maximum concentration of 0.5% with the limitation "Authorized in products rinsed off after use, forbidden in products for oral hygiene".

Back in 1984 (17/12/1984) the Scientific Committee on Cosmetology (SCC) concluded in its opinion XI/389/84 concerning the use of pyrithione zinc in hair-care preparations not rinsed off after use: "The Committee notes that the use of pyrithione zinc is allowed as a preservative in products rinsed off after use at a maximum concentration of 0.5% in the finished product.
The Committee finds that the substance is highly toxic, and cannot agree recommending any extension of its use unless percutaneous absorption in man can be shown not to occur in normal skin, nor under conditions of inflammation or abrasion."

Submission I for Zinc pyrithione was submitted in July 2000 by COLIPA 1.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted its opinion (SCCNFP/0671/03) on 17th December 2002 with the conclusion: "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."

Zinc pyrithione is currently regulated as a preservative in rinse-off products (excluding oral hygiene products) in a concentration up to 0.5% in general and up to 1.0% in hair products (Annex VI/1, 8). Furthermore zinc pyrithione is also allowed in a concentration up to 0.1% in leave-on hair products (Annex III/1, 101).

In the present submission by COLIPA (submission II), which is a supplemental dossier to submission I, the applicant applies for an extension of the authorised concentration from 1.0% to 2.0% in rinse-off antidandruff hair care products.

2. TERMS OF REFERENCE

1. *Based on the scientific data provided, does the SCCS consider that zinc pyrithione, when used in a concentration up to 2.0% as an anti-dandruff agent in rinse-off hair care products, is safe for the consumer? [The request should be seen as additional to the currently authorized use].*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1 Introduction

In submission II the applicant provided comprehensive toxicokinetic data, data on physiologically-based pharmacokinetic (PBPK) modelling of ZPT as well as data on a human pharmacokinetic study where disposition and excretion of ZPT after different 4-day treatment regimes with shampoo containing up to 2 % ZPT and tonic containing up to 0.25 % ZPT were investigated. Further, representative samples of HRIPT study reports were provided.

Apart from the data provided in submission II, after submission I and finalisation of opinion SCCNFP/0671/03 on 17th December 2002, further data on ZPT has become available, as the substance is also used as a biocide and as the substance has been registered under Regulation (EC) No 1907/2006 (REACH regulation) in a tonnage band of 1000 – 10000 tpa.

Information on Registered Substances is publicly available at the website of the European Chemicals Agency (ECHA) (http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances) and comes from registration dossiers which have been assigned a registration number. **The assignment of a registration number does however not guarantee that the information in the dossier is correct or that the dossier is compliant with Regulation (EC) No 1907/2006 (the REACH Regulation). This information has not been reviewed or verified by the Agency or any other authority.**

SCCS contacted ECHA in order to be able to cite third party data on ZPT published at ECHAs website. ECHA advised the SCCS to directly contact the owner of the data. Subsequently, the SCCS requested Cosmetic Europe to get additional Zn-pyrithione data, which was submitted to ECHA but not to the SCCS. However, these data were not provided to the SCCS.

In part, studies published at ECHAs website had been described and taken up in two publicly available scientific opinions on ZPT:

1) HSE (The Health and Safety Executive) (2003): Advisory committee on pesticides No 208. Evaluation on: Zinc pyrithione: use as a booster biocide in antifouling products (available at: http://www.pesticides.gov.uk) (reference D8)

2) MAK (2012): Zinkpyrithion. The MAK Collection for Occupational Health and Safety (available at http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb1346341d0052/pdf) (reference D19).

Therefore, SCCS utilised information given in these two documents to supplement the toxicological information on ZPT.

However, as a considerable number of studies have not been made available to the SCCS, the SCCS considers submission II as incomplete. The present evaluation is based on submission I and submission II to the SCCS and on the reviewed information from HSE and MAK evaluations on Zn-pyrithione.

3.2. Chemical and Physical Specifications

3.2.1. Chemical identity

3.2.1.1. Primary name and/or INCI name

Zinc pyrithione (INCI name)

3.2.1.2. Chemical names

Bis [1-hydroxy-2(1 H)-pyridinethionato-O,S] (T-4) zinc (IUPAC)
Pyrithione zinc
Zinc bis(2-pyridylthio)-N-oxide
Zinc pyridinethione
Zinc 2-pyridinethione-l-oxide
Bis (N-oxopyridine-2-thionato) zinc (II)
ZP, ZnPT, ZnPTO, BOTZ

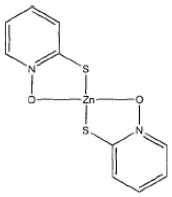
3.2.1.3. Trade names and abbreviations

Zinc Omadine
Vancide ZP

3.2.1.4. CAS / EINECS number

CAS: 13463-41-7
EINECS: 236-671-3

3.2.1.5. Structural formula



3.2.1.6. Empirical formula

Formula: $C_{10}H_8N_2O_2S_2Zn$

3.2.2. Physical form

White to slightly yellow crystals

SCCS comment
Additional information is available at ECHAs website (http://echa.europa.eu/)

3.2.3. Molecular weight

Molecular weight: 317.7 g/mol

3.2.4. Purity, composition and substance codes

Zinc pyrithione is commercially supplied as a 24-26% aqueous solution

3.2.5. Impurities / accompanying contaminants

No data submitted

3.2.6. Solubility

Very low solubility in most solvents

Water:	0.0015 w/w %
Ethanol:	0.031 w/w %
Acetone:	0.07 w/w %
Chloroform:	0.34 w/w %
Mineral oil, light:	0.0001 w/w %

Water solubility at 20°C, reported at ECHA Website: 4.93 mg/L (EU Method A.6),
6.3 ppm (OECD 105)

SCCS comment
Concerning solubility in ethanol and acetone, further information is available at ECHAs website (http://echa.europa.eu/). HSE gives a water solubility of 8 g/l.

3.2.7. Partition coefficient (Log Pow)

Log P_{ow}: 0.9 (HSE, 2003)
0.97 (MAK, 2012)
0.883 (EU Method A.8, ECHA Website) 0.9 (OECD 107, ECHA Website)

3.2.8. Additional physical and chemical specifications

Melting point:	240 °C (Decomposition at 240°C)
Boiling point:	/

Flash point:	/
Vapour pressure:	< 0.000001 Pa at 25°C (OECD 104)
Density:	1.782 at 25 °C
Viscosity:	1.76 g/cm^3 (OECD 109)
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	/

SCCS comment

Concerning melting point, ECHAs website (http://echa.europa.eu/) gives a slightly different value, whereas in HSE 2003 it is stated that the substance decomposes before melting (200°C).

HSE (2003) gives a vapour pressure of < 0.532 Pa at 21°C, MAK (2012) gives a vapour pressure of 2.49 x 10^{-9} hPa.

3.2.9. Homogeneity and Stability

Homogeneity and stability of Zn pyrithione in test solutions has been reported for some of the studies performed. In the toxicokinetic investigations described in section 3.3.9.3. stability of zinc pyrithione in frozen rat plasma was evaluated by analyzing stability samples stored under the same conditions as study samples. Results indicate a frozen-state stability of approximately 377 days at -70 °C.

SCCS general comments to physico-chemical characterisation

Concerning some physico-chemical properties, diverging values are available from different sources of information.

3.3. Function and uses

Zinc pyrithione (ZPT) is currently regulated as a preservative in rinse-off products (excluding oral hygiene products) in a concentration up to 0.5% in general and up to 1.0% in hair products (Annex VI/1, 8). Furthermore zinc pyrithione is also allowed in a concentration up to 0.1% in leave-on hair products (Annex III/1, 101).

In the present submission, the applicant applies for an extension of the authorised concentration from 1.0% to 2.0% in rinse-off antidandruff hair care products.

According to the EC Commission Regulation (No. 1451/2007), Zink pyrithione is also used as a biocide in biocidal product categories 2, 6, 7, 9, 10, 11, 12 and 22 of Annex V of the EU Biocide Directive (Directive 98/8/EC).

3.4. Toxicological Evaluation

3.4.1. Acute toxicity

3.4.1.1. Acute oral toxicity

Ingredient based data taken from SCCNFP/0671/03
LD_{50} 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. Six hundred mg/kg was found to be the LD_{50} when administered orally to dogs.

Ref.: B6, B10, B33, B57, B71, B73

New ingredient based data
Information on further acute oral studies performed with ZPT is available at ECHAs website (http://echa.europa.eu/).

Product derived data taken from SCCNFP/0671/03
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. In addition, Snyder et al (1965) studied the acute oral toxicity of the cream shampoo product with higher levels of ZPT and estimated the LD_{50}. The results showed that increasing the level of ZPT increases acute oral toxicity.

Ref.: B61

Emetic studies in dogs and pigeons showed that zinc pyrithione in a cream shampoo product is a potent emetic (ED_{50} app. 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, indicating gastric irritation. 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 was concluded in SCCNFP/0671/03 that it is unlikely that a human accidentally ingesting shampoo could retain a hazardous amount.

Ref.: B14, B15, B38.

SCCS comment
In addition to acute oral toxicity studies evaluated in SCCNFP/0671/03 further studies have been performed. The data is not available for evaluation. SCCS notes, however, that classification as Acute Tox 3; H301 (toxic if swallowed) according to CLP (Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures) is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/).

3.4.1.2. Acute dermal toxicity

Ingredient-based data taken from SCCNFP/0671/03
Dermal LD_{50} values for albino rabbits range from < 2,000 mg/kg to 10,000 mg/kg.

Ref.: B66*

New ingredient based data:
Information on further acute dermal studies performed with ZPT is available at ECHAs website (http://echa.europa.eu/). HSE (2003) describes an acute dermal toxicity study

(limit study) performed in 5 male and 5 female New Zealand White rabbits. The animals received a single dermal application of 2000 mg/kg ZPT moistened with water which was held in place for 24 hrs under semi-occlusive patch. The LD_{50} was > 2000 mg/kg. A single death was noted, but it was unclear, whether this was treatment-related.

Product derived data taken from SCCNFP/0671/03
A shampoo containing 2% ZPT 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 ZPT alone indicating that its incorporation into a shampoo formulation does not significantly enhance penetration.

Ref.: B61, B67

3.4.1.3. Acute inhalation toxicity

At ECHAs website information on acute inhalation studies with ZPT is available. In HSE (2003), two studies are described. In a nose only study in male and female Sprague-Dawley rats, a LC_{50} > 0.6 mg/l was derived.
In a whole body inhalation study performed in male and female Sprague-Dawley rats, a LC_{50} value of 0.14 mg/l was derived.

SCCS comment
The studies were not available for evaluation.
SCCS notes, however, that classification as Acute Tox 3; H331 (toxic if inhaled) according to CLP (Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures) is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/).

3.4.1.4. Acute toxicity – other routes

Ingredient based data taken from SCCNFP/0671/03

Intraperitoneal
Intraperitoneal injection of ZPT resulted in LD_{50} values of 36 mg/kg for rats and 500 mg/kg for mice.

Ref.: B71, B73

Intravenous
Generally, 25 mg/kg of ZPT was fatal to both dogs and monkeys within 24 hours and produced cholinergic-like effects prior to death. 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 20 mg/kg and 10 mg/kg was a lethal dose for rabbits. Intravenous doses of 5 mg/kg or less produced only transient effects.

Ref.: B2, B7, B72

3.4.2 Irritation and corrosivity

3.4.2.1. Skin irritation

Product derived data taken from SCCNFP/0671/03

A study evaluated the effect of ZPT in a marketed shampoo base 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 used the cream and lotion shampoos did not produce any skin irritation, nor did they change the skin pigmentation level in Caucasian or black skin.

Ref.: B37

A case report described a reaction by a patient to a shampoo containing 2% ZPT. The patient had had a similar reaction after using a hair cream with a lower level seven years before. Another report described a case of eczema of the scalp and face after using a shampoo containing 2% ZPT for a short period.

Ref.: B4, B23

Ingredient based data

From ECHAs website (http://echa.europa.eu/), the SCCS is aware, that skin irritation studies have been performed with ZPT. The studies are not available for evaluation. A total of four skin irritation studies have been described in MAK (2012):

6 male New Zealand rabbits were exposed occlusively for 4 hrs towards 0.5 g moistened ZPT. Skin reactions were assessed after 0.5, 1, 24, 48 and 72 hrs. After 0.5- 1 hrs, slight erythema of grade 1 (of 4) was observed in three animals and oedema (grade 2 of 4) was observed in two animals. After 24 hrs, erythema have disappeared and oedema were scored 1. After 48 hrs, also oedema have disappeared. From this study, ZPT was considered mildly irritating (below classification level) (Cerven, 1991, taken from MAK (2012)).

The dermal irritancy of a 20 % suspension of ZPT was examined in three animal models (rabbits, guinea pigs and mice). In open patch tests involving five daily applications, ZPT was slightly irritant and induced a marginal epidermal hyperplasia and increased hair growth.
(Lansdown, 1991; cited from MAK (2012)).

In a Buehler-Test performed on 10 male guinea pigs, occlusive 6-hr treatment with 0.4 ml of a 48 % dispersion did not cause skin irritation. (Newcomb, 1996; cited from MAK (2012)).

MAK (2012) also lists the publication Collum and Winek, 1967 in section "skin irritation". However, this study gives no information on skin irritation. It describes the influence of the vehicle on dermal penetration/dermal toxicity of pyrithiones.

In some human repeat insult patch test studies (described in section 3.3.3 – skin sensitization), slight irritation was observed in some volunteers.

SCCS comment on skin irritation
Skin irritation studies performed with ZPT were not available for evaluation. However, from product based data evaluated in SCCNFP/0671/03, from the description of skin irritation studies performed with ZPT and from human HRIPT tests it can be inferred that ZPT is – at least - a mild skin irritant.

3.4.2.2. Mucous membrane irritation

Product derived data taken from SCCNFP/0671/03

The eye irritation potential of ZPT has been evaluated in a number of product types: Instillation of a soap solution containing 0.25% ZPT to rabbit eyes 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 ZPT (2%) were tested. Undiluted solutions produced extensive damage to the eyes of rabbits which was characterised 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% ZPT shampoo was instilled undiluted. Instillation of the shampoo formulation diluted to 10% (0.2% ZPT) resulted in no ocular irritation. Conclusion: The irritation potential of shampoo in rabbit eyes was not increased by the incorporation of ZPT.

Ref.: B61

Ingredient based data

From ECHAs website (http://echa.europa.eu/), the SCCS is aware that eye irritation studies have been performed with ZPT. The studies are not available for evaluation. Two eye irritations performed with ZPT are described in MAK (2012) and HSE (2003):

In an eye irritation study 0.1 ml of powdered zinc pyrithione (95.6 %) was instilled into the conjunctival sac of 6 New Zealand White rabbits, with the other eye serving as a control. Observations were carried out at 1, 24, 48 and 72 h. The mean scores (24, 48 and 72h) were 3 for corneal opacity and conjunctival redness, 4 for conjunctival chemosis and 1.2 for iridial effects (only 4 animals being scored due to excessive discharge making iris scoring difficult in 2 animals). From this study, the substance was considered as a severe eye irritant.
(Olin, 1991b, cited from MAK (2012)).

In a further eye irritation study 0.1 ml of a 48% aqueous dispersion of zinc pyrithione was instilled into the conjunctival sac of 6 New Zealand White rabbits, with the other eye serving as a control. Treated eyes were rinsed 24 h post instillation. Observations were carried out at 24, 48 and 72 h. The mean scores (24, 48 and 72h) were 2.5 for corneal opacity and conjunctival redness, 3 for conjunctival chemosis and 1 for iridial effects.
(HSE (2003), MAK (2012)).

SCCS comment
In SCCNFP/0671/03 only product based information was evaluated. It was concluded, that the irritation potential of shampoo in rabbit eyes was not increased by the incorporation of ZPT. Pure ZPT has also been investigated in eye irritation tests, but the studies are not available for evaluation. HSE (2003) concludes that ZPT is a severe eye irritant, MAK (2012) states, that ZPT is corrosive to the eye. SCCS notes, that classification as Eye Damage 1; H318 (causes serious eye damage) according to CLP is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/)).

3.4.3. Skin sensitisation

Animal data

Taken from SCCNFP/0671/03

ZPT was evaluated for its potential to induce contact hypersensitivity to guinea pigs. Using the procedure of Buehler (1965) to detect contact hypersensitivity, 40 animals were exposed to a 50% aqueous slurry of ZPT. No reactions indicative of contact hypersensitivity were seen in any of the animals at challenge.

Ref.: B25

A 0.1% solution of the ZPT (1 % ZPT) 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 sensitisation.

Ref.: B61

New animal data

Further skin sensitization studies with ZPT have been performed which are not available for evaluation. They are described at ECHAs website (http://echa.europa.eu/), in MAK (2012) and HSE (2003).
ECHA mentions a Guinea pig maximisation test performed according to OECD 406 using 20 female animals, where two animals were regarded as sensitized; thus it is concluded that ZPT may cause sensitization by skin contact.

MAK (2012) describes a Buehler Test using 10 animals and a 48 % ZPT suspension, where no positive reactions were observed.

In further Buehler test performed with ZPT of 97% purity in 10 test and 5 control male Hartley guinea pigs ZPT was not sensitizing. HSE states that this study had been adequately conducted (HSE (2003)).

An earlier study performed according to an unusual protocol in guinea pigs using a suspension of 50 % ZPT in mineral oil was considered as not adequate for evaluation because of insufficient documentation and methodological deficits (MAK (2012)).

Human data

Taken from SCCNFP/0671/03

The work by the Danish Contact Dermatitis Group was described in which ZPT (1%) was added to the European Standard Patch Test series. 1652 consecutive dermatitis patients were tested. Only three positive reactions were found. The authors state that in only one of these was the ZPT reaction interpreted with certainty as being of present relevance. They also point out the wide use of ZPT 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 ZPT in the sensitisation area.

Ref.: B4

A multi-centre investigation was conducted in France in order to evaluate the risk of sensitisation by a number of preservatives. 465 subjects were tested. They were suffering from an eczema for which the anecdotal circumstances pointed to an allergy to cosmetics, medicine, industrial products or clothing accessories. Only two patients (0.4%) gave positive patch tests to ZPT.

Ref.: B38*

Two separate closed-patch test studies on human volunteers were conducted. A 1% aqueous solution of shampoo containing 2% ZPT 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 sensitisation. 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 sensitised, and if so, what the offending material was. The remaining subjects gave only a transient erythematous response indicative of irritation.

Ref.: B61

Cream and lotion shampoo products were tested in two separate HRIPT's. Both studies were conducted according to a modified Draize procedure, in which 0.25% shampoo was patched. No sensitisation 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.

Ref.: B26, B65

A hair dressing cream containing 0.5% ZPT was used to patch test 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 sensitisation in humans.

Ref.: B46*

Marketing experience with a commercially available formulation has conclusively demonstrated that ZPT is, at worst a very weak sensitiser. Few reports of sensitisation have appeared in the literature.
Ref.: B4, B17, B23, B40

Further data

Further information on skin sensitizing studies in humans has become available. The information comprises (1) representative samples of HRIPT study reports provided by the applicant, (2) information from MAK (2012) and (3) information at ECHAs website (http://echa.europa.eu/).

Ad 1)

Study 1:
The test substance was a dilution of shampoo with added perfume. The test report has been audited in compliance with the principles of Good Clinical Practice. Informed consent was obtained from the participants. In a pre-test, the irritating potential was investigated by three semi-occlusive 24 hour exposures of the upper outer forearm on days, 1, 3 and 5 to 5% [w/v] and 10 % [w/v] dilutions of the shampoo with added perfume in deionised water. Based on the outcome of the pretest, a dilution of 10% [w/v] shampoo with added perfume (final concentration of perfume: 1.20 %) was chosen for the main study.
The main study involved 9 semi-occlusive 24 hour induction exposures of the upper outer arm over a period of three weeks in 93 subjects from which 87 completed induction and challenge. After the induction period, there was a 14 day rest, followed by the application of the final induction patch prior to duplicate challenge exposures at both original and virgin sites. For most subjects, skin was assessed approximately 48 or 72 hr after induction applications and 48 and 96 hr after challenge application.
Under the conditions of this test, there was no evidence of skin sensitisation, but slight irritation was seen in some of the volunteers.

Ref.: A13

Study 2:
The test protocol was approved by the North West Ethical Committee and the study was performed in accordance with the principles of Good Clinical Practice. A total of 101 volunteers participated in the study, 93 of them fully completed the study. The substance was applied using 2 x 2 cm Webril pad Semi-occlusive Micropure tape. The test material was initially applied at 10 % [w/v] (aq.) to a Webril square for the first four induction patches. This concentration provoked an unacceptably high level of irritation during this period resulting in a reduction to 5% [w/v] for the remainder of the test. A total of 9 induction patches were applied and scored. 48 and 98 hrs after challenge, readings were undertaken. Irritant response of both dermal and epidermal nature with pronounced edge effects were observed which were in some cases more prominent at the 96 hr after-challenge reading when compared to the 48 hr after-challenge reading. Despite of the delayed nature of some of these responses, the investigators concluded that there was no evidence of skin sensitisation on the 93 subjects who completed the test.

Ref.: A14

Study 3:
The study adhered to QAU principles. Information on informed consent/ involvement of an ethical committee is not given. The study panel consisted of 84 volunteers who received occlusive patches with the substance during a three week induction period each Monday, Wednesday and Friday. Each patch was left in place for 24 hrs and then removed. Fourteen days after the last induction application, duplicate challenge patches were applied for 24 hrs. 81 volunteers completed the study (the dropping out of volunteers was unrelated to the test). Mild erythematous reaction was observed at many occasions during the induction phase, but the test material showed no evidence of skin sensitisation.

Ref.: A15

Study 4:
The study was performed according to the principles of good laboratory practice. Informed consent was obtained from the 92 volunteers participating in the study (86 volunteers completed the study). The test material was used in a concentration of 0.15 % [w/v] and applied in 0.4 ml amounts to a $2cm^2$ Webril pad and fixed on the lateral surface of the upper arm. In the induction phase, patches were applied on Monday, Wednesday and Friday for three weeks. Test sites were scored before application of each subsequent patch and on the fourth Monday after the final insult patch. After a 2-week rest, challenge patches were applied to both arms of each subject and results were graded after 48 and 96 hrs. During the induction phase, mild erythematous reaction was observed in many occasions. At challenge, none of the volunteers showed reactions greater than that of a mild erythema. It was concluded that the substance did not produce reactions indicative of skin sensitization.

Ref.: A16

Study 5:
The study adhered to QAU principles. A total of 96 volunteers took part in the study, 92 of them fully completed the study, the reasons for withdrawal of volunteers were unrelated to the test/test material. A 0.15 % [w/v] dilution in distilled water was used. There were 9 induction applications. Results for sensitising effects were graded 48 and 96 hrs after challenge application. The 0.15 % aqueous dilution of test material caused an acceptable level of irritation during the study. There was no evidence of skin sensitizing potential observed in the 92 subjects who fully completed the study.

Ref.: A17

Study 6:
The study adhered to QAU principles. The test substance was used in a concentration of 10 % [w/v] in distilled water. The study consisted of nine semi-occluded induction patches over

a three week period followed by a 14 – 20 day rest period. The induction patch sites were evaluated at 48 hrs after patch application (72 hrs for weekends). Panellists were challenged on the original and naïve sites and evaluated at 48 and 72 or 96 hrs after patch application. 102 panellists from a total of 117 (informed consent was obtained) completed the study. None of the 102 panellists completing the study exhibited responses during the challenge phase of the study. Thus, it was concluded that there was no clinical evidence indicative of delayed contact hypersensitisation to the substance.

Ref.: A18

SCCS comment on studies 1 – 6 described above

The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

Ad 2)

In a modified Draize test in 10 human volunteers, there was no evidence of skin sensitization. For induction, either a 3% ZPT suspension in petrolatum or a 1 % solution of ZPT in DMSO was used. Challenge consisted of either a 3% ZPT suspension in petrolatum or of a 0.5 % solution of ZPT in DMSO.

Ref.: D20 Marzulli and Maibach, 1973.

In addition, MAK 2012 lists reports on reactions in epicutaneous tests on zinc pyrithione or sodium pyrithione in patients with suspected contact allergy which are presented in table 1 and 2; grading was apparently performed according to the guidelines of the European Society of Contact Dermatitis. With respect to the utilization of data from sodium pyrithione it should be noted that it is claimed that rather the organic moiety than the Zn ion is responsible for sensitizing effects of ZPT. Therefore, studies using sodium pyrithione are included in the case reports listed in table 1 and 2. Further, worker data from the use of ZPT as a cooling lubricant are also included in the overview.

Table 1: reports on reactions in epicutaneous tests on zinc pyrithione or sodium pyrithione in patients with suspected contact allergy (according to MAK 2012); grading was apparently performed according to the guidelines of the European Society of Contact Dermatitis.

Tested individuals	Test substance, concentration, vehicle	Result	Remarks	Reference
Metal worker with hand eczema	Sodium pyrithione, 0.5% (petrolatum)	1 + and 2 + (after 48 and 96 h)	less than 1% sodium pyrithione in the cooling lubricant concentrate	Le Coz, 2001 (D18)
Patient with facial and scalp eczema*)	zinc pyrithione, 0.05%, 0.2%, 0.5%, 1% (petrolatum)	1 +, 2 +, 3 +, 3 + (at 48 and 72 h)	Shampoo containing 0.5% and 2% zinc pyrithione; no reaction in 20 control persons exposed to 0.1% and 0.5% zinc pyrithione	Goh and Lim, 1984 (B23)
2 patients with scalp eczema	zinc pyrithione, 1%	2 + / 3 + and - / 2 + (after 48	zinc pyrithione-containing	Gonzalez Perez et al., 1995

	(petrolatum)	and 96 h)	shampoos, in both patients sensitization to p-phenylenediamine, and numerous other substances	(C3)
Patients with dermatitis of the scalp, face, neck and hands	zinc pyrithione, 1% (petrolatum)	2 + (after 48, 72 and 168 h)	Shampoo containing 0.45% zinc pyrithione; patients also have a positive reaction to the shampoo (tested at 2% and 5%)	Hsieh et al., 2010 (D9)
Lathe operator with dermatitis on the bac	zinc pyrithione, 1% (petrolatum) and sodium pyrithione, 0.1% (water)	negative; 1+ and 3+ when re-tested after 48 h	coolant with 0.1-1% sodium pyrithione; questionable reaction towards the used coolant and 2 + response to the coolant concentrate (5% in buffer)	Isaksson, 2002 (D11)
Female patient with pustular psoriasis	zinc pyrithione, 1% and 2% (petrolatum)	1 + / 2 + (after 48 and 96 h, respectively)	within 20 days after application of a zinc pyrithione-containing shampoo, pustular psoriasis with Koebner phenomenon occurred in the patient with stable psoriasis	Jo et al., 2005 (D12)
2 patients (tested only in one case) with eczema on the scalp, face and upper body / neck, arms and hands[*)]	zinc pyrithione, 1% (water), (tested only in one case)	1 + (after 48 and 96 h)	zinc pyrithione-containing anti-dandruff preparations; in the second case no testing of zinc pyrithione, but positive patch test with zinc pyrithione-containing product and no response to zinc pyrithione-free formulation	Muston et al., 1979 (B40)
Female patient with dermatitis of the scalp zinc	Zinc pyrithione (no further information on solvent)	2 + (after 48 h)	zinc pyrithione-containing shampoo, at rechallenge: Koebner phenomenon with the exacerbation	Nielsen and Menné, 1997 (C4)

			of psoriasis	
Patient with dermatitis of the scalp, face and neck	zinc pyrithione, 0.2% and 0.5% (petrolatum)	1 + and 3 + (72 h)	shampoo containing 2 % zinc pyrithione	Nigam et al., 1988 (C5)
Female patient with dermatitis on forehead, neck and hands	zinc pyrithione, 1% (petrolatum)	2 + (after 48 and 96 h)	eczema after treatment of pityriasis capitis, with a shampoo containing 1% zinc pyrithione, no reaction in 14 control subjects at 1% Zinc pyrithione	Pereira et al., 1995 (C1)
Patient with dermatitis of the scalp, face and neck	zinc pyrithione, 5% (petrolatum)	2 + / 3 + (after 48 and 96 h)	no response to 5% zinc pyrithione in 10 control subjects	Yates and Finn, 1980 (D23)

Table 2: Results of patch testing in larger populations

Tested individuals	Test substance, concentration, vehicle	Result	Remarks	Reference
1652 consecutive patients*)	zinc pyrithione, 1% (petrolatum)	1+, 2+ and 3+	zinc pyrithione-containing shampoo; 2+ and 3+ result questionable	Brandrup and Menné, 1985 (C2; B4)
183 metal workers	sodium pyrithione, 0.1% (water)	2 of 183 positive (1 +, after 72 h)	Individuals overlap with Geier et al., 2006	Geier et al., 2004 (D5)
135 metal workers	sodium pyrithione, 0.1% (water)	1 of 135 positive (after 72 h)	Individuals overlap with Geier et al., 2006	Geier et al., 2006 (D6)
181 metal workers	sodium pyrithione, 0.1% (water)	0 of 181 positive		Gruvberger et al. 2003 (D7)
465 patients*)	zinc pyrithione 1% (petrolatum)	2 of 465 positive	no information on the clinical relevance	Meynadier et al., 1982 (B38*)

*) data already considered in section "Taken from SCCNFP/0671/03"

Ad 3) A human patch test is described at ECHA's website (http://echa.europa.eu/). The study is not available for evaluation.

Conclusion on sensitizing potential

ZPT is not sensitizing in most animal studies; one animal study (which is not available for evaluation) concluded that ZPT might be a sensitizer. Concerning human data, ZPT (or better: the PT moiety) has a low potential to induce contact hypersensitivity when tested per se or as part of a cosmetic formulation. However, in some human HRIPT studies, evaluation was partly hindered by the erythematous reactions observed.

3.4.4. Dermal / percutaneous absorption

3.4.4.1. *In vitro* dermal absorption

An *in vitro* dermal absorption study using full-thickness human skin, performed according to OECD 428 is described at ECHAs website (http://echa.europa.eu/). The study is not available for evaluation.

3.4.4.2. *In vivo* dermal absorption

Taken from SCCNFP/0671/03

Based on the submitted *in vivo* animal data it was concluded that percutaneous absorption of ZPT varies from approximately 0.03 to 3.4% (see study descriptions in SCCNFP/0671/03).

Further data

Animal studies

By using Albino rabbits of both sexes who received daily occlusive doses of 400 mg/kg ZPT as a 10 % suspension in a 2 % solution of methylcellulose in either water or DMSO for 14 days it could be demonstrated that DMSO enhances percutaneous absorption of ZPT with the production of toxic signs and, in most cases, death.

Ref.: D2 Collum and Winek, 1967.

Three *in vivo* dermal absorption studies are mentioned at ECHAs website (http://echa.europa.eu/). The studies are not available for evaluation.

Further, in submission II the applicant provided data on the *in vivo* dermal toxicokinetics of ZPT in female CD rats. In order to develop a physiologically-based pharmacokinetic model for ZPT, pharmacokinetics of ZPT was investigated in two phases in the rat. A detailed study description is given in section 3.3.9 Toxicokinetics and PBPK modelling.
Phase 1 of the study was exploratory in nature and therefore not conducted in strict accordance with GLP regulations (and it did not adhere to OECD TGs 427 or 417). The conclusion of this part of the study was: "Dermal exposure to ZPT at levels of 10 mg/kg or greater resulted in plasma pyrithione concentrations that were measurable (>0.5 ng/mL). A lag time to reach these concentrations of about 8−12 hr from exposure initiation was observed. Decreases in both hind-limb muscle mass and muscle tone were observed in animals exposed daily for 10 days to 100 mg/kg ZPT. The bioavailability of dermally applied ZPT was estimated to be about 3−8%. Since concentrations of pyrithione in plasma were high enough to measure in only a few samples and only at the later time points, these estimates should be considered only to be crude estimates of bioavailability."

Ref.: A19

Phase 2 of the dermal exposure studies included three dose levels (10, 30, and 100 mg/kg) and a vehicle control. Bioavailabilities of the dermal doses were calculated from pyrithione concentrations in plasma (0−24 h) using data from the pilot study for the intravenous dose. These calculated bioavailabilities were **2.3, 8.6, and 0.3%**, respectively, for the **10, 30, and 100** mg/kg/day exposures.

SCCS comment
In principle, study was performed in line with OECD TGs 427 and 417, although TG adherence was not explicitly mentioned. The study was performed according to GLP. The

applicant states that the calculated bioavailabilities should be used with caution: the AUC(0−24h) values for dermal administration do not capture the total AUC, and use of the AUC(0-∞) values are fraught with the difficulties for parameters based on k_{el} as a consequence of high variabilities in various TK parameters.

Ref.: A19

Human studies

Taken from SCCNFP/0671/03

A clinical pharmacokinetic study has investigated deposition, absorption and excretion of ^{14}C radiolabelled ZPT resulting from the use of a ZPT containing shampoo alone (1 % ZPT) and in combination with a ZPT containing leave on hair tonic (0.1% ZPT). This study demonstrated that systemic loading of ZPT was increased significantly less than could be expected from the corresponding skin deposition in those subjects using the shampoo/tonic combination compared with those using the shampoo alone. Additionally, absorption of ZPT in patients with compromised scalps was not found to be statistically different to normal scalps patients.
Deposition, absorption and excretion parameters were measured in 20 volunteers (10 patients using ZPT containing shampoo alone (Group A) and 10 using the ZPT containing shampoo and ZPT containing tonic combination (Group B)) over a 4 day treatment period. Each treatment group was comprised of 5 patients with healthy scalps and 5 patients with compromised scalps with either severe dandruff or seborrheic dermatitis. All patients used 10 g of shampoo per day and those in Group B also used 4 g of tonic per day during the 4 day treatment period.
Measurements of ZPT 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 ZPT is excreted in the urine within 24 hours, thus for the purposes of this study the level of ZPT excreted was taken to represent the level of ZPT absorbed (i.e. systemic dose).
Mean ^{14}C-ZPT Systemic Load results are shown in table 3.

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

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

Analysis of the dermal deposition data indicated:
* ZPT deposition on the hands between the treatment groups A and B was not significantly difference [sic] throughout the study except on day 2
* ZPT 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
* ZPT deposition on the hands was determined to be approximately half the level deposited on the scalp
* ZPT 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.

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 ZPT 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 ZPT across the skin.

SCCS comment
From the data it can be seen, that systemic exposure to ZPT increases with increasing amount of ZPT applied. Systemic exposure loads up to 3.43 µg/kg/d were obtained from the study.
In a follow-up study to the above mentioned study (which is therein declared as study # CRB9907-083) it is stated that deposition in the study described above was higher than the deposition in the later study. It was discussed, that this could - apart from interstudy variation - be due to the fact that deposition is lower, when tonic is applied to dry hair. In the first study, tonic was applied to wet hair only, whereas in the subsequent study, only the first application of tonic was on wet hair.

Study provided in submission II

As the new submission intends to increase of the authorised concentration of ZPT from 1.0% to 2.0% in rinse-off antidandruff hair care products, a new clinical study has been performed to determine the deposition, systemic absorption and excretion of ^{14}C-radiolabeled ZPT resulting from the repeated application of treatment regimens including 2% ZPT containing antidandruff shampoo in a 7-day randomized, parallel group comparison study in which a 4-day treatment period was followed by a 3-day wash-out period. The study adhered to GLP and Quality assurance principle, informed consent was obtained from the participants.
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 per 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 are given in table 6.

Table 4: Treatment regimens of the clinical dermal absorption study provided in submission II

Treatment	Test Products

Regimen	
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 the 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 blow-dried. 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 hrs later. Subjects assigned to treatment regimens B and C applied 3 doses of the tonic. The 1st dose was also applied immediately after 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 hrs 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 hr urine specimens were collected during the entire study period. Subjects were dismissed on day 8 upon completion of the 24 hr urine collection on day 7.

Results: The measured scalp deposition did not increase with repeated application. 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% 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 5-10 times 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.

Mean values from excretion measurements calculated on day 4 were used to approximate daily ZPT absorption and excretion during longer term consumer use. The 24 hr 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.

Ref.: A20, A21

SCCS comments

(1) Calculated recoveries were lower than expected (61 % to 86 %). The study authors attributed low recoveries to low values obtained from rinse water measurements due to insufficient stirring of rinse water during sampling. After further regression analysis the

authors came to the conclusion that the mean recovery was highly correlated with rinse water measurements but not with skin deposition or urinary analysis. It was therefore concluded that the low recovery rates do not raise questions about the validity of the disposition data. As the calculation/regression analysis performed by the study authors has not been made transparent, the SCCS cannot reproduce the conclusions. Therefore, the value of systemic exposure load from treatment C (4.66 µg/kg/day) + 1 standard deviation (SD) (0.59 µg/kg/d) will be taken for risk characterisation which is 5.25 µg/kg/d.

(2) The applicant states, that "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." SCCS compared the systemic loads obtained from the study provided in submission I (using a shampoo containing 1% ZPT and a tonic containing 0.1 % ZPT) with systemic loads from the study provided in submission II. A graphic overview is given in figure 1. From this figure it can be seen that indeed, as the applicant states, apparent steady states could be inferred from the data. However, the respective levels of the individual "apparent" steady states increase with increasing amounts ZPT applied. Therefore, it cannot be excluded that higher systemic exposure loads than the maximal levels derived from the studies submitted might be obtained in consumers, in case that ZPT containing products will be applied frequently and in considerable amounts. This is further supported by the results of a dermal toxicokinetic study in female rats, which suggest that maximal absorption of ZPT and therefore pyrithione exposure was achieved at 30 mg/kg (see section 3.3.9). These considerations further support the use of the maximal systemic load + 1 SD.
(3) The applicant uses a 4 day treatment regimen to extrapolate to long term consumers use. SCCS considers this as not appropriate.

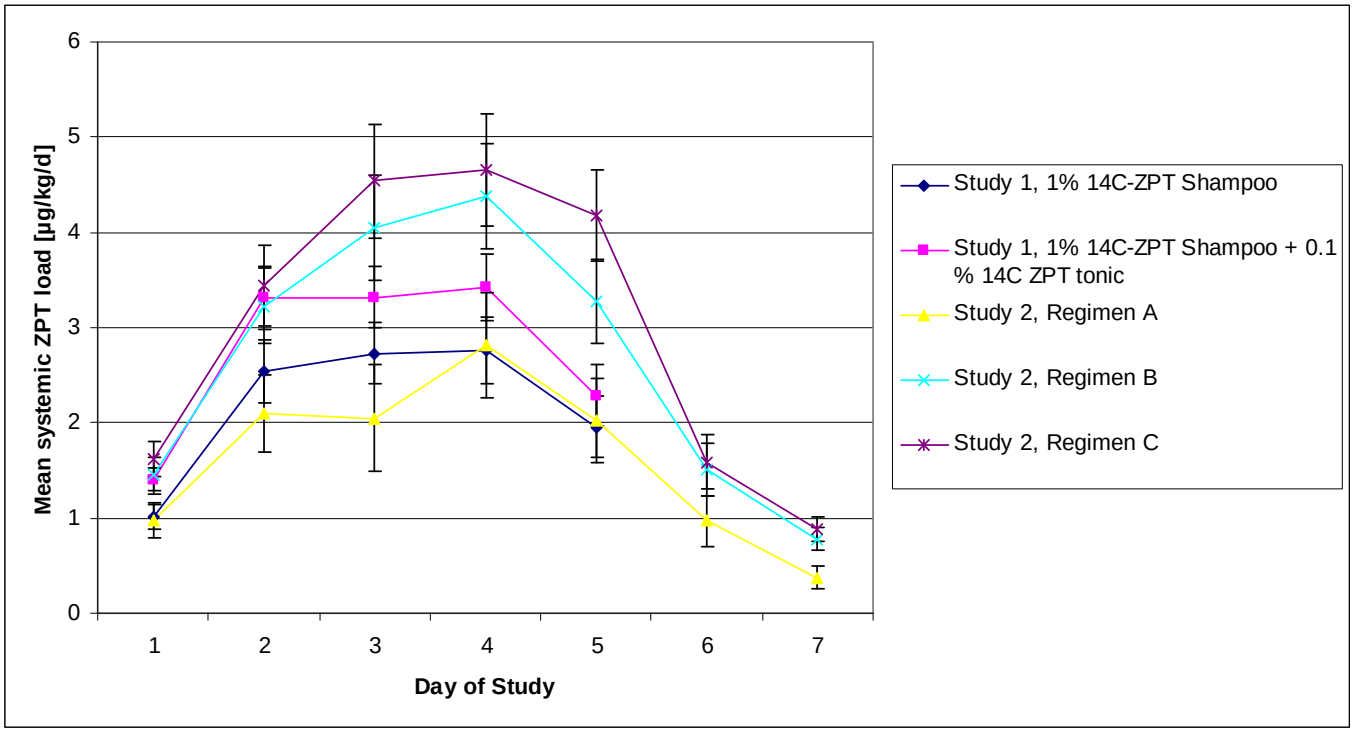


Figure 1: comparison of systemic ZPT loads obtained from two clinical studies performed with shampoo and lotion containing ZPT as provided in submission I and II. Regimen A, B and C of study 2 were as follows: Regimen A: Shampoo with 1% ^{14}C-ZPT + Leave-on Tonic with 0.1% ^{14}C-ZPT (2 applications); Regimen 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); Regimen C: Shampoo with 2% ^{14}C-ZPT + Leave-on Tonic with 0.25% ^{14}C-ZPT (3 applications).

Summary dermal absorption:
From the animal studies available in submission I, it was concluded in SCCNFP 0671/03 that percutaneous absorption of ZPT varies from approximately 0.03 to 3.4%. Further studies on dermal absorption of ZPT have been performed thereafter.
One *in vitro* dermal absorption study using human skin and three *in vivo* animal studies were not available for evaluation.
From dermal toxicokinetic studies (not fully OECD compliant) performed in female CD rats in order to build up a PBPK model for ZPT, absorption percentages of 2.3, 8.6 and 0.3 were derived after single dermal administration of 10, 30 and 100 mg/kg ZPT. However, these values are associated with uncertainties (see study description).
Dermal absorption values obtained from animal studies were not taken for MOS calculation as two clinical human studies were performed. One investigated the systemic absorption of a shampoo containing 1 % ZPT (with or without combination with a leave-on tonic containing 0.1 % ZPT) in a 4-day treatment regimen. In this study, a systemic load of ZPT up to 3.43 µg/kg/d was derived. In the second clinical study, the systemic absorption of a shampoo containing 2 % ZPT (either in combination with leave-on tonics containing 0.1 and 0.25 %ZPT or with a leave-on tonic containing 0.25% ZPT only) was investigated in a 4-day treatment regimen. Systemic exposure loads up to 4.66 µg/kg/d were derived. As in the new submission the applicant applies for an extension of the authorised concentration from 1.0% to 2.0% in rinse-off antidandruff hair care products, a systemic exposure load of 4.66 µg/kg/d (the highest exposure load obtained in a clinical study from a shampoo containing 2% ZPT) + 1 SD yielding 5.25 µg/kg/d is taken for MOS calculation. The reason for the addition of 1 SD is based on the fact that (a) low recoveries were obtained and (b) even higher systemic amounts of exposure cannot be excluded after repeated prolonged exposure to ZPT containing products.

3.4.5. Repeated dose toxicity

General Remark on Repeat Dose toxicity
The repeat dose toxicity of ZPT has been investigated in several studies using the oral (gavage and diet), dermal and inhalation route. Further studies are available for sodium pyrithione from which read across to ZPT may be performed.

3.4.5.1. Repeated Dose oral / dermal / inhalation toxicity (of different duration)

Oral studies

Summary taken from SCCNFP/0671/03

Concerning ZPT:
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 ZPT 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. With respect to paralysis, a NOEL of 0.5 mg/kg/day (500 µg/kg/d) has been determined in two year feeding studies in rats. At this dose no toxic effects were observed over the two year study period.

Concerning shampoo formulations containing ZPT:

Orally administered ZPT in a shampoo formulation produces a reversible paralysis in rats and rabbits within one to two weeks at levels of 10 mg/kg/day. A dose level of 10 mg/kg/day of ZPT in shampoo was used in a monkey gavage study which lasted 16 weeks. No adverse effects were observed. Because of the emetic potential of shampoos, oral dosing of dogs has not produced any toxic symptoms, including ocular effects.

New / additional data
Further repeat dose studies performed in rats or monkeys are given at ECHAs website (http://echa.europa.eu/). The studies are not available for evaluation.

In addition to repeat dose studies performed with ZPT, HSE (2003) derives a NOEL of 0.5 mg/kg/d based on hind limb muscle atrophy from an oral 90 day study in Sprague-Dawley rats performed with **sodium pyrithione,** which is described in HSE (2003), therein cited as Unpublished, 1988. A read across from sodium pyrithione to ZPT is considered appropriate based on the following reasoning: data on metabolism of ZPT (see section "toxicokinetics" and see also SCCNFP/0671/03) demonstrate that Zn is cleaved from the molecule after uptake and that ADME of the metal ion and the pyrithione moiety is different. Studies performed in pigs using NaPT and ZPT pointed to a common metabolic pathway (references B.68, B69. B.70). Further, both Zn^{2+} and Na^{+} ions are not considered to be neurotoxic. Thus, it can be assumed that neurotoxic effects observed after ZPT exposure are due to the pyrithione moiety. It can thus be concluded that results from other pyrithione-liberating salts might support the findings obtained with ZPT.

Dermal studies

Taken from SCCNFP/0671/03

Larson (1957) conducted a 90-day percutaneous toxicity study with ZPT (2 ml of water per gram of 50% wettable ZPT powder) using albino rabbits. Doses of 125, 250, 500, 1000 and 2000 mg/kg were applied daily (5 days per week) to groups of three or four animals for 13 weeks. The animals were harnessed during application and remained so until the material dried, at which time the animals were washed. None of the rabbits receiving 1000 or 2000 mg/kg survived the 90-day test period, the longest survival being 21 days. Four of twelve animals dosed at the lower levels survived, and those were necropsied at that time. Focal necrosis of, either the brain or spinal cord in three of the four surviving animals [sic]. There were no histological changes in other organs.
Nelson et al (1965) also conducted studies on the toxicity of ZPT applied topically to rabbits. The material was left in contact with the skin during the periods between application and no effort was made to preclude ingestion of the ZPT. Dosage was daily, and ranged from 50 to 480 mg/kg. After 7 to 15 daily treatments, the rabbits developed hind-limb weakness and diarrhoea. With continued treatment, weakness of both forelegs and death occurred. Treatment was discontinued in three instances coinciding with the onset of severe quadraparesis in two animals and mild quadraparesis in the third rabbit. All three animals recovered. Histopathological observation after autopsy of severely paralysed rabbits revealed no significant structural alterations in the brains, spinal cords, peripheral nerves, muscles, and abdominal or thoracic viscera in 8 of 12 rabbits. In the other four rabbits various alterations in CNS tissue were seen that the author associated with a protozoan infection.
In work conducted at Food and Drug Research Laboratories, cited by Snyder et al. (1965), two groups of six rabbits received daily topical applications, five days a week, of 5 ml of a 20% aqueous paste of a commercial soap, with or without 1 % ZPT (on a soap basis). This amounts to 10 mg/kg/day. The animals were kept in stocks for six hours after treatment, at which time the skin was washed and dried, and the animals were returned to their cages. There was a total of 65 applications, equivalent to 50 mg/kg/week. No difference was noted in skin effects or in gross or microscopic pathology between the test group and the control

group treated with soap alone. No effects on the eye were seen in either group. Histopathologic study of the brains of both groups revealed changes frequently seen in laboratory rabbits that are thought to be associated with the protozoan organism Encephalitozoon cuniculi. This condition is spontaneous, is seen with great frequency, and is mild chronic in nature. There were no compound-related lesions. In addition to the rabbit studies, two sub-chronic mouse percutaneous toxicity studies were conducted and are summarised below. A six-week study by Dobbs and Nixon (1973) was conducted to determine dose levels of ZPT for an eighteen-month dermal carcinogenicity bioassay study that would not cause systemic toxicity from oral ingestion during grooming. Ten female mice were used per test material and dose level; five were group-housed, and five were housed individually. Application of 0.1 ml of undiluted test material was made five times per week to a 2 x 2 cm clipped area of the interscapular skin for six weeks. Test materials were ZPT at 0.08, 0.4 and 2.0% in a 1 % surfactant (triethanolamine lauryl sulfate)/ 0.5% thickener (Methocel) aqueous slurry, and a vehicle control. These levels correspond to approximately 0.28, 1.4, and 7.0 mg/kg/day. Four animals, two group-housed and two single-housed, treated with the mixture containing 2% ZPT were necropsied after six weeks of treatment, and no gross abnormalities were observed. None of the treatments produced local or systemic effects after a total of 30 applications (six weeks). The study was terminated at this point. Another mouse study to determine maximum tolerable cutaneous doses of ZPT in a 1% surfactant/0.5% thickener vehicle was conducted by Gargus (1974). Groups of 30 mice (15 male and 15 female), individually housed, were treated topically three time weekly for four weeks with 0.1 ml doses of 0.4, 2.0 and 10.0% (10, 50 and 250 mg/kg/application) ZPT. The high dose group had the 10% concentration applied for one week, and since no toxicity was observed, a 20% concentration (500 mg/kg/application) was substituted for an additional three weeks. After four weeks no skin irritations or other toxicity was observed for animals treated with 0.4 and 2.0% concentration of ZPT. Animals treated with 20% ZPT showed thickening of the skin and erythema. No hind-limb paralysis as observed in rats or rabbits was seen in any of the groups.

From the Snyder et al (1965) study, one can conclude that a dose level of 10 mg/kg/day of ZPT applied topically to rabbits is a no-effect level. Interpretation of the rabbit studies by Larson (1957) and Nelson et al (1965) summarised above, is complicated by several factors. Depending on what Larson (1957) used to wash the animals, some material probably remained on the skin and was subsequently ingested. Nelson et al (1965) study, and the uncertainty regarding ingestion in these studies make any conclusions tentative, at best. That ingestion of ZPT occurred in these studies can be supported by examining dose levels used in teratology studies conducted by Nolen et al (1975, 1979). He reported that application of 25 to 100 mg/kg/day of ZPT to the backs of rabbits produced no adverse effects when ingestion was meticulously prevented. Therefore, 100 mg/kg/day instead of 10 mg/kg/day is a better estimate of a no-effect level for topical administration to rabbits. The no-effect level in mice for percutaneous toxicity is approximately 100 mg/kg/day, and the effect level (local irritation) is approximately 200 mg/kg/day.

New / Further Data

MAK (2012) describes two dermal repeat dose studies which address mechanistic aspects. After dermal application of 100 mg ZPT for 10 days to Sprague-Dawley rats, all of 5 treated animals showed reduced amplitude of the evoked compound muscle action potential (CMAP), 4 animals showed signs of a reduced muscle tone.

SCCS comment

Apparently the same study was described shortly in an Addendum to the proposal for Annex III listing of zinc pyrithione provided by Procter and Gamble in January 2002. Therein the following was stated: after 10 day repeated dermal administration of 100 mg/kg/d ZPT to female rats (strain not given) the muscle evoked potential in the hindlimb (measured as M-wave amplitude) was significantly reduced when compared to untreated controls (22.95 ± 11.61 mV in treated animals versus 46.56 ± 5.95 mV in controls).

In a 28-day dermal neurotoxicity study groups of 5 Sprague-Dawley rats received daily dermal doses of 0, 50, 150 and 200 mg ZPT/kg/d (male animals) or 0, 10, 25, 50, 75 and 100 mg ZPT/kg/d (female animals). The vehicle was 0.1 % triethanolamine-lauryl sulfate, the treatment site was protected by a fixed convex piece of plastic shielding. Low muscle tone was observed at 150 and 200 mg/kg/d in male animals beginning on day 8 and day 11 that continued throughout the study duration. Hindlimb and forelimb grip strength as well as muscle tone and body weight were decreased in male animals of the two highest doses on days 14 and 28. No significant changes in plasma, RBC or brain cholinesterase was observed at any dose tested for any time point measured. Decreases in the electrophysiological measurements measured as the maximum amplitude were observed in males at 150 mg/kg/d. In female animals low muscle tone was observed at 50, 75 and 100 mg/kg/d beginning on day 8 in the 100 mg/kg/d group, on day 15 in the 75 mg/kg/d group and on days 22-28 in the 50 mg/kg/d group. On day 14 grip strength was reduced in the 75 and 100 mg/kg/d group and on day 28 grip strength was reduced in the three highest dose groups. No consistent decreases or dose dependent changes were apparent in plasma, RBC or brain cholinesterase at any dose tested. Decreases in the electrophysiological values measured as the maximum amplitude were observed in the 50 and 75 mg/kg/d group (electrophysiological measurements not taken at the 100 mg/kg/d dose level).

Ref.: D1 Arch Chemicals 2003 (Link given in MAK (2012))

SCCS comment
A dermal NOEL of 8.33 mg/kg/d might be derived from this study (NOEL of 25 mg/kg/d after 28d, to be adjusted to subchronic study, i.e. divided by 3= 8.33 mg/kg/d)

A further subchronic toxicity study is mentioned at ECHAs website (http://echa.europa.eu/). The study is not available for evaluation. A NOAEL of 100 mg/kg/d was derived from that study.

SCCS comment to studies with dermal administration of ZPT
The fact that hindlimb weakness after dermal administration was not observed in some of the studies might be due to the dosing regimen and the vehicle used. In oral studies it could be demonstrated, that in contrast to continuous administration, effects were less pronounced or not observable, when there were discontinuities in the dosing (e.g. 5 days per week). Further, when using water or DMSO as vehicle, toxic effects were more pronounced with DMSO as vehicle.

Inhalation studies
ECHAs website mentions a 21 d nose-only study in Sprague-Dawley rats from which a NOAEL of 2.0 mg/m^3 was derived (http://echa.europa.eu/). Further, a 90 d whole-body study performed in Sprague-Dawley rats is mentioned at ECHAs website. From this study, a NOAEL of 0.5 g/m³ air was derived. The studies are not available for evaluation.

In MAK (2012), a 28 d inhalation study is described: groups of 15 male and female Sprague-Dawley rats were head-nose exposed to ZPT aerosol (MMAD 1.3 – 1.8 µm) concentrations of 0.0, 0.5, 1,5 and 5.0 mg/m^3 for 6 hr/d, 5d/week for 4 weeks. The study was mainly aimed at determining lung toxicity of ZPT. Body weight and body weight gain were reduced from 1.5 mg/m^3 onwards. Even at the lowest exposure concentration bronchio-interstitial pneumonitis and hypertrophy of smooth muscles of the lungs were observed as well as increased lung weights. In broncho-alveolar lavage fluid (BALF) eosinophilic cells, neutrophils, lymphocytes, cell lysis, erythrophagocytosis, mucus (in males only), lactate-dehydrogenase (study day 5 and 12) and protein (study day 5 and 12) were increased. In some animals of the two higher doses further lesions (enlargement of the bronchial lymph nodes, enlargement of the mediastinal lymph nodes) could be observed which were classified as slight to moderate hyperplasia. In two female animals of the

highest dose group minimal to slight degeneration of sceletal muscles were observed. As lung effects were present even at the lowest concentration tested, no NOAEC could be derived for local effects in the lung. For systemic effects, a NOAEC of 1.5 mg/m^3 was derived.

A summary on repeat dose toxicity is given in section 3.4.5.2.

3.4.5.2. Chronic (> 12 months) toxicity

Taken from SCCNFP/0671/03

A two year feeding study was conducted by Larson (1958). Young Wistar rats in groups of ten males and ten females were fed diets containing ZPT 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 ZPT. 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.
From this study, an oral NOAEL of 0.5 mg/kg/d (500 µg/kg/d) was derived.

<u>Further data</u>
Two oral chronic studies performed with ZPT are mentioned at ECHAs website (http://echa.europa.eu/), one is apparently the study by Larson (1958) already evaluated for SCCNFP 0671/03. The other study (GLP-compliant and performed according to OECD 453 in male and female Spague-Dawley rats) is not available for evaluation and concluded that there were no dose-related or significantly increased incidences of tumours in the dose groups compared to the controls.

Data on metabolism of ZPT (see section "toxicokinetics" and see also SCCNFP/0671/03) demonstrate that Zn is cleaved from the molecule after uptake and that ADME of the metal ion and the pyrithione moiety is different. Studies performed in pigs using NaPT and ZPT pointed to a common metabolic pathway (references B.68, B69. B.70). Further, both Zn^{2+} and Na^+ ions are not considered to be neurotoxic. Thus, it can be assumed that neurotoxic effects observed after ZPT exposures are due to the pyrithione moiety. It can thus be concluded that results from other pyrithione-liberating salts might support the findings obtained with ZPT. In this respect chronic studies performed with sodium pyrithione can be used as supporting studies.
A chronic toxicity study performed with sodium pyrithione is described in HSE (2003), therein cited as Unpublished, 1991g. From that study it was concluded that there was no evidence for a carcinogenic potential of sodium pyrithione. A NOAEL of 1.5 mg/kg/d was established from this study based on muscle atrophy and sciatic nerve degeneration.

(Comment: the study is also mentioned at ECHAs website (http://echa.europa.eu/) in section "Carcinogenicity").

Summary on repeat dose toxicity and chronic toxicity

Oral:
Several oral repeat-dose studies of different durations have been performed with ZPT. In addition, one sub-chronic and a chronic oral studies performed with sodium pyrithione can be considered adequate to assess repeat-dose effects of ZPT. Not all of these studies were available for evaluation. Neither of the non-available studies resulted in NO(A)ELs lower than 500 µg/kg/d – the value derived in SCCNFP 0671/03. However, as the studies were not available for evaluation, it cannot be ruled out, that SCCS would arrive at different NOAELs after thorough assessment. As long as studies won't be made transparent to the SCCS, SCCS will take the NOAEL of 500 µg/kg/d (the value derived in SCCNFP 0671/03) obtained from a chronic oral study (Larson, 1958) performed with ZPT based on paralysis/hind-limb weakness for MOS calculation.
The SCCS is aware that HSE (2003) considered the Larson 1958 study as inadequate due to insufficiently large group sizes to ensure statistical power. However, a 90 d oral study performed with sodium pyrithione which was considered adequate by HSE, also lead to a NOAEL of 500 µg/kg/d, supporting the outcome of the Larsson study. Or in other words: 500µg/kg/d is considered as an adequate oral NOAEL for neurotoxic effects of pyrithiones.

Dermal:
Several dermal repeat-dose studies have been performed. Interpretation of the findings is partly hampered by the fact that grooming was not always prevented and that intermittent exposure regimens (causing recovery) have been applied. From a 28-day dermal neurotoxicity study in which grooming was prevented, an NOEL of 8.33 mg/kg bw/d can be derived based on reduced electrophysiological parameters and muscle tone which might be taken for MOS calculation.

Inhalation:
Three inhalation studies of different durations have been performed which are not available for evaluation. In a 90d study, animals were whole-body exposed and oral intake cannot be excluded. In a study focusing on lung toxicity, no NOAEC could be derived for local effects in the lung and an NOAEC of 1.5 mg/m^3 was derived for systemic effects.

3.4.6. Mutagenicity / Genotoxicity

Based on an Ames test, an *in vitro* CHO/HGPRT gene mutation assay, an *in vivo* mouse bone marrow micronucleus test and an UDS-Assay (references B58 and B60), SCCNFP/0671/03 concluded that ZPT has shown no mutagenic effect in any of the *in vitro* and *in vivo* studies conducted.
Since publication of SCCNFP/0671/03, further *in vitro* and *in vivo* studies on mutagenicity/genotoxicity of ZPT have been performed. However, the data are only partly available for evaluation and described in the following two sections.

3.4.6.1 Mutagenicity / Genotoxicity *in vitro*

An Ames test with Salmonella strains TA98, TA 100, TA 1535 and TA 1537 using ZPT concentrations from 0.03 – 33 µg/plate was negative in the absence or presence of metabolic activation (rat liver S9-mix from Aroclor 1245-treated rats); no information on GLP- and guideline-adherence (Zeiger et al., 1987, taken from MAK 2012).

An Ames test with Salmonella strains TA98, TA 100, TA 1535 and TA 1538 used aqueous 48 % ZPT concentrations from 0.03 – 5.0 µg/plate in the absence of S9 and concentrations from 10 – 333 µg/plate in the presence of rat liver S9 mix. Cytotoxicity was observed from 3.3 µg/plate without metabolic activation and from 333 µg/plate with activation. No mutagenicity was observed (MAK, 2012).

In an in vitro Comet Assay using keratinocytes from human epidermis and human melanocytes from epidermis, DNA strand-breaks were induced after treatment with 100 or 500 nM ZPT. ZPT treatment at 500 nM induced comets with average tail moments that were increased approximately 3-fold over untreated controls within 1 hr of exposure. Significant comet formation was even observed at 100 nM, a dose that does not impair the viability of the cells. In a modified protocol using bacterial formamidopyrimidine-glycosylase, no oxidative DNA-damage was induced after treatment with ZPT.

Reference: D15 (Lamore et al., 2010).

ECHAs website ((http://echa.europa.eu/) mentions further *in vitro* assays which are not available for evaluation:

- a GLP-compliant Ames test performed according to OECD TG 471 with Salmonella strains TA98, TA100, TA1535 and TA1537. ZPT was considered negative in the absence and presence of metabolic activation (S9 mix not further specified) under the test conditions used.

- a GLP compliant mammalian cell gene mutation assay performed according to OECD TG 476 using Chinese hamster lung fibroblasts with and without metabolic activation. The test substance was considered not to induce gene mutations in this system.

- a GLP-compliant *in vitro* mammalian chromosome aberration test in Chinese hamster lung fibroblasts (V79) performed according to OECD TG 473. It was concluded that ZPT induced chromosomal aberrations under the test conditions used.

- a GLP-compliant mammalian cell gene mutation assay performed according to EPA OPP 84-2 using Chinese hamster ovary cells. ZPT was considered negative in the absence or presence of metabolic activation (S9) under the test conditions applied (comment: this study is also described in HSE (2003), therein cited as unpublished, 1990b.).

- a GLP-compliant mammalian cell gene mutation assay performed according to OECD TG 476 using Chinese hamster ovary cells. ZPT was considered negative in the absence or presence of metabolic activation (S9) under the test conditions applied.

- a GLP-compliant bacterial reverse mutation assay performed according to EPA OPP 84-2 using Salmonella strains TA98, TA100, TA1535, TA1537 (with and without metabolic activation; microsomal enzymes from Aroclor induced rat liver as metabolic system). ZPT was considered negative under the conditions applied.

- a GLP-compliant *in vitro* mammalian chromosomal aberration test using human peripheral lymphocytes from 2 volunteers (apparently not performed according to a guideline). It was concluded that ZPT did not induce chromosomal aberrations under the test conditions applied (comment: the study might be identical to the study cited as unpublished 1992 in HSE (2003)).

- a GLP-compliant DNA damage and repair assay (unscheduled DNA synthesis) in mammalian cells *in vitro* according to US EPA 84-4 using rat hepatocytes. No conclusions were drawn from this study.

3.4.6.2 Mutagenicity/Genotoxicity *in vivo*

ECHAs website (http://echa.europa.eu/) mentions three *in vivo* genotoxicity assays which are not available for evaluation:

- a GLP-compliant *in vivo* mammalian erythrocyte micronucleus test performed according to OECD TG 474 in male and female Crl:NMRI BR mice. It was concluded that ZPT did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes after oral gavage of doses up to 1300 mg/kg.

- a GLP-compliant micronucleus assay performed according to EPA OPP 84-2 using Sprague-Dawley mice*) and intraperitoneal administration. ZPT was concluded to be negative in this assay. (Comment: this study appears to be identical with a study described in HSE (2003), therein cited as unpublished, 1990c. HSE concluded that ZPT did not increase micronucleus formation and considered this study as adequate).
*) the SCCS is not aware that such a mouse strain exists; the mouse strain was not specified in HSE (2003)

- a GLP-compliant chromosome aberration assay performed similar to EC method B.10 and OECD 473 in male and female Cynomolgus monkeys applying oral (capsule) ZPT treatment once daily for 28 days. It was concluded that ZPT is not clastogenic under the conditions of this test. (Comment: the study appears to be identical with a study described in HSE (2003), therein cited as unpublished, 1992e. HSE (2003) concluded that this study is of limited value in determining the potential of ZPT to produce chromosomal aberrations, as no positive control had been included).

Summary on Genotoxicity
From the studies available for SCCNFP/0671/03, it was concluded that ZPT is not mutagenic. Since then, further *in vitro* and *in vivo* genotoxicity/mutagenicity studies have been performed. Apart from two studies published in the open literature, the studies are not available for evaluation.
Therefore, no firm conclusion with respect to genotoxicity/mutagenicity can be drawn by the SCCS. The SCCS, however, is aware that HSE (2003) and MAK (2012) considered ZPT as non-genotoxic and non-mutagenic.

3.4.7. Carcinogenicity

Oral:
See section 3.3.5.3

Dermal:

Taken from SCCNFP/0671/03

Since the highest dose level of 0.1 ml of 10% ZPT was well tolerated in mouse pilot studies it was deemed suitable for use in the dermal carcinogenicity bioassay conducted by Patterson and Gargus (1979). ICR Swiss mice (730 animals) were selected at random and assigned to the following groups. Each received the noted treatment on a 6 cm² clipped area. No attempt was made to control ingestion of the applied material. The two dose levels represent approximately 20 and 100 mg/kg/day.

Table 5: Treatment regimen of the dermal carcinogenicity bioassay by Patterson and Gargus (1979).

Group	Treatment	Number of mice	
		male	female
1	Negative controls, no treatment	139	141
2	Vehicle controls, 0.1 ml	75	75
3	Low dose, 0.1 ml vehicle (2 mg ZPT/application)	75	75
4	High dose, 0.1 ml vehicle (10 mg ZPT/application)	75	75

All animals were housed in individual hanging wire-mesh cages. Individual body weights were recorded initially and at monthly intervals. Observations were made daily for mortality and at each treatment period for evidence of systemic effect and skin lesions in the area of treatment. Treatment was continued for 18 months, at which time it was discontinued, and the mice were maintained until each group mortality reached 75%. The group was then necropsied.
Only the males in the high-dose group experienced a mortality rate of 75% prior to the end of the study. For this group the average survival time was 409 days as compared to 492 days for the untreated males, which suggests a relationship to ZPT toxicity. However, neither gross examination of the animals and tissues, revealed any consistent lesion that would explain the reduced life span. The skin of all three treated groups exhibited changes consistent with exposure to a low-level chemical irritant.
There were no significant differences in the types or incidence of tumours or abnormal tissue masses between any of the groups that could be related to the administration of ZPT.
The chronic study summarised above reveals no evidence of a carcinogenic response when ZPT was applied topically (up to 100 mg/kg/d) in lifetime studies using mice and rats.

New data:
At ECHAs website (http://echa.europa.eu/) a GLP compliant dermal life-time carcinogenicity study performed according to EPA 83-2 is mentioned, which investigated the dermal administration of sodium pyrithione to the mouse over 80 weeks. Sodium pyrithione did not affect tumour formation adversely. The only observed lesion, which appeared to be related to the treatment, was epidermal hyperplasia (dermal irritation) at the application sites of high and mid dose animals. The study is not available for evaluation.

Conclusion on carcinogenicity
From chronic oral and dermal studies available in submission I, SCCNFP 0671/03 concluded: "no evidence of a carcinogenic response was seen when ZPT 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."
Since that, further chronic (lifetime) studies performed with ZPT and sodium pyrithione (from which read across to ZPT is considered adequate) using the oral and dermal uptake pathway have become available.
These studies are not available for evaluation. Therefore, no firm conclusion with respect to oral and dermal carcinogenicity of ZPT can be drawn by the SCCS. The SCCS, however, is aware that HSE (2003) and MAK (2012) considered ZPT as non-carcinogenic.
Carcinogenicity of ZPT has not been investigated by the inhalation route.

3.4.8. Reproductive toxicity

3.4.8.1. Fertility

In GLP- and guideline-preceding study, fertility effects of ZPT after dermal administration were investigated in Sprague-Dawley rats. Animals were treated with a 48% ZPT slurry at doses of 1.2, 7.5 and 15.0 mg/kg/d for 8 weeks (grooming not prevented) or only on days 6-15 of gestation (grooming prevented). In animals receiving 8 week treatment, either treated males were mated with untreated females of untreated males were mated with treated females. ZPT dosed rats from the 8 wk treatment scheme did not differ significantly from controls in either growth or reproductive characteristics except a statistically significantly lower lactation index in females of the highest dose group. No toxic signs such as paralysis and no test-related histopathology was seen in the males. Further, neither reproduction nor neonatal viability was affected after topical administration of ZPT on GD 6—15. A NOAEL of 7.5 mg/kg/d can be derived from this dermal study.

Reference: B43

Comment

This study was already available for SCCNFP 0671/03, but therein it was discussed together with the developmental/teratogenic studies.

3.4.8.2. Two generation reproduction toxicity

Both HSE (2003) and MAK (2012) describe a two generation reproduction toxicity study performed with **sodium pyrithione**, from which read-across to ZPT is considered appropriate (see section 3.3.5.3). The study is not available for evaluation.

Groups of male and female Sprague Dawley received aqueous suspensions of 0, 0.5, 1.5 and 3.5 (the latter dose was reduced from 4.5 mg/kg/d due to toxicity) mg/kg/d sodium pyrithione by gavage. Parental animals were treated for 11 weeks, and then mated. Dosing of females continued through mating, gestation and lactation. In parental females at the highest dose, body weight was decreased by approximately 10% at the end of gestation and during lactation. A single female of this dose group was killed *in extremis* due to hind limb impairment. No further mortalities or clinical signs of toxicity were noted in parental animals. At necropsy of parental animals, atrophy of hind limb skeletal muscle (27/50) was found at the top dose, characterised by a reduction and variation in the diameter of muscle fibres. In male parental animals, copulation and fertility indices were both decreased by 26 % at the highest dose group.

In F1 pups there were no effects on gestation success or duration, number of pups born, live births, indices of viability and lactation, cumulative survival or sex ratio. No effects on pup weight were observed. No pup abnormalities were found following necropsy. No effects on development of ear opening, righting reflex or eye opening were observed. However, at the highest dose a reduced incidence of startle response at 15 d was observed (by 10%).

In the P_1 generation, 2 females of the highest dose group were killed *in extremis* due to hind limb impairment. No further mortalities or clinical signs of toxicity were noted. No adverse effects on food consumption or body weight were noted. No adverse effects on the indices of copulation or fertility were observed in the P_1 animals. At necropsy of P1 animals, abnormal findings among P1 animals were confined to atrophy of skeletal muscle (29/50) at the top dose.

There were no effects on the success or duration of gestation, number of live births, viability, lactation and the cumulative survival score in the F2 generation. In F2 pups no effects on pup weight were observed. No abnormalities were found following necropsy. No effects on development of ear opening, righting reflex or eye opening were observed. However, in the top dose a reduced incidence of startle response (by 10%), an indicator of delayed development, was observed on day 15.

From this study, a NOAEL of 1.5 mg/kg/d was established for parental toxicity based on hind limb impairment and skeletal muscle atrophy. An NOAEL of 3.5 mg/kg/d was derived for fertility effects.

Ref.: MAK (2012), HSE (2003)

Two-2-generation studies are mentioned at ECHAs website (http://echa.europa.eu/). These studies are not available for evaluation:

- a 2-generation study performed according to EPA OPPTS 870.3800 in male and female Sprague-Dawley rats receiving 0, 0.7, 1.4, and 2.8 mg/kg/d aqueous substance by gavage. For parental animals, NOAELs of 1.4 and 0.7 mg/kg/d were derived for male and female animals, respectively. For F1 animals NOAELs of 1.4 and 0.7 mg/kg/d were derived for male and female animals, respectively.

- a further 2-generation study. This study appears to be identical with the 2-generation study performed with sodium pyrithione (described above). However, the material is not described and it is not stated, whether sodium pyrithione had been used instead of ZPT. The following NOAELs were derived:
NOAEL parental animals (male/female): 1.5 mg/kg/d
NOAEL F1: 1.5 mg/kg/d (male) and 0.5 mg/kg/d (female)
NOAEL F2: (male/female): 3.5 mg/kg/d

3.4.8.3. Teratogenicity

Taken from SCCNFP/0671/03

Several teratology/reproduction studies have been conducted using rats and rabbits, in which ZPT 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 ZPT, 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 summarised in the table below, followed by a brief description of each of the studies.

Table 6: Summary of Teratogenicity Studies performed with ZPT

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	B24
Rat	Oral	7.5 15.0	7.5 – none 15.0 – increased incidence of fused or forked ribs	B43
Rat	Topical with ingestion of applied material	2.5 7.5 15.0	2.5 - none 7.5 - none 15.0 - none	B43

Rat	Topical	2.5 7.5 15.0	2.5 - none 7.5 - none 15.0 - none	B43
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	B43
Rabbit	Oral	1.0 2.5 5.0	1.0 – none 2.5 – none 5.0- none	B43
Rabbit	Topical	25.0 50.0 100.0	25.0- none 50.0 – none 100.0- none	B43

Oral, Ingredient based data

In the teratology study conducted by Haley et al (1971) groups of 19, 16, and 20 albino rats were administered dose levels of 0, 7.51 or 15.0 mg/kg/day, respectively, of ZPT for the sixth through the fifteenth day of gestation. The material was dosed as a solution in corn oil by oral intubation. All animals were allowed food and water ad libitum. Maternal body weights were depressed in the groups given ZPT. The mean weight gain per animal for the control group was 132 mg, for the group receiving 7.5 mg/kg, 69 mg; and for the group receiving 15.0 mg/kg, 89 mg. An increased incidence of skeletal abnormalities, particularly fused and forked ribs, was seen in the foetuses in the higher dose group, but not in the group receiving 7.5 mg/kg/day.

Nolen and Dierckman (1979) in a series of studies evaluated the embryotoxic/teratogenic effects of ZPT in rats and rabbits. Initially they confirmed the report by Haley et al. (1971) in that pregnant rats dosed orally with 15 mg/kg/day produced litters with an increased incidence of skeletal abnormalities. They also confirmed that 7.5 mg/kg day did not cause a statistically significant increase in the number of foetal abnormalities. ZPT administered orally to pregnant rabbits from day 6 through day 18 of pregnancy was lethal to 6/15 at the 5 mg/kg level, 10/15 at the 19 mg/kg level and 15/15 at the 20 mg/kg level. The surviving animals lost weight and had significantly higher incidences of embryonic resorption.

However, there was no evidence of teratogenicity. In another experiment, rabbits were dosed orally with 1, 2.5, or 5 mg/kg of ZPT. Animals receiving 5 mg/kg lost a significant amount of weight, but none died. In addition, the number of resorptions was significantly increased compared to the water control. Dams dosed with 2.5 mg/kg ZPT gained less weight than controls and had a higher incidence of resorptions, neither observation being statistically significant, while the data from dams treated with 1 mg/kg were similar to those of the control. None of the ZPT doses had any adverse effects on foetal development. Thus, 2.5 mg/kg/day is a no-effect level for orally administered ZPT in teratology studies.

Dermal, Ingredient based data

No deleterious effects were seen in rabbits treated topically with 25, 50, or 100 mg ZPT/kg/d with oral ingestion controlled by a leather harness, and as in the other rabbit studies, no teratogenic effects were observed in this study (Nolen and Dierckman (1979)).

In a separate study using rats, Nolen and Dierckman (1979) topically applied a 48% aqueous slurry of ZPT to three groups of 10 animals at levels of 2.5, 7.5, and 15 mg/kg/day. The animals were dosed from eight weeks before mating until day 15 of gestation. No attempt was made to control ingestion during grooming in that the material was not washed off, and additional applications were made over the previous treatment. Three other groups of rats were treated at the same dose levels, but ingestion of the ZPT was prevented by means of plastic domes glued to their backs. The animals in this portion of the study were treated from day 6 through day 15 of gestation. In the rat reproduction portion of the study, ZPT produced no adverse effects on growth, pathology, or conception in the parents, or on viability, post-weaning growth, or pathology in the neonates. Ingestion of the material during grooming did cause hind-limb paralysis in 2/10 dams dosed at 7.5 mg/kg/day and in 5/10 dams dosed at 15 mg/kg day. There was no evidence of any adverse effects in any group in either the dams or foetuses when ingestion was prevented by the plastic dome. No teratogenic or adverse effects on reproduction have been seen when the material is applied topically to rats and rabbits at levels up to 15 and 100 mg ZPT/kg/day respectively (highest doses tested).

Product based data

Teratology studies have been conducted using rabbits and pigs in which ZPT was incorporated into a product base and applied topically. Jordan and Borzelleca (1975) studied the effects of 1%, 2% or 6% ZPT applied as part of a shampoo formulation to the backs of pregnant Yorkshire swine at levels equivalent to 50, 100, or 300 mg/kg/day. Product was applied from day 12 through day 36 of gestation. There was no evidence of embryotoxicity nor teratogenic effects in the foetuses.

In a study reported by Wedig et al (1976), Yorkshire pigs were again used as the test species. In this test, a 50% (w/v) suspension of ZPT in Aquafor Cream (commercial product) was applied to a 380 cm^2 area of the backs of the animals. Eight dose sites were used in rotation to prevent irritation at the dosing area. An untreated group and a group treated with Aquafor Cream without ZPT were used as controls. Dose levels of ZPT were 30, 100, and 400 mg/kg/day. Material was left on the skin for eight hrs/day from the eighth through the thirty-second day of gestation. During the treatment period each animal was individually confined in such a manner as to prevent oral ingestion of the test materials. Slight erythema was observed in some animals during the dose administration period, but all lesions were reversible and were not apparent at sacrifice (day 100). No signs of systemic toxicity were observed in any of the animals. Maternal body weights were not depressed by administration of ZPT. No evidence of teratogenic effects was observed in the foetuses from the ZPT-treated animals either grossly following examination of internal organs or upon skeletal examination.

Nolen et al (1975) have reported the results of a percutaneous teratology study of ZPT in rabbits. A lotion shampoo containing 2% ZPT was applied for two hours each day at either 1 or 2.5 g/kg (20 or 50 mg/kg of ZPT) from the seventh to the eighteenth day of gestation to groups of 15 rabbits. A third and fourth group of animals received either no treatment or the shampoo base without ZPT. These were control groups. Oral ingestion was prevented by harnessing the animals and cleaning the cages. ZPT had no effect on maternal weight gains and was not teratogenic under these conditions.

No teratogenic effects were seen in rabbits topically treated from the seventh to the eighteenth day of gestation with shampoo containing up to 50 mg/kg ZPT. Neither were effects seen in pigs topically treated from the eighth to the thirty sixth day of gestation with a shampoo containing up to 300 mg/kg ZPT.

Further data

Since publication of SCCNFP 0671/03, further studies have been performed. The studies are not available for evaluation.

- in a GLP-compliant study performed according to US EPA83-3, the developmental toxicity of a 48 % suspension of ZPT was investigated in groups of 20 female rabbits at 0, 0.5, 1.5 and 3.0 mg/kg bw/d after oral gavage. Based on post implantation losses, reduced number of viable foetuses increased number of foetuses with developmental variations and abnormalities a NOAEL of 0.5 mg/kg/d was derived for teratogenicity/embryotoxicity. Based on decreased body weight gain and uterus weights, a NOAEL of 0.5 mg/kg/d was derived for maternal toxicity (the study is mentioned in MAK (2012), HSE (2003) and at ECHAs website (http://echa.europa.eu/)).

- in a GLP-compliant study performed according to US EPA83-3, the developmental toxicity of a 48 % suspension of ZPT was investigated in groups of 30 female Sprague-Dawley rats at 0, 0.75, 3.0 and 15.0 mg/kg bw/d after oral gavage. Based on increased post implantation loss and rib malformation in foetuses, a NOAEL of 0.75 mg/kg/d was derived for teratogenicity/embryotoxicity. For maternal toxicity, also a NOAEL of 0.75 mg/kg/d was derived based on e.g. uterine effects and decreases in weight gain. In high dose animals, delated pupils were observed in half of the animals before and after dosing (the study is mentioned in MAK (2012), HSE (2003) and at ECHAs website (http://echa.europa.eu/)).

- in a GLP-compliant study performed according to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study), the developmental toxicity of dermal doses of 0, 15.0, 30.0 and 60 mg/kg/d ZPT was investigated in female Sprague-Dawley rats. A maternal NOAEL of 15 mg/kg/d was derived. Based on reduced fetal weight, reduced skeletal ossification and increases in skeletal variations at the highest dose, a NOAEL of 30 mg/kg/d was derived for teratogenicity/embryotoxicity (this study is only mentioned at ECHAs website (http://echa.europa.eu/)).

Conclusion on Reproductive toxicity

In SCCNFP 0671/03 the following conclusions were drawn with respect to Reproductive toxicity of ZPT:
- 2.5 mg/kg/d administered orally to rats is a no effect level for teratological effects
- no reproductive effects have been observed when ZPT was applied topically to rats and rabbits at levels up to 15 and 100 mg ZPT/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 ZPT/kg/d respectively.

Since that, two generation as well as developmental toxicity studies have been performed with ZPT. Further, a 2 generation study with sodium pyrithione (from which read across to ZPT is considered adequate) has been performed. These studies were not available for evaluation by SCCS. Therefore, no firm conclusion with respect to toxicity to reproduction and development can be drawn by the SCCS .The SCCS, however, is aware that HSE (2003) did not identify any potential concern to humans regarding adverse effects on fertility. Further, both MAK (2012) and HSE (2003) concluded that adverse effects on development were most likely attributable to maternal toxicity.

3.4.9. Toxicokinetics and PBPK modeling

3.4.9.1 Summary of toxicokinetics taken from SCCNFP/0671/03

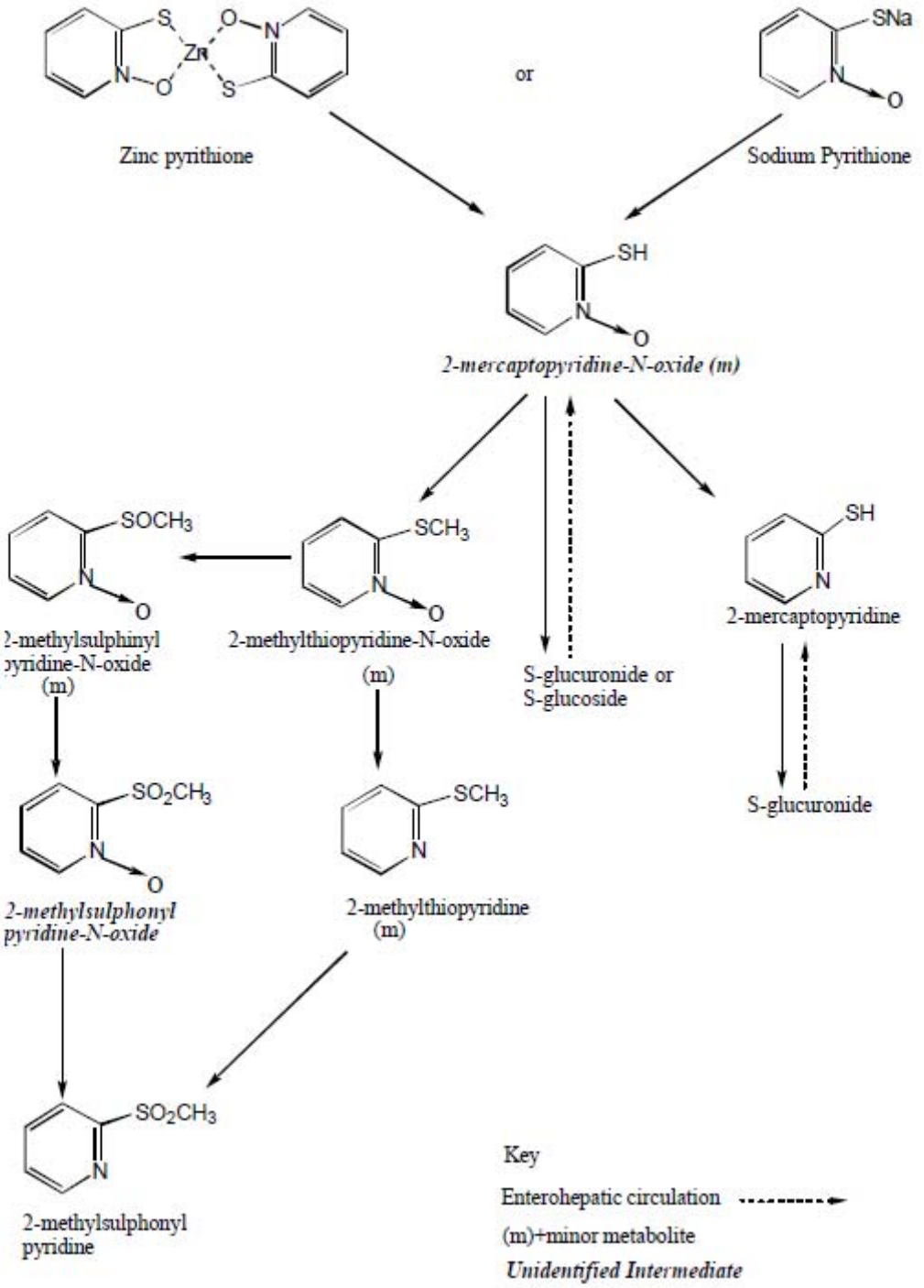
- percutaneous absorption of ZPT varies from approximately 0.03 to 3.4%
- the distribution of radioactivity in tissues after oral administration of labelled ZPT showed that the radioactivity rapidly disappeared from the blood, and the primary route of excretion was via the urine. The residual radioactivity was low (4.5% of dose), ZPT was distributed throughout the body, and was not concentrated in any particular tissue.
- all animal species investigated (rat, rabbit, dog, and monkey) biotransformed ZPT in qualitatively similar ways. This similarity with regard to ZPT metabolism suggests that human metabolism is likely to be similar. This has been confirmed by Wedig et al. (1984).

3.4.9.2. General information on Metabolism and Toxicokinetics of ZPT

The metabolism and toxicokinetics of ZPT have been well investigated in different species. An overview on toxicokinetic studies performed with ZPT and NaPT in animals and humans is given in HSE (2003) where ADME studies are summarised as follows and a metabolic scheme is given:

"Studies have been presented in experimental animals to address the toxicokinetics of zinc pyrithione and sodium pyrithione following oral administration, and zinc pyrithione only following dermal administration. Studies are also presented to address the toxicokinetics of zinc pyrithione and the related compound sodium pyrithione in a number of experimental animal species following i.v. administration. No specific information was identified to address the toxicokinetics of zinc pyrithione following single inhalation exposure or repeated exposure by any route. However, from the information available it was possible to conduct an adequate assessment of the toxicokinetics of zinc pyrithione. No further toxicokinetic information is required at the present time.
Following oral dosing, absorption of ^{14}C labelled zinc pyrithione and sodium pyrithione was high, around 75-90% of the administered radiolabel. Studies performed with ^{65}ZnP indicate that following oral administration, zinc pyrithione disassociates to liberate Zn and the pyrithione moiety which are then absorbed independently. Dermal absorption of zinc pyrithione was found to be minimal. Short term tissue retention of radiolabel was confined to erythrocytes. The metabolic profiles of orally administered zinc pyrithione and i.v. administered sodium pyrithione were extensive, and qualitatively similar in rats, rabbits, dogs and monkeys. In all species, independent of route or pyrithione salt, a common terminal metabolite, 2-methylsulphonylpyridine was identified, which was also identified in workers involved in pyrithione manufacture. The identification of a common terminal metabolite suggests a common metabolic pathway. Data from studies performed in the pig with both zinc pyrithione and sodium pyrithione support the suggestion of a common metabolic pathway. Excretion of zinc pyrithione and sodium pyrithione was found to be rapid, principally via the urine, with faecal excretion being a minor route. It was found that biliary excretion and enterohepatic circulation are both important in the rat.
As zinc pyrithione is rapidly metabolised, the potential for bioaccumulation of the parent molecule is low. However, it was found that 2-methylsulphonylpyridine levels rise with time after dosing (up to 72 hours), although insufficient information was available to indicate bioaccumulation potential. As the zinc pyrithione metabolites are anticipated to be water soluble, then distribution of these metabolites is expected to be extensive. Thus, exposure of the developing embryo/fetus to zinc pyrithione metabolites is likely. However, given the very high lipid content of breast milk, it is thought unlikely that postnatal exposure could occur via this route.
Given the detection of 2-methylsulphonyl pyridine in human plasma, it can be concluded that the metabolic pathways are qualitatively similar across a range of species. However, as the molecular weight cut off for biliary excretion in humans is higher than in rats, this route of excretion is likely to be of diminished importance, in humans."

Figure 2: metabolic scheme for ZPT (according to HSE (2003))


Zinc pyrithione
or
Sodium Pyrithione
2-mercaptopyridine-N-oxide (m)
2-methylsulphinyl pyridine-N-oxide (m)
2-methylthiopyridine-N-oxide (m)
S-glucuronide or S-glucoside
2-mercaptopyridine
S-glucuronide
2-methylsulphonyl pyridine-N-oxide
2-methylthiopyridine (m)
2-methylsulphonyl pyridine
Key
Enterohepatic circulation
(m)+minor metabolite
Unidentified Intermediate

3.4.9.3. New data on metabolism and toxicokinetics of ZPT

(1) Toxicokinetics of ZPT after single (i.v.) and repeat dose oral (gavage, diet) and dermal administration (Experiments performed in a first phase to assist in the parameter selections for the main repeat dose studies described below)

Ref.: A22

The experiments consisted of a series of single dose experiments involving two animals per experiment (pilot studies) and several repeat dose studies involving five animals per experiment (satellite studies). Experiments of this first phase were exploratory in nature and therefore not conducted in strict accord with GLP regulations. Further, data obtained after i.v. administration in the first phase was used to calculate bioavailability in the main study.
The following major results were obtained from the first phase:
• Urine is the primary route of excretion of radiolabeled compounds derived from ZPT following administration of ZPT to female rats by intravenous injection, dermal exposure, oral gavage or in fortified feed.
• The estimated elimination half-life of pyrithione, the bioactive, organic moiety of ZPT, after intravenous administration of ZPT is about 2 hrs.
• Dietary exposure to feed containing 1 or 10 ppm of ZPT (actual consumption of 0.03−0.4 mg/kg), produced pyrithione concentrations that were either near or below the lower limit of quantitation of 0.5 ng/mL. Exposure to feed containing 50 or 250 ppm of ZPT (actual consumption of 0.7−2.7 mg/kg) produced measurable pyrithione concentrations in plasma for ca. 12 h. Repeated exposure for 10 days to feed containing 250 ppm of ZPT resulted in weight loss and decreased hind-limb muscle mass.
• Dermal exposure to ZPT at levels of 10 mg/kg or greater resulted in plasma pyrithione concentrations that were measurable (>0.5 ng/mL). A lag time to reach these concentrations of about 8−12 hrs from exposure initiation was observed. Decreases in both hind-limb muscle mass and muscle tone were observed in animals exposed daily for 10 days to 100 mg/kg ZPT.
• Bioavailabilities of ZPT administered either by oral gavage or dietary via ZPT-fortified feed were greater than 70%. The bioavailability of dermally applied ZPT was estimated to be about 3−8%. Since concentrations of pyrithione in plasma were high enough to measure in only a few samples and only at the later time points, the estimates of dermal bioavailability should be considered only to be crude estimates of bioavailability.
• ZPT-derived compounds do not appear to be concentrated in any tissue/organ examined.
• Concentrations of total radiolabel in blood were higher and much more persistent in blood than concentrations of pyrithione in plasma. This is consistent with previous studies that identified pyrithione metabolites as the main constituents in serum.

2) Toxicokinetics of ZPT – main repeat dose studies

Oral gavage study:

Guideline:	not mentioned, but experiments were in accordance with OECD TG 417
Species/strain/sex:	Rat (CD), female
Group size:	designed to obtain data from 6 animals per treatment group[*)]
Test substance:	a) non-labelled: ZPT powder from Arch Chem lot #0108244691, Master Log I.D. # 74-01B10CuZPT; purity 98,3 % b) radiolabelled ZPT from Perkin Elmer, lot #3547-059, specific activity 11.0 mCi/mmol, radiochemical purity 97.3 %
Dose levels:	1.25, 3 and 10 mg/kg/d (3 treatment groups per dose)

Controls:	vehicle control (0 mg/kg/d) (3 treatment groups)
Vehicle:	suspensions of ZPT in appropriate volumes of 0.1% aqueous triethanolamine lauryl sulfate
Exposure:	gavage, volume: 5 ml/kg Treatment groups A and B: 10 daily doses; treatment group C: 3 daily doses
GLP statement:	Yes
Date:	2005- 2007

*) this could not always be achieved, therefore the study plan was amended and sometimes only data from 5 animals were available

Treatment group A consisted of animals that had a jugular cannula installed to facilitate the collection of serial blood samples. These animals were dosed daily for 10 days (9 days for the highest oral gavage group), and 8−9 serial blood samples were taken over 24 hr following the first dose and following the last dose of ZPT. Additional blood samples were taken 48 and 96 hr following the last dose of ZPT, at which time the animals were sacrificed. Blood volume in these animals was maintained by injections of plasma taken from donor animals. Serial blood samples were not taken from groups B and C. Animals of group B were sacrificed 24 hr following nine daily doses of ZPT while the animals in group C were sacrificed 24 hr following three daily doses of ZPT. Animals were housed individually in glass metabolism chambers equipped for separate collection of urine and feces. Excreta were collected daily from animals in all treatment groups, necropsies were performed at sacrifice. Biological samples were assayed for radioactivity by liquid scintillation spectrometry. Pyrithione (PT) was measured in plasma samples using LC/MS. Several functional assessments were conducted prior to sacrifice to ascertain the extent of hind-limb weakness in the animals. These measurements consisted of "muscle mass" and "muscle tone". Necropsies were performed on each animal following sacrifice, with specific tissues being collected for radiochemical analysis. Pharmacokinetic analyses were conducted using WinNonlin.

Results

Concentrations of PT in plasma reached maximal levels within 0.4−0.9 hrs of ZPT administration. PT concentrations then declined with mean half-lives that ranged from 2.6 to 6.9 hrs with no difference between half-lives of the first dose and the last dose at any dose level. Estimates of PT half-life in plasma were considerably shorter than those for total radioactivity in blood (40−60 h). Maximal concentrations of PT increased in a dose-related manner following both the first and the last dose of ZPT.

Systemic exposures (expressed as AUC) also increased in a dose related manner following the first and the last dose of ZPT as administered dose increased from 1.25 to 10 mg/kg. Ratios of study day 10 (study day 9 for the highest dose group) to study day 1 for both Cmax and AUC were less than 3 at all doses, suggesting that accumulation of pyrithione was minimal after 9−10 days of oral administration to rats.

The majorities of the oral gavage doses were excreted in urine at all dose levels with more than half of this occurring within 24 hrs of dosing. Overall, urine accounted for about 73% of the administered dose, and excretion in urine (as a percentage of dose) was independent of dose level or length of dosing.

Excretion of total radioactivity in feces was much lower, accounting for 5−10% of the dose. For animals sacrificed 96 hrs after their final dose of ZPT, only about 1% of the total dose remained in the carcass at sacrifice. Much higher percentages of the dose (up to 17% of the highest dose) were still in the carcasses of animals sacrificed 24 hrs following three daily doses of ZPT. Highest concentrations of radioactivity were found in livers, followed by kidneys.

In general, animals administered the vehicle control and the two lower doses maintained or slightly increased body weight throughout the study. Animals at the highest dose lost approximately 15% of their study day 1 body weight at the end of nine days of dosing. Muscle mass and muscle tone decreased with the length of dosing and with increasing dose

levels of ZPT. Reductions of both muscle mass and muscle tone were also found in animals in the vehicle control Treatment Group, in which serial blood samples were drawn.

Oral dietary study:

Guideline:	not mentioned, but experiments were in accordance with OECD TG 417
Species/strain/sex:	Rat (CD), female
Group size:	designed to obtain data from 6 animals per treatment group*)
Test substance:	a) non-labelled: ZPT powder from Arch Chem lot #0108244691, Master Log I.D. # 74-01B10CuZPT; purity 98,3 %
	b) radiolabelled ZPT from Perkin Elmer, lot #3547-059, specific activity 11.0 mCi/mmol, radiochemical purity 97.3 %
Dose level:	250 ppm [^{14}C] ZPT in feed (3 treatment groups)
Controls:	vehicle control (feed meal without ZPT) (3 treatment groups)
Vehicle:	suspensions of ZPT in appropriate volumes of 0.1% aqueous triethanolamine lauryl sulfate
Exposure:	oral via diet; treatment groups A and B: 10 daily doses; treatment group C: 3 daily doses
GLP statement:	Yes
Date:	2005- 2007

*) this could not always be achieved, therefore the study plan was amended and sometimes only data from 5 animals were available

Dietary studies involved daily doses of ZPT in feed meal (target concentration 250 ppm) or of feed meal alone (vehicle control) for periods up to 10 days. Animals were placed in one of three treatment groups (A, B and C) for the ZPT feed and vehicle control, respectively. Each animal in the A treatment groups had a cannula implanted into a jugular vein for serial blood sampling. Serial blood samples were removed from these animals at specified times following access to dosed feed on study day 1 and study day 10. Animals in Treatment Groups B and C were sacrificed at intermediate time points. Group C animals were sacrificed on study day 4 and group B animals were sacrificed on study day 10, 24 hrs following their final exposure to ZPT-fortified feed. Excreta were collected daily from animals sacrificed at the intermediate time points.
Blood, urine, feces, and tissue samples were analyzed for total radioactivity, 12 urine samples and 12 feces samples, were chosen at random from animals in the vehicle control treatment groups for analysis. Tissues from animals administered ZPT and from two animals in each of the vehicle control treatment groups were analyzed. Pyrithione was measured in plasma, prepared from selected blood samples.
Several functional assessments were conducted prior to sacrifice to ascertain the extent of hind-limb weakness in the animals. These measurements consisted of "muscle mass" and "muscle tone". Necropsies were performed on each animal following sacrifice, with specific tissues being collected for radiochemical analysis. Pharmacokinetic analyses were conducted using WinNonlin.

Results
Animals given ZPT-fortified feed for 9−10 days lost an average of 19% of their study day 1 body weights. Concentrations of PT reached maximal levels within 2.4−4.1 hrs of introduction of ZPT-fortified feed. Following Cmax, plasma levels of PT declined with a half-life that ranged from 3.7 (study day 1) to 11.0 hrs (study day 10). Both PT C_{max} and AUC increased significantly from study day 1 to study day 10 as would be expected for increased ZPT intake on study day 10. When Cmax and AUC were normalized for daily dose, neither parameter showed large changes from study day 1 to study day 10. Thus the data suggest that accumulation of pyrithione was minimal after 10 days of ZPT administration to rats via dosed feed.

Excretion in urine accounted for 84−92% of the ingested ZPT. Animals exposed to 250 ppm ZPT in their diets for 9−10 days exhibited slightly to greatly reduced muscle mass and moderate to no muscle tone.

Dermal study:

Guideline:	not mentioned, but experiments were in accordance with OECD TG 417
Species/strain/sex:	Rat (CD), female
Group size:	designed to obtain data from 6 animals per treatment group
Test substance:	a) non-labelled: ZPT powder from Arch Chem lot #0108244691, Master Log I.D. # 74-01B10CuZPT; purity 98,3 % b) radiolabelled ZPT from Perkin Elmer, lot #3547-059, specific activity 11.0 mCi/mmol, radiochemical purity 97.3 %
Dose level:	10, 30 and 100 mg/kg bw/d ZPT (3 treatment groups)
Controls:	vehicle control (0 ppm) (3 treatment groups)
Vehicle:	suspensions of ZPT in appropriate volumes of 0.1% aqueous triethanolamine lauryl sulfate
Exposure:	dermal; treatment groups PK and 10-day intermediate sacrifice group: 10 daily doses; 4-day intermediate sacrifice group: 3 daily doses
GLP statement:	Yes
Date:	2005- 2007

A dose area of 12 cm^2 was clipped free of fur in order to apply the dose to bare skin; rats were fitted with rodent jackets in order to prevent grooming, an appliance was created to surround the application site. Calculated daily target doses of 10, 30 and 100 mg/kg bw/d based on the actual body weight were applied via syringe and a non-occlusive cover was attached to the top of the appliance which was then secured by a metal screen in order to prevent removal of the appliance. Six hours after application of the dermal doses, the cover was removed and treatment sites were washed. Since the dose sites were uncovered except for the 6-h period each day when the animals were being exposed dermally to ZPT, oral ingestion of any small amounts of ZPT remaining on the surface of the skin during the interval between dermal exposures cannot be ruled out.
The animals of the PK group were dosed daily for 10 days and 8 serial blood samples were taken over 24 hrs following the beginning of the first dermal exposure, on study days 2, 3, 4 und 5 and up to 96 hrs following the last 6 hr exposure to ZPT. 96 hrs following the last exposure to ZPT the animals were sacrificed. Blood volume in these animals was maintained by injections of plasma taken from donor animals. Serial blood samples were not taken from the other two exposure groups. Animals of the 10-day intermediate sacrifice group were sacrificed 24 hrs following nine daily doses of ZPT while the animals of the 4-day intermediate sacrifice dose were sacrificed 24 hrs following three daily doses of ZPT.
Animals were housed individually in glass metabolism chambers equipped for separate collection of urine and feces. Excreta were collected daily from animals in all treatment groups, necropsies were performed at sacrifice. Biological samples were assayed for radioactivity by liquid scintillation spectrometry. Pyrithione (PT) was measured in plasma samples using LC/MS. Several functional assessments were conducted prior to sacrifice to ascertain the extent of hind-limb weakness in the animals. These measurements consisted of "muscle mass" and "muscle tone". Necropsies were performed on each animal following sacrifice, with specific tissues being collected for radiochemical analysis.
Approximately 24 hrs following application of the last dose, the dose sites were washed again (2% soap in water followed by deionized water) and dried. The dose site was then left uncovered for the remainder of the study. Unabsorbed dose (total radioactivity) recovered from the collections of the protective appliances, cloth coverings, and gauzes were measured. Necropsies were performed on each animal following sacrifice, with specific

tissues being collected for radiochemical analysis. Pharmacokinetic analyses were conducted using WinNonlin.

Results

Radioactivity was only slowly absorbed following dermal exposure. Concentrations of pyrithione reached maximal levels after 12 −26 hrs of administration of dermal ZPT doses. On study day 1, pyrithione concentrations were too low to measure at the time points shorter than 8 hrs; thus, the lag time required for transiting skin is longer than the 6-hr exposure period. High variability in study day 1 pyrithione Cmax and AUC point to variability in the penetration of ZPT. Study day 10 AUC increased in a roughly proportional manner between rats receiving 10 and 30 mg/kg daily. However, between 30 and 100 mg/kg dose groups, study day 10 AUCs were similar. This suggests that maximal absorption of ZPT and therefore pyrithione exposure was achieved at 30 mg/kg.

Because of variability caused by apparent delayed absorption of ZPT estimates of tmax and parameters based on on k_{el} ($t_{1/2}$, AUC(0-∞), Vd/F and CL/F) should be used with caution. Bioavailabilities of the dermal doses can be calculated from pyrithione concentrations in plasma (0−24 hr) using data from the pilot study for the intravenous dose (concentrations of pyrithione in plasma following intravenous administration of ZPT were measurable for less than 24 hrs). These calculated bioavailabilities are 2.3, 8.6, and 0.3%, respectively, for the 10, 30, and 100 mg/kg/day exposures. However, the AUC (0−24hrs) values for dermal administration do not capture the total AUC, and use of the AUC (0-∞) values are fraught with the difficulties described above for parameters based on kel. Only small proportions of the administered doses were excreted in urine (1−7% of the total dose for animals kept 4 days following the last dose of [^{14}C]ZPT), and even smaller proportions were excreted in feces. The potential exists that even these small proportions are inflated due to contamination by residual dose present in the dose site.

Animals exposed to the lowest dose of ZPT excreted the largest percentages of the dose in urine and feces. The lowest percentages of the dose were excreted by animals exposed to the highest doses of ZPT. As opposed to urinary excretion following oral gavage administration of ZPT, the rate of urinary excretion of total radioactivity following dermal administration did not decrease during the four days following the last dermal exposure.

For animals carried 96 hrs after their final dose of ZPT, about 1−3% of the total dose remained in the carcass at sacrifice, over half of which was in the dose site skin. Somewhat larger percentages of the dose (up to 9% of the lowest dose) were still in the carcasses of animals sacrificed 24 hrs following their last dermal exposure to ZPT. The largest contributor to the residual radioactivity, in general, was the dose site skin and the second highest concentrations were in the liver.

In general, body weights decreased during the first few days of the study for animals in all dermal treatment groups, including the vehicle control groups. Weights then remained relatively constant during the period of daily dermal exposure. Hind limb muscle mass and muscle tone both decreased with increasing exposure to ZPT. For the animals exposed to 100 mg/kg of ZPT for 10 days, muscle mass was greatly reduced with low to no muscle tone.

SCCS comment

Oral intake cannot completely be excluded based on the study design

3.4.9.4. PBPK modelling

Toxicokinetic data has been used to build up physiologically-based-pharmacokinetic (PBPK) models for ZPT. At first a preliminary model has been developed based on data mainly obtained from rabbit studies. The development of this model and its informative value has been described and discussed in the document, "Feasibility Study for a Physiologically-based Pharmacokinetics Model of Zinc Pyrithione (August 2003) which was provided by the applicant (Ref. A23).

Afterwards, a preliminary rat model was developed by using data obtained from Wedig et al., 1978 (Ref.: A26), from which a first rat model was successfully parameterized and optimized (no data have been provided for the preliminary and the first rat model). The first rat model failed to predict the multi-dose kinetics of pyrithione carbon (PTC) as observed in the experiments described above (Ref. A22 / Annex I of submission II) and could not simulate the kinetics of pyrithione, as parameters for PT metabolism were not developed for the first rat model. Therefore, in addition to data already used for the older models, data from the experiments described above (Ref. A22 / Annex I of submission II) were used parameterize and calibrate the second rat model (Ref. A24 / Annex I of submission II).

Data used

Data from reports compiled in a Procter and Gamble document "Zinc pyrithione Studies of Absorption, Distribution, Metabolism, and Excretion" prepared by J.F. Nash, 2004 (the document is not available to SCCS, extracted data were given in tabulated form in Annex I of submission II) formed the basis for the second rat model. Data on tissue masses, tissue volumes and blood and plasma flows were from ILSI (1994). Data on tissue:plasma partition coefficients and on plasma – red blood cell mass transfers mainly based on Wedig et al., 1978 (Ref. A26).

Data used for model calibration were (1) plasma PT concentrations for the first 24 hours following the first dose of ZPT (1.25 mg/kg) from Ref. A22 /Annex II of submission II; (2) blood PTC concentrations for the first 24 hours following the first dose of ZPT (1.25 mg/kg) from Ref. A22 /Annex II of submission II; (3) relative abundance of MSP and SG in blood at 1, 4, and 16 hours following a single oral dose of ZPT (1 mg/kg), from Gibson and Turan (1978) (this study is an internal Procter and Gamble report. Data utilized from this study were given in tabulated form in Ref. A24); and (4) cumulative urinary and fecal excretion (% of dose) of ^{35}S (pyrithione sulfur, PTS) up to 72 hours after a single oral dose of ZPT (25 mg/kg) from Ziller et al. (1977) (Ref. B74). Data used for calibration, evaluation and parameter estimation were from Ref. A22 /Annex II of submission II, from the Gibson and Turan (1978) study, from Ref. B69 and from Ref. B74.

All data were compiled in tabular form. Models were constructed and implemented in Advanced Continuous Simulation Language (acslXtreme, v. 2.0.1.6). Parameter estimation was performed in acslXtreme Optimum using the Nelder-Mead and/or conjugate gradient algorithms, set to minimize relative error for each parameter. No attempt was made to weight observations for measurement error or uncertainty. Goodness of fit was judged by visual inspection of model predictions compared to observations. Univariate sensitivity analysis consisted of running the model after perturbing values for single parameters by a factor of 0.01, in the up and down directions.

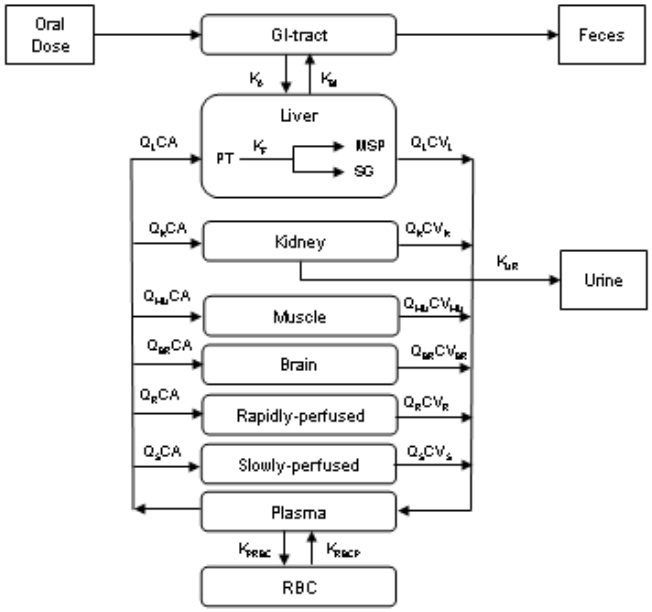
PBPK model Structure

The model structure is depicted in Figure 2. The model simulates the kinetics of absorption, distribution, and excretion of PT and products of its two major metabolic pathways, the 2-(methylsulfonyl)pyridine (MSP)-pathway and the S-glucuronide (SG)-pathway. The central distributing compartment is plasma. Gastrointestinal tract (GI-tract) absorption processes for PT and metabolites are simulated as first-order kinetic processes of unlimited capacity. These are represented with absorption (i.e., bioavailable) fractions and absorption rate constants, with mass flow from the GI-tract to liver, conceptually representing the hepatic portal flow. 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 assumed 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). Transfers between plasma and RBC 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. Two excretory pathways are simulated: first-order transfer from kidney to

urine, and first-order transfer from liver to GI-tract, the latter representing biliary secretion; both pathways have unlimited capacity. Distinct absorption parameters for PT and metabolites provide a means of simulating reabsorption of chemical secreted in bile. The above specification of the model does not include simulations of binding or sequestration of PTC in tissues. The model is implemented as a series of first-order differential equations.

SCCS comment
The SCCS notes, that skeletal muscle is assigned to slowly-perfused tissue but at the same time as an individual compartment.

Figure 2: Structure of the PBPK model for ZPT



Model calibration
In the model calibration phase, sequential optimizations were performed in order to achieve a good representation of the data obtained from the studies submitted in Annex II of submission II (Ref. A22, described in 3.3.9.3). The adequacy of the outcome of the optimizations was assessed by visual inspection of model simulations against the observations from the multi-dose oral phase II study (Ref. A22).

Results
Comparisons between simulated and observed values were performed. It should be noted that partly overlapping data have been used to build up the model and to optimize and check the model.
A comparison between observed and simulated kinetics was performed with respect to the following aspects:
- kinetics of plasma PT
- kinetics of blood pyrithione carbon
- kinetics of urinary and fecal excretion of pyrithione carbon
- kinetics of tissue pyrithione carbon
- kinetics of metabolites in blood

The comparisons demonstrated that the model quite reasonably replicates the observed short-term elimination kinetics of PT and pyrithione carbon following single doses and

longer-term temporal patterns of blood concentrations of pyrithione carbon 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 latter is the major pyrithione carbon species in blood within several hours following a dose of ZPT.
It was stated that physiological parameters and most chemical parameters that are highly influential in simulation blood and tissue pyrithione carbon levels are scaled to body weight and could therefore be scaled to represent different species.
In a submitted poster (Ref. A25) it is stated that the rat model has been allometrically scaled to humans.

By using the rat model allometrically scaled to humans, the applicant provided a calculated external human concentration which would correspond to that AUC in the rat, which is obtained from an oral dose of 500 µg/kg/d. This human equivalent external dose was 2170 µg/kg/d (or 2260 µg/kg/d - two slightly differing values are given in reference A 25; in the supplemental submission the applicant gives the value of 2170 µg/kg/d as the human equivalent external dose).
The applicant states in his supplemental submission:
(1) "The predictive validity of the ZPT PBPK model was verified in repeat dose pharmacokinetic studies conducted in female rats. Toxicologically-relevant doses of 14C-ZPT were administered by gavage, in the diet, topically, or intravenously. Radiolabel (PTC from 14C-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% 14C-ZPT thereby linking PBPK estimates to the data obtained in human subjects."
(2) "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."
However, no information has been given how the rat model was allometrically scaled to humans (i.e. which parameters have been used to scale the model to humans in terms of e.g. partition coefficients, organ masses and volumes) and whether and to which extent species differences (e.g. with respect to enterohepatic circulation, with respect to metabolic parameters) have been considered when scaling the rat models to humans. Further, no description has been provided how the rat model had been verified by human clinical study results.

Therefore, it cannot be reproduced, whether the human external dose of ZPT equivalents, that would correspond to the rat NOAEL of 500 µg/kg/d (2260 and 2170 µg/kg/d) are sound and reliable values to base MOS calculation on.
Thus, solely from the information available from the SOT 2008 poster (Ref. A25) and the statements in the submission (see citations (1) and (2) above) the human model cannot be used for deriving quantitative threshold values, as there is no transparent information on the human model and its validation.
This is in line with the REACH guidance on information requirements and chemical safety assessment, Chapter R-8, where it is stated: "Furthermore, if a PBPK model is used to extrapolate from animals to humans, the proposed model should be validated by data from humans if these are available, and extrapolations from the model should be within or close to the range of experimental measurements used to validate the model. If there is no validation of the model by data from humans, PBPK models may be used to support an interpretation of toxicodynamic data or toxicological findings rather than as a basis for the derivation of a DN(M)EL."

Ref.: D24

The SCCS acknowledges that PBPK modelling is a straightforward way to perform various types of extrapolation in risk assessment and that it might assist in reducing uncertainties

associated with conventional extrapolation procedures. However, when the model is not sufficiently documented, confidence in the model is not given.
This means in case of submission II for ZPT that without more detailed information especially with respect to the human model the data cannot be accepted for the assessment of ZPT and for the derivation of a safe exposure level.

3.4.10. Photo-induced toxicity

3.4.10.1. Phototoxicity / photoirritation and photosensitisation

No data available

3.4.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data available

3.4.11. Human data

See section 3.3.3. Skin sensitization and section 3.3.4.2. *In vivo* dermal absorption

3.4.12. Special investigations

3.4.12.1. Mode of Action and Neurotoxicity studies

Taken from SCCNFP/0671/03

(a) Work by Snyder et al (1977, 1979), Dejesus et al (1978), Chrisman and Ross (1978) and Sahenk and Mendell (1979, 1979a, 1980) has provided considerable information concerning ZPT induced paralysis.
Snyder et al (1977) determined that the hind-limb muscle wasting reported by Dearwester and Johnson (1974) was a disuse atrophy secondary to neurologic effects. Using in situ sciatic nerves Snyder et al (1977) found conduction velocities were normal, but they observed a decrease in the force of muscle contraction. Effects on serum cholinesterase were rejected as a possible cause, since levels were measured and found to be normal.
Dejesus et al (1978) in a later study confirmed their finding of normal conduction velocities using the sural nerve, but in addition he found a reduction in the amplitude of the sensory potential and a reduction in duration of the evoked response.
Similar studies by Chrisman and Ross (1978) investigated the clinical and electrophysiologic response to several dose levels of ZPT using rats. The animals were fed diets containing 0, 10, 50, 250, 500, 750 and 1000 ppm ZPT for 12 weeks. At a concentration of 10 ppm in the diet (0.6 mg/kg/day), there were no changes in neurologic signs or electrophysiologic function during the course of the study. However, at 50 ppm in the diet (4.0 mg/kg/day for females and 2.6 mg/kg/d for males) severe neurologic deficits and electrophysiologic abnormalities were noted. Reduction in the electrophysiologic response began after about one week on the diet, and neurologic deficit was grossly apparent about a week later. These changes became progressively more pronounced and were most severe at six weeks. After eight weeks on the diet, the animals began to improve, and some of the rats completely recovered clinically. Animals receiving 250 ppm of ZPT in the diet died prior to termination of the study. All of the rats were severely affected, and no recovery was observed prior to death. Concentration of 500 ppm and greater in the diet produced mild or no neurologic deficit or electrophysiologic changes prior to death.

Milligram/kilogram equivalents have not been provided for dietary concentration of ZPT above 50 ppm because of excessive body weight loss and extreme variability in the amount of chow eaten.
(b) it seems likely that a critical systemic level of ZPT or a metabolite must be attained and maintained for a sufficient period of time to produce paralysis. Gibson (1979) has shown that animals partially recover over the weekend when they are dosed 5 days/wk. Intermittent reduced food consumption or food avoidance on some days could produce a similar effect that in turn affects the systemic level.
(c) During the course of many studies, the animals failed to gain weight, and some even lost weight. This was due to reduced food consumption, either because of progressing paralysis (part of this weight loss was due to a significant decrease in food consumption, since the animals had difficulty reaching their food) or because of reduced palatability of chow with higher levels of ZPT added.

Further data

In vivo studies

A study was performed in order to investigate the electrophysiological correlates of ZPT-induced neuromuscular dysfunction in rats after oral (dietary) intake of ZPT. Male Fischer F344 rats (6-8 animals/group) received diet containing 50 ppm ZPT of unspecified impurity (equivalent to approximately 2.5 mg/kg/d) for 14 d, followed by a 42 d recovery period. Control animals received diet without ZPT. A further group of animals received diet without ZPT but at reduced levels, in order to determine whether reduced food intake could account for the observed neuromuscular deficit. Electrophysiological, observational and direct measurements were used to monitor the progress of changes in neuromuscular function. No toxicologically significant alterations in food consumption were observed during the study; body weights were reduced (by 13%) in animals receiving the reduced diet.
In ZPT-treated animals, mean hind limb strength was decreased by approximately 40 % from day 8 of dosing to day 8 of the recovery period. Forelimb strength was reduced by approximately 25 % from day 12 to day 4 of the recovery period. Electrophysiological changes, consistent with the observed decreases in hind limb function were also noted. Electrophysiological alterations (M wave changes) remained evident for 42 d of the recovery period, although hind limb functionality returned after 8 d. There were no apparent changes in nerve conduction velocity following ZPT treatment. There was no apparent neuromuscular deficit in the group receiving reduced amount of food. The data indicate that the earliest neuromuscular deficits after oral ZPT administration are observed in hind limbs. Animals regained hind limb function following removal of treatment, although electrophysiological dysfunction remained detectable.

Ref.: D21 Ross, J.F. and Lawhorn, G.T. (1990)

2 studies already mentioned in section 3.3.5.1 (data summarized from MAK 2012)

After dermal application of 100 mg ZPT for 10 days to Sprague-Dawley rats, all of 5 treated animals showed reduced amplitude of the evoked compound muscle action potential (CMAP), 4 animals showed signs of a reduced muscle tone.

In a 28-day dermal neurotoxicity study groups of 5 Sprague-Dawley rats received daily dermal doses of 0, 50, 150 and 200 mg ZPT/kg/d (male animals) or 0, 10, 25, 50, 75 and 100 mg ZPT/kg/d (female animals). The vehicle was 0.1 % triethanolamine-lauryl sulphate, the treatment site was protected by a fixed convex piece of plasic shielding. Low muscle tone was observed at 150 and 200 mg/kg/d in male animals beginning on day 8 and day 11 that continued throughout the study duration. Hindlimb and forelimb grip strength as well as muscle tone and body weight were decreased in male animals of the two highest doses

on days 14 and 28. No significant changes in plasma, RBC or brain cholinesterase was observed at any dose tested for any time point measured. Decreases in the electrophysiological measurements measured as the maximum amplitude were observed in males at 150 mg/kg/d. In female animals low muscle tone was observed at 50, 75 and 100 mg/kg/d beginning on day 8 in the 100 mg/kg/d group, on day 15 in the 75 mg/kg/d group and on days 22-28 in the 50 mg/kg/d group. On day 14 grip strength was reduced in the 75 and 100 mg/kg/d group and on day 28 grip strength was reduced in the three highest dose groups. No consistent decreases or dose dependent changes were apparent in plasma, RBC or brain cholinesterase at any dose tested. Decreases in the electrophysiological values measured as the maximum amplitude were observed in the 50 and 75 mg/kg/d group (electrophysiological measurements not taken at the 100 mg/kg/d dose level).

Ref.: D1 Arch Chemicals, 2003

In vitro studies

Knox et al. (2004) performed *in vitro* studies using bag cell neurons of a marine snail (Aplysia) in order to investigate the mechanisms underlying the reversible neurotoxicity of pyrithiones by using sodium pyrithione. It could be demonstrated that NaPT caused intracellular Ca^{2+} elevation. Several hypotheses which could build the molecular basis for this calcium entry have been tested. It could be demonstrated that the elevation of intracellular Ca^{2+} results from influx of calcium ions from the external medium, which is not affected by blockage of voltage gated Ca^{2+} channels. Intracellular Ca^{2+} elevation was also unaffected by inhibition of voltage-gated Na^+ channels or a Na^+/K^+ ATPase inhibitor. It could be demonstrated that the NaPT-induced elevation of Ca^{2+} was attenuated by two potential inhibitors of store-operated Ca^{2+} entry (SKF 96365 and Ni^{2+}) suggesting that NaPT activates a form of calcium influx in these invertebrate neurons. From the studies performed the authors concluded, that pyrithione-evoked Ca^{2+} entry into the cells might also trigger activation of this nonselective cation current and depolarize neurons, thus explaining at least in part the neurotoxic effects of pyrithiones in rodents.

Ref.: D13 Knox et al. (2004)

In a follow-up study, the same group investigated whether pyrithione is also capable of inducing influx of Ca^{2+} into mammalian neurons and whether there would be any difference between cells obtained from rats (strain not mentioned) and Rhesus monkeys.
It could be demonstrated that in isolated rat as well as in isolated monkey motor neurons NaPT produced an increase of intracellular Ca^{2+}. The study authors discussed that elevation of intracellular Ca^{2+} can be used as an explanation for the accumulation of tubovesicular profiles within the terminals of rodent motor neurons following exposure to NaPT *in vivo.* In rat motor neurons, the NaPT induced Ca^{2+} entry was unaffected by nefedipine, a blocker of L-type voltage-dependent calcium channels or by tetrodotoxin, which blocks voltage-dependent sodium channels. Ca^{2+} elevation was also unaffected by ouabain, an inhibitor of the plasma membrane Na^+/K^+ ATPase. As has been demonstrated for bag cell neurons of Aplysia in a precedent study, SKF 96365, an antagonist of certain store-operated plasma membrane Ca^{2+} channels, inhibited NaPT induced Ca^{2+} entry into the cells in rat as well as in Rhesus monkey motor neurons, suggesting that PT targets such channels and suggesting that the mechanism of action of NaPT in motor neurons is conserved across species. Despite the qualitative similarities, quantitative differences in NaPT induced intracellular Ca^{2+} elevation were observed between rat and Rhesus monkey motor neurons. In rat motor neurons, the NaPT concentration that produces an increase in intracellular Ca^{2+} corresponding to 50 % of the maximum response (EC_{50}) is 0.31 µM, whereas in Rhesus monkey motor neurons the EC_{50} value for NaPT induced Ca^{2+} elevation is 10 µM, i.e. 30 times higher compared to the rat. This finding might explain the species differences between rats and monkeys observed after ZPT administration *in vivo*.

In addition to NaPT, the authors also investigated the effects of 2-MSP (2-methylsulfonylpyridine, the terminal serum metabolite of NaPT or ZPT) on intracellular Ca^{2+} elevation. In rat as well as monkey motor neurons, this metabolite did not induce Ca^{2+} elevation (which is in line with studies demonstrating that this metabolite fails *in vivo* to produce neurotoxicity in rats).

Ref.: D14 Knox et al., (2008)

In a study aiming at systematically identifying potassium channel modulators, Xiong et al. discovered by using cell lines stably expressing potassium channels KCNQ2 and KCNQ2/3 that ZPT activates both recombinant and native KCNQ m currents (remark: an M-current is a non-inactivating potassium current found in many neuronal cell types. In each cell type, it is dominant in controlling membrane excitability by being the only sustained current in the range of action potential initiation. It can be modulated by a large array of receptor types, and the modulation can occur either by suppression or enhancement. Modulation of M-current has dramatic effects on neuronal excitability. (Marrion N.V. (1997): Annu. Rev. Physiol. 59, 483 – 504)). The activation of KCNQ potassium channels is reversible and not mediated by Zn^{2+} ions but consistent with ZPT directly binding to KCNQ channels. In the same study it could be demonstrated that ZPT is able to upregulate mutants of KCNQ channels exhibiting reduced currents which are thought to be associated with benign familial neonatal convulsions.

Ref.: D22. Xiong, Q., Sun, H. and Li, M. (2007)

Lamore et al. (2010) demonstrated that cultured primary human skin keratinocytes and melanocytes display an exquisite vulnerability to nanomolar concentrations of ZPT resulting in pronounced induction of heat shock response gene expression and impaired genomic integrity. In keratinocytes treated with nanomolar concentrations of ZPT, expression array analysis revealed massive upregulation of genes encoding heat shock proteins (HSPA6, HSPA1A, HSPB5, HMOX1, HSPA1L, and DNAJA1) further confirmed by immunodetection. Moreover, ZPT treatment induced rapid depletion of cellular ATP levels and formation of poly (ADP-ribose) polymers. Consistent with an involvement of poly(ADP-ribose) polymerase (PARP) in ZPT-induced energy crisis, ATP depletion could be antagonized by pharmacological inhibition of PARP. This result was independently confirmed using PARP-1 knockout mouse embryonic fibroblasts that were resistant to ATP depletion and cytotoxicity resulting from ZPT exposure. In keratinocytes and melanocytes, single-cell gel electrophoresis and flow cytometric detection of γ-H2A.X revealed rapid induction of DNA damage in response to ZPT detectable before general loss of cell viability occurred through caspase-independent pathways. Combined with earlier experimental evidence that documents penetration of ZPT through mammalian skin, the authors point out that their findings raise the possibility that this topical antimicrobial may target and compromise keratinocytes and melanocytes in intact human skin.

Ref.: D15 Lamore, S.D., Cabello, C.M. and Wondrak, G.T. (2010)

Lamore et al. (2011) further demonstrated that ZPT causes rapid accumulation of intracellular zinc in primary keratinocytes as observed by quantitative fluorescence microscopy and inductively coupled plasma mass spectrometry (ICP-MS), and that PARP activation, energy crisis, and genomic impairment are all antagonized by zinc chelation. In epidermal reconstructs (EpiDerm™) exposed to topical ZPT (0.1–2% in Vanicream™), ICP-MS demonstrated rapid zinc accumulation, and expression array analysis demonstrated upregulation of stress response genes encoding metallothionein-2A (*MT2A*), heat shock proteins (*HSPA6*, *HSPA1A*, *HSPB5*, *HSPA1L*, *DNAJA1*, *HSPH1*, *HSPD1*, *HSPE1*), antioxidants (*SOD2*, *GSTM3*, *HMOX1*), and the cell cycle inhibitor p21 (*CDKN1A*). Immunohistochemistry analysis of ZPT-treated EpiDerm™ confirmed upregulation of Hsp70 and TUNEL-positivity.

Thus, ZPT impairs zinc ion homeostasis and upregulates stress response gene expression in primary keratinocytes and reconstructed human epidermis, activities that may underlie therapeutic and toxicological effects of ZPT.

Ref.: D16 Lamore, S.D. and Wondrack, G.T. (2011) (only abstract available)

3.3.12.2. Ocular Toxicity

Ocular effects of ZPT as observed in Snyder (1965) and Cloyd et al., (1978) after oral administration are species specific and are not considered of relevance for humans: studies performed in various species provide evidence that the tapetum lucidum is the target tissue for the ocular toxicity of ZPT, since the eyes of animals lacking this choroidal structure are not affected. Most humans and non-human primates do not possess a tapetum lucidum.

Ref.: B61; D3 Cloyd, G.G. et al., (1978)

Conclusions on Special Investigations

Reversible hind limb paralysis is the most prominent effect observed in rats after repeated oral administration of ZPT. In the first instance dermal administration was considered to not cause hind limb paralysis. However, mechanistic studies have demonstrated that dermal administration of ZPT caused electrophysiological changes and decreases in hindlimb and forelimb grip strength and muscle tone. So far, no conclusions can be drawn on probable neurotoxic effects after uptake by the inhalation route.
The loss of hind limb function is mediated by peripheral axonopathy. Thus, muscle atrophy is considered as a secondary event due to underlying nerve damage.
Species differences have been observed with respect to loss of hind limb function with monkeys being appreciably less sensitive to ZPT induced loss of hind-limb function.
In vitro studies so far contributed to a mechanistic understanding of ZPT-induced neurological effects: Pyrithione stimulates an influx of calcium into both rat and Rhesus monkey motor neuron preparations. This influx is mediated via pyrithione-stimulated Ca^{2+} release-activated Ca^{2+} channels. Although quantitative differences in Ca^{2+} influx were observed between rat and monkey motor neurons *in vitro*, which might be used to explain differences in sensitivities to the neurotoxic effects of ZPT in rats and monkeys, no conclusions with respect to human sensitivity can be drawn from these studies.
Furthermore, it has to be kept in mind that in the *in vitro* studies no attempt had been made to relate the *in vitro* pyrithione concentration to *in vivo* blood levels.
Apart from an interaction with Ca^{2+} channels, ZPT is also able to activate KCNQ potassium channels. In cultured human primary keratinocytes, ZPT caused an upregulation of heat shock proteins and other stress response genes, depletion of cellular ATP levels, formation of poly (ADP-ribose) polymers and impairment of zinc homeostasis. Further, ZPT induced DNA damage in keratinocytes and melanocytes as shown by single cell gel electrophoresis.

3.4.12.3 Market experience

Taken from SCCNFP/0671/03

ZPT has been used as an anti-dandruff active in shampoo formulations at levels of 1.0 and 2.0% since the 1940's. During this time there has been little evidence of any serious adverse effects from this usage, and those few effects that have been recorded are limited to eye and skin irritation as can be expected for surfactant-based formulations.
The effective use of shampoos containing this ingredient involves regular (2-3 times a week at least) usage.

Recent tracking of consumer marketplace complaints continue to indicate that ZPT containing shampoos have a very similar, low problem incidence profile compared to conventional shampoos which do not contain the material.

3.4.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

As the systemic exposure has been determined in clinical studies in humans using shampoo/lotions with the intended concentrations of ZPT, dermal absorption percentages are not necessarily required. The highest value of the systemic exposure dose derived from a human clinical study using shampoo and leave-on tonic with the intended concentration of ZPT was 4.66 µg/kg/d. Due to low recoveries in the study and in order to account for repeated long-term use, SCCS will take 4.66 µg/kg/d + 1 SD for MOS calculation. The mean + 1 SD is 5.25 µg/kg/d.

The NOAEL of 500 µg/kg/d as derived from the Larsson (1958) study, which was already used in SCCNFP 0671/03 is taken for MOS calculation. Although HSE (2003) expressed major criticism on that study, NOAELs derived from further studies (e.g. a subchronic study and a chronic study performed with sodium pyrithione) support the value of 500 µg/kg/d. However, these further studies are not available to the SCCS.

Roughly, the oral bioavailability of ZPT is considered to be between 80 and 95 %.

By using a PBPK model for ZPT (i.e. by using a rat model allometrically scaled to humans), the applicant provided a calculated external human concentration which would correspond to that AUC in the rat, which is obtained from an oral dose of 500 µg/kg/d. This human equivalent external dose was 2170 µg/kg/d or 2260 µg/kg/d (these two slightly differing values were the only information provided concerning the human PBPK model). By using a human equivalent external exposure dose of 2170 µg/kg/d and a human systemic exposure value of 4.66 µg/kg/d, the applicant results in a MOS of 465.7. However, as explained in section 3.3.9.4, without further information on the human PBPK model, it cannot be accepted for quantitative risk assessment.

The calculation of the margin of exposure only considers the exposure from the use of shampoo and leave-on tonic. Other exposure/products are not considered.

Systemic exposure dose (4.66 µg/kg/d+ 1 SED)		**= 5.25 µg/kg/d**
No observed effect level (mg/kg) (oral 2 year study, rat)	**NOAEL**	**= 500 µg/kg/d**
90 % bioavailability		**= 450 µg/kg/d**
80% bioavailability		**= 400 µg/kg/d**
Margin of Safety (90 %bioavailability)	**NOAEL / SED**	**= 86**
Margin of Safety (80 %bioavailability)	**NOAEL / SED**	**= 76**

Physico-chemical properties
Concerning some physico-chemical properties, diverging values are available from different sources of information.

Toxicity
In addition to acute oral toxicity studies evaluated in SCCNFP/0671/03 further studies have been performed. The data is not available for evaluation. SCCS notes, however, that classification as Acute Tox 3; H301 (toxic if swallowed) according to CLP is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/).
The acute dermal toxicity of ZPT appears to be higher than 2000 mg/kg.
Acute inhalation studies have been performed with ZPT. The studies were not available for evaluation. SCCS notes, however, that classification as Acute Tox 3; H331 (toxic if inhaled) according to CLP is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/).
Several oral repeat-dose studies of different durations have been performed with ZPT. In addition, one sub-chronic and a chronic oral studies performed with sodium pyrithione can be considered adequate to assess repeat-dose effects of ZPT.
The SCCS is aware that HSE (2003) considered the Larson 1958 study as inadequate, due to insufficiently large group sizes to ensure statistical power. However, a 90 d oral study performed with sodium pyrithione which was considered adequate by HSE, also lead to a NOAEL of 500 µg/kg/d, supporting the outcome of the Larsson study. Or in other words: 500µg/kg/d is considered as an adequate oral NOAEL for neurotoxic effects of pyrithiones.

Skin/eye irritation and sensitisation
Skin irritation studies performed with ZPT were not available for evaluation. However, from product based data evaluated in SCCNFP/0671/03, from the description of skin irritation studies performed with ZPT and from human HRIPT tests it can be inferred that ZPT is – at least - a mild skin irritant.
ZPT has been investigated in eye irritation tests, but the studies are not available for evaluation. HSE concludes that ZPT is a severe eye irritant, MAK (2012) states, that ZPT is corrosive to the eye. SCCS notes, that classification as Eye Damage 1; H318 (causes serious eye damage) according to CLP is suggested by the registrant(s) under REACH (ECHA website (http://echa.europa.eu/)).
ZPT is not sensitizing in most animal studies; one animal study (which is not available for evaluation) concluded that ZPT might be a sensitizer. Concerning human data, ZPT (or better: the PT moiety) has a low potential to induce contact hypersensitivity when tested per se or as part of a cosmetic formulation.

Percutaneous absorption
From the animal studies available in submission I, it was concluded in SCCNFP 0671/03 that percutaneous absorption of ZPT varies from approximately 0.03 to 3.4%. Further studies on dermal absorption of ZPT have been performed thereafter.
One *in vitro* study dermal absorption studies using human skin and three *in vivo* animal studies were not available for evaluation.
From dermal toxicokinetic studies (not fully OECD compliant) performed in female CD rats in order to build up a PBPK model for ZPT, absorption percentages of 2.3, 8.6 and 0.3 were derived after single dermal administration of 10, 30 and 100 mg/kg ZPT. However, these values are associated with uncertainties (see study description).
Dermal absorption values obtained from animal studies were not taken for MOS calculation as two clinical human studies were performed. One investigated the systemic absorption of a shampoo containing 1 % ZPT (with or without combination with a leave-on tonic containing 0.1 % ZPT) in a 4-day treatment regimen. In this study, a systemic load of ZPT up to 3.43 µg/kg/d was derived. In the second clinical study, the systemic absorption of a shampoo containing 2 % ZPT (either in combination with leave-on tonics containing 0.1 and 0.25 %ZPT or with a leave-on tonic containing 0.25% ZPT only) was investigated in a 4-day treatment regimen. Systemic exposure loads up to 4.66 µg/kg/d were derived. 1 SD is added yielding 5.25 µg/kg/d, which is taken for MOS calculation based on the fact that (a) low recoveries were obtained and (b) even higher systemic amounts of exposure cannot be excluded after repeated prolonged exposure to ZPT containing products.

Mutagenicity/genotoxicity
From the studies available for SCCNFP/0671/03, it was concluded that ZPT is not mutagenic. Since then, further *in vitro* and *in vivo* genotoxicity/mutagenicity studies have been performed. Apart from two studies published in the open literature, the studies are not available for evaluation.
Therefore, no firm conclusion with respect to genotoxicity/mutagenicity can be drawn by the SCCS. The SCCS, however, is aware that HSE (2003) and MAK (2012) considered ZPT as non-genotoxic and non-mutagenic.

Carcinogenicity
From chronic oral and dermal studies available in submission I, SCCNFP 0671/03 concluded: "no evidence of a carcinogenic response was seen when ZPT was applid topically (up to 100 mg/kg/d) or given orally (up to 5 mg/kg/d) in lifetime studies using mice and rats."
Since that, further chronic (lifetime) studies performed with ZPT and sodium pyrithione (from which read across to ZPT is considered adequate) using the oral and dermal uptake pathway have become available.
These studies are not available for evaluation. Therefore, no firm conclusion with respect to oral and dermal carcinogenicity of ZPT can be drawn by the SCCS. The SCCS, however, is aware that HSE (2003) and MAK (2012) considered ZPT as non-carcinogenic.
Carcinogenicity of ZPT has not been investigated by the inhalation route.

Reproductive toxicity
In SCCNFP 0671/03 the following conclusions were drawn with respect to Reproductive toxicity of ZPT:
- 2.5 mg/kg/d administered orally to rats is a no effect level for teratological effects
- no reproductive effects have been observed when ZPT was applied topically to rats and rabbits at levels up to 15 and 100 mg ZPT/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 ZPT/kg/d respectively.

Since that, two generation as well as developmental toxicity studies have been performed with ZPT. Further, a 2 generation study with sodium pyrithione (from which read across to ZPT is considered adequate) has been performed. These studies are not available for evaluation. Therefore, no firm conclusion with respect to toxicity to reproduction and development can be drawn by the SCCS .The SCCS, however, is aware that HSE (2003) did not identify any potential concern to humans regarding adverse effects on fertility. Further, both MAK (2012) and HSE (2003) concluded that adverse effects on development were most likely attributable to maternal toxicity.

4. Conclusion

SCCS confirms the previous opinion (SCCNFP/0671/03) on the safe use of zinc pyrithione for preservative purposes in cosmetic rinse-off hair care products at a maximum concentration of 1.0 %.

SCCS considers that zinc pyrithione, when used in a concentration up to 2.0% as anti-dandruff agent in rinse-off hair care products, is **not** safe for the consumer. This conclusion is based on the fact that a MOS below 100 was obtained based on the information available Data provided by the applicant is incomplete in order to enable risk assessment based on PBPK modelling.

Aggregate exposure to Zinc Pyrithione from non-cosmetic sources has not been considered.

5. Minority opinion

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7. List of abbreviations

A:	amount
ADME:	Absorption, distribution, metabolism, excretion
ATP:	Adenosine triphosphate
AUC:	area under the curve
BALF:	broncho-alveolar lavage fluid
Br:	brain
CA:	arterial concentration
C_{max}:	maximal concentration
Cl:	clearance
CLP:	Classification, Labelling, Packaging
DMSO:	demethyl sulphoxide
ECHA:	European Chemicals Agency
F:	fraction
GLP:	good laboratory practice
HRIPT:	Human Repeat Insult Patch Test
HSE:	Health and Safety Executive
ICP-MS:	Inductively coupled plasma mass spectrometry
K:	rate constant
K_{el}:	elimination rate constant
LC/MS:	liquid chromatography/mass spectrometry
MAK:	Maximale Arbeitsplatz Konzentration (maximum workplace concentration)
MMAD:	mass median aerodynamic diameter
MSP:	2-(methyl)sulfonyl pyridine
Mu:	muscle
NaPT:	sodium pyrithione
PBPK:	physiologically-based pharmacokinetic modeling
PK:	pharmacokinetic
PT:	pyrithione
PTC:	pyrithione carbon
Q:	plasma flow
QAU:	Quality assurance unit

1	R:	rapidly perfused tissue
2	RBC:	red blood cells
3	REACH:	Registration, Evaluation, Authorisation and Restriction of Chemicals
4	S:	slowly perfused tissue
5	SCCNFP:	Scientific committee on cosmetic products and non-food products
6	SG:	S-glutathione
7	T_{max}:	time of maximal concentration
8	UDS:	unscheduled DNA synthesis
9	UR:	urine
10	V:	volume
11	Vd:	distribution volume
12	ZPT:	zinc pyrithione