Clinical Study Protocol

A Randomized, Controlled, Investigator- Assessor Blinded, Comparative Study to Evaluate the Safety and Efficacy of a Water-Soluble Head Lice Suffocation Product (X92001666; Elimax Ultra®) VS Rid® Shampoo (Pyrethrin) in Subjects with Head Lice.

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TABLE OF CONTENTS

Contents

1. ABBREVIATIONS

ADR	Adverse Drug Reaction	IFRA	International Fragrance Association
AE	Adverse Event	IMP	Investigational Medicinal Product
BMI	Body-Mass Index	ITT	Intention-to-Treat
C	Concentration	l/L	Liter
CAS	CAS Registry Number	mg	Milligram
CI	Confidence Interval	mL	Milliliter
CRF	Case Report Form	p.a.	post administrationem
CRO	Contract Research Organization	PTSS	Pre-treatment Sign and Symptom
GCP	Good Clinical Practice	QAU	Quality Assurance Unit
GLP	Good Laboratory Practice	SAE	Serious Adverse Event
GMP	Good Manufacture Practice	SD	Standard Deviation
g	Gramm	SOP	Standard Operating Procedure
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use	TMF	Trial Master File
		OTC	Over-The-Counter
IEC	Independent Ethics Committee	V	Visit

2. INTRODUCTION

Head lice (*Pediculus humanus capitis*) are parasitic insects that can be found on the head, of humans, particularly school age children. Head lice are tiny, wingless insects (2-3 mm in length) which live among the warmth of human hair, usually close to the scalp, and feed from the blood in the scalp. They crawl but cannot fly or hop. Although small, head lice can be seen with the naked eye. However, a magnifying glass may facilitate observation. When head lice bite the scalp to feed on the blood, it may cause itching; when lice crawl, it can cause a tickling/itching sensation. Lice are also more active in the dark, disrupting the infested person's sleep.
Female lice lay 3-5 eggs (also called nits) per day, which take 1-2 weeks to hatch. Nymphs need 7-10 days to become adult lice. Head lice infest the head and neck and the nits attach to the base on the hair shaft, generally within ¼ inch of the scalp. Head lice survive on the human host for up to 30 days.

Lice are spread by direct contact with an infested individual or contact with clothing, worn by the infested individual, or with personal items (e.g., hair brushes, hair ties, towels, bed linens, etc.). Personal hygiene or cleanliness has nothing to do with getting lice. There is no evidence that lice prefer clean or dirty hair or whether the length of the hair increases the risk for being infested with lice. Head lice do not carry diseases, nor can they survive on non-humans (e.g. pets do not transmit). When removed from the human host, head lice usually die within 24 hours. Also, when lice are in water, they become extremely inactive but remain firmly attached to the hair. For this reason, lice are generally not transmitted in the swimming pool; however, sharing the towel, used to dry the hair of the infested individual, may contribute to transmitting head lice.

Head lice are very common; about 10% of the population are infested. From 6 to 12 million infestations occur in the United States each year in children aged 3 to 12 years of age. It occurs more frequently in girls than boys. It occurs less frequently in African Americans than other races; this might be explained by the shape and thickness of the hair shaft.

Diagnosis is made by observing the live louse/lice on the head of the infested individual. Generally, the person looking for live lice and/or nits should use a magnifying lamp. The hair should be divided into small sections to facilitate detection. The areas around the top of the ears and at the neck line are the most common locations for nits [1].

Treatment is recommended if nits are found on the hair. The American Academy of Pediatrics recommends the use of Nix® (1% permethrin) with a second treatment applied 7-10 days later. However, because of increasing resistance and safety concerns with classic treatments, alternative treatments are taking over the European and US markets during the last years, including silicone-based head lice products with a physical mode of action. For some of these products, both *in vitro* and clinical studies have confirmed their mode of action, efficacy and safety [2-5].

3. STUDY RATIONALE

Treatment of head lice infestations remains a clinical relevant problem because of increasing resistance and safety concerns, observed with classic insecticidal pediculicides. For this reason, alternative treatments have been put on the market, relying on a physical mode of action. Some examples are dimethicone- and mineral oil-based products, of which clinical efficacy and safety has been extensively proven [2-5]. Although very efficient, rinseability may be impaired, especially in products containing high molecular weight silicones. For this reason, Oystershell has developed a new water-dispersible, [redacted] head lice treatment (X92001666; Elimax Ultra®; further referred to as "test product"), combining high efficacy (as confirmed by the results of adulticidal and ovicidal experiments) and improved rinseability (and thus user convenience). The test product is classified as medical device and elicits its effect via a physical mode of action (more details are presented in chapter 4.1).

The present study is set-up to compare *in vivo* clinical performance and safety of the test product versus an in the US commercially available, pyrethrum-based product (RID® shampoo). According to US regulations, the rules for clinical trials with drug products and the rules for clinical trials with medical devices must be followed. Before the trial can start, approval according to drug regulation and medical-device regulation must be granted. The study will be performed in subjects ≥2 year of both genders with confirmed diagnosis of head lice infestation. To support safety, local and global tolerability, skin and ocular irritation will be assessed and adverse events (AEs) will be registered.

4. CHARACTERISTICS INVESTIGATIONAL PRODUCTS

4.1. Test product: X92001666 (Elimax Ultra®)

The sponsor will provide test product and the lice comb (OYS internal code: 07080021), which will be incorporated in the final packaging. The site will be providing the Rid Shampoo. The comb will be used following application of either product. The test product X92001666 (Elimax Ultra®) is a medical device for the treatment of head lice infestations. [redacted]

[redacted] acts as a suffocant by penetrating the respiratory system. Consequently, water and gas exchange is impaired, finally resulting in death. In addition, head lice and nits, treated with this product showed severe symptoms of dehydration (in house experiments). Because of its physical mode of action, no resistance is expected to occur.

4.2. Reference product: RID® shampoo

The active ingredient of RID® shampoo, the reference product in this study, is pyrethrum extract (equivalent to 0.33% pyrethrins), combined with piperonyl butoxide (4%). Pyrethrum has been used for centuries as an insecticide and as a lice remedy. This natural insecticide is made from dried flower heads, primarily of *Chrysanthemum cinerariifolium.* The main active ingredients are pyrethrins, also cinerins and jasmolins. Pyrethroids are synthetic, or man-made, versions of pyrethrins, one common example is permethrin. Pyrethrin is usually combined or synergized with piperonyl butoxide. Both chemicals act synergistically to kill lice by acting on nerve cell membranes and interrupting signal travelling between brain and muscles. The lice become paralyzed and die because they are unable to breath.

Pyrethrum has been extensively studied for its effects on people and the environment. Like all insecticides, pyrethrum is used to have a toxic effect on insects. Pyrethrum has a very good toxicity profile. [8]. For mammals, doses that elicit toxic reactions are significantly larger than the exposures people typically experience in using pyrethrum based products.

On broken skin, pyrethrum produces irritation and sensitization, which is further aggravated by sun exposure. Absorption of pyrethrum through the stomach and intestines and through the skin is slow [9]. However, humans can absorb pyrethrum more quickly through the lungs during respiration. Pyrethrum (as 100%) has an acute oral toxicity in rats of LD_{50} 3500 mg/kg and an acute skin toxicity in rabbits of LD_{50} 19000 mg/kg [10]. Piperonyl butoxide (as 100%) has an acute oral toxicity in rats of LD_{50} 6150 mg/kg and an acute skin toxicity in rabbits of LD_{50} 1880 mg/kg.
Response appears to depend on the pyrethrum compound used. Inhaling high levels of pyrethrum may bring about asthmatic breathing, sneezing, nasal stuffiness, headache, nausea, lack of coordination, tremors, convulsions, facial flushing and swelling, and burning and itching sensations [10]. The lowest lethal oral dose of pyrethrum is 750 mg/kg for children and 1000 mg/kg for adults [11]. At high doses, pyrethrum can be damaging to the central nervous system and the immune system [12].

Pyrethrins and their metabolites are not known to be stored in the body nor excreted in the milk. The urine and feces of people given oral doses of pyrethrum contain chrysanthemumic acid and other metabolites [13]. In mammals, tissue accumulation has not been recorded.

Overall, pyrethrins have low chronic toxicity to humans and the most common problems in humans have resulted from the allergenic properties of pyrethrum. Pyrethrum can produce skin irritation, itching,

pricking sensations and local burning sensations [10]. Pyrethrins appear to have low reproductive toxicity [14].

5. OBJECTIVES & ENDPOINTS

5.1. Primary objective

The primary objective of this study is to demonstrate that the cure rate after local application of test product is better than a predefined limit. The test product must achieve a cure rate superior to 70% (at the end of day 10, corrected for re-infestation).

To compare efficacy and safety of test product against reference product in subjects, infested with head lice (*Pediculus humanus capitis*).

5.2. Secondary objectives

Secondary objectives refer to comparison of the cure rate of test product with those of reference product using the following endpoints:

Secondary efficacy objectives:

a) To show that the cure rate at the end of day 10 (corrected for re-infestation) for the reference product for all baseline infestations is better than 70%.

b) To show that the test product has a cure rate superior to that of the reference product.

c) In case that superiority cannot be shown: to show that the test product is non-inferior to reference product regarding cure rate. A non-inferiority margin of 7.5% will be used.

Further secondary objectives refer to objectives c and d, however evaluated in subgroups or other time points.

d) Efficacy of the investigational products for head lice for mild and moderate baseline infestations (cure rate at the end of the study day 10, corrected for re-infestation).

e) Efficacy of the investigational products for head lice for all baseline infestations (cure rate at day 1).

f) Efficacy of the investigational products for head lice for mild and moderate baseline infestations (cure rate at day 1).

As further secondary objectives, safety and tolerability of test product or reference as well as the acceptance of the investigational products will be evaluated.

Secondary safety objectives;

g) To evaluate local tolerability by subject: subjective symptoms (burning, paraesthesia, pruritus): 0h, 1h, 24h, 7d and 10d p.a. (descriptive evaluation).

h) To evaluate global tolerability by the subject and study staff (number of subjects with global tolerability ratings of “very good”, “good”, “moderate”, “poor”, descriptive evaluation) at 10d

after application.

i) To evaluate skin irritation by study staff (secondary infection, erythema, excoriation) on day 0, 1, 7 and 10.

j) To evaluate eye irritation by study staff on day 0, 1, 7 and 10.

k) To evaluate characterization of safety and tolerability of the investigational products considering Adverse Events in the study population.

5.3. Endpoints

The primary endpoint is the cure rate at the end of day 10 (corrected for re-infestation) for the test product for all baseline infestations.

The secondary endpoint is the difference of cure rates (as defined as primary endpoints) between both treatments.

Safety endpoints are the secondary characteristics of the lice infestation (see Section 9.4) and reported adverse events.

6. INVESTIGATIONAL PLAN

6.1. Study Design

This dual-centre, randomized, controlled, investigator-blinded, comparative study will be conducted in 70 subjects (male and female subjects; age >=2) with confirmed diagnosis of head lice infestation. All subjects will be randomized in a 1:1 ratio in both treatment groups.

In this study, a repeated treatment will be performed, as described in the literature. For two-treatment protocols, it is recommended that the second treatment should be applied on day 7 to day 8 and the final assessment should be on day 10 [15]. In total, 4 visits are planned:

Visit 1 – Baseline visit (Day 0; first treatment)

Visit 2 – Post treatment evaluation visit (Day 1: 24hrs +/- 3 hrs.)

Visit 3 – 2nd treatment visit/evaluation (Day 7 + 1 day)

Visit 4 – Final evaluation visit (Day 10 + 2 days)

Subjects complying with the in- and exclusion criteria will receive the study medication according to a subject number, allocated in ascending order in correspondence with their inclusion into the study. Both investigational products will be tested as described in the product leaflets in compliance with the above-mentioned treatment recommendations.

6.2. Screening/Treatment Day (Day 0)

Subjects with suspicion of head lice infestation will be recruited for this study. Written informed consent will be obtained from adult subjects or the legal representative of minors. Children, must give their assent in written. The assent of younger children will be given verbally as far as appropriate. Subject's eligibility for participation in the trial will be established by the investigator prior to enrolment. Screening assessments will include recording of demographic data, medical history and current medication usage, an assessment of the head, scalp and eyes, vital signs, and a urine pregnancy test in females of child-bearing potential. A subject must meet all inclusion criteria, and none of the exclusion criteria, to be eligible.

Diagnosis of a head lice infestation will be confirmed by identification of at least five living adult head lice or nymphs and five apparently live eggs. The severity of the infestation will be judged on a 4-point severity scale:

- 0 = no relevant infestation (0-4 lice and/or nymph present)
- 1 = mild (5-9 lice and/or nymphs present)
- 2 = moderate (10-24 lice and/or nymphs present)
- 3 = severe (≥25 lice and/or nymphs present)

Subjects who meet the inclusion criteria will be randomized to receive test product or reference product. Prior to the study, a block randomisation (block sizes of 2) will be performed by the external statistician using statistical software (R version 3.4.2; R-development core team, 2017). The randomization schedule will be send to the sites prior to study conduct. All subjects will be enrolled into the study sequentially beginning with Subject Number 1-70.

Following the instructions for use, subjects will have the test product applied by clinic personnel to ensure consistent application and consequently combed with a lice comb (also see chapter 7). Date and times of start and end of application of the investigational product and times of tolerability and efficacy assessments will be documented. Post-treatment assessments will be performed directly after start of treatment, as well as 1 hour after removing the investigational product. This will be considered Day 0 of the study.

If household members exhibit infestation with lice or nits, they will be offered the opportunity to participate in the study or will receive the standard of care if they choose not to participate in the study. In addition, household members will be advised to wash bed linens, towels, and brushes or combs used on the hair to prevent reinfestation.

To sum up, at visit 1, the following operations will be performed and/or parameters controlled by the study team:

- Age-appropriate subject information and obtaining of written informed consent
- Documentation of demographic data
- Documentation of medical history
- Documentation of pre-existing or concomitant medication
- Verifying of head lice infestation, incl. severity

- Checking of inclusion and exclusion criteria including pregnancy test if applicable
- Decision regarding study inclusion, randomisation
- Baseline assessments (including local tolerability before treatment)
- First application of the investigational product

- Post-treatment assessments (local tolerability, skin and eye irritation, adverse events) directly after start of treatment, as well as 1 hour after removing the investigational product
- Questionnaire on the satisfaction with esthetical effect of the products

After the assessments, the subject is released from the clinical ward and advised to follow the instructions / restrictions made in the protocol and to come back on the next day for the second visit.

6.3. Evaluation Visit (24 ± 3hours)

24 hours (± 3h) after end of study drug administration, the subjects will attend the study site. After the 24-hour interval, the clinical evaluator will perform the efficacy and tolerability assessments.
These data, any concomitant medication taken during the study period, adverse events and any change in concomitant diseases will be recorded in the Case Report Form (CRF). Therefore, at visit 2 the following operations will be performed:

- Hair scalp assessment for living lice (all stages) by study staff (see chapter 9.4.8)
- Assessments of skin and eyes irritation, local tolerability
- Questioning on general well-being
- Registration and documentation of any adverse event and/or changes regarding concomitant diseases, concomitant medication, withdrawal criteria
- Vital signs

In case that the examination of the hair and scalp (diagnostic combing; see chapter 9.4.8) will show active head lice infestation, the life stage of the lice will be documented. Subject will not be removed from the study and will continue until study end.

6.4. Second Treatment (Day 7 + 1 day)

On study day 7 (+1 day), the scalp and hair will be examined for live head lice by blinded study staff. The life stage of the lice will be documented. The assessments of skin and eyes irritation will be performed within 1h prior to administration. Subjects without severe skin or eyes irritations will receive a second treatment with the assigned test product. The subjects will be asked for any concomitant medication taken during the study period and adverse events that occurred.

At visit 3 the following operations are being performed by the study team:

- Hair scalp assessment for living lice (all stages) by study staff (see chapter 9.4.8)
- Second application of the investigational products by the study staff
- Assessments of skin and eyes irritation, local tolerability
- Questioning on well-being
- Registration and documentation of any adverse events and/or changes regarding concomitant diseases, concomitant medication, withdrawal criteria
- Vital signs

6.5. Final evaluation visit (Day 10 + 2 days)

The final visit will be performed on study day 10 (+2 days). The final visit examination is performed to verify if the subject is cured. A subject will be considered cured, if no living head lice could be detected. If living lice present on day 10, the life stages of the lice will be documented. If any other pediculicidal agents was employed during the observation period, the subject will be considered a treatment failure (no efficacy analysis will be performed).

The following examinations will be performed within the final visit:

- Hair scalp assessment for living lice (all stages) by study staff (see chapter 9.4.8)
- Assessments of skin and eyes irritation, global tolerability, local tolerability
- Questioning on well-being
- Registration and documentation of any adverse events and/or changes regarding concomitant diseases, concomitant medication, withdrawal criteria
- Questionnaire on the satisfaction with the esthetical effect of the products
- Vital signs

In case that the examination (diagnostic combing) will show active head lice infestation (living nymph or imago), the subject will be considered treatment failure or re-infestation and will receive the standard of care at the site.

Subjects exhibiting either subjective or objective abnormalities when the trial has been completed will be followed up. Any AE which remains unresolved after completion of the trial requires detailed evaluation and follow-up until the AE has been resolved or a reasonable explanation for its persistence is found. If the subject refuses to follow the instructions of the study staff, the latter is released from responsibility.

6.6. Restrictions

- No parallel use of any other anti-head lice therapy.
- Subject agrees not to cut or chemically treat their hair while participating in the study
- Female subjects are to be informed that they must apply adequate contraceptive methods (e.g. uterine tube sterilization, sexual abstinence or vasectomized partner or combination of anti-conceptive medication and condom, spermicidal products, diaphragm, temperature method).
- Subjects agree not to use any other anti-lice treatment for the duration of the study.

6.7. Time and Events Table

The actual date and time should be recorded for all procedures and the Investigator should make every effort to perform procedures as required by the protocol.

VISIT NUMBER	Visit 1			Visit 2	Visit 3	Visit 4
VISIT DAY	**Day 0**			**Day 1 ± 3 hrs.**	**Day 7 + 1d**	**Day 10 +2d**
VISIT NAME	Screening visit (baseline)			Evaluation Visit	Second treatment	Final Evaluation
	-1H	0	+1H			
Informed Consent	x					
Demographics (ethnic group, gender, age, hair length, height…)	x					
Medical history and medication usage	x					
Concomitant medication usage during study				x	x	x
Vital signs (blood pressure, pulse, respiratory and temperature)	x			x	x	x
Urine pregnancy test – child bearing potential	x					x
Physical examination of the scalp to verify head lice infestation by study staff	x					
Inclusion/exclusion	x					
Randomization study product		x				
Skin irritation – baseline assessment prior to treatment by blinded study staff	X				X	
Ocular irritation – baseline assessment prior to treatment by blinded study staff	X				X	
Application of study medication by unblinded study staff		x			X	
Global tolerability assessment by blinded study staff and subject						X
Local tolerability assessment (subjective symptoms: burning, paraesthesia, pruritus)	X		x	X	X	X
Skin and scalp irritation –by blinded study staff		x	x	X	X	X
Ocular irritation – post treatment assessment by blinded study staff		x	x	X	X	X
Adverse Events – questioning by blinded staff			x	X	X	X
Check of restrictions				x	x	x
Questioning on satisfaction with the esthetical effect			x		x	
Discharge from study						x

7. INVESTIGATIONAL PRODUCTS

7.1. Labeling

Both test products will be labeled as shown below.

7.1.1. Product 1 (Elimax Ultra®)

Sponsor: OYSTERSHELL

[redacted]

Principal Investigator: Elisabeth Rivera
Phone 754 304 9462 in case of emergency:
Clinical trial ref.: OYS007-0017

Subject n#:
Use / zone: Topical use on hair and scalp
[redacted].
Posology: First treatment: D0; second treatment: D7
Storage: room temperature, keep away from light, close bottle tightly, **shake well before application.**
For personal use

[redacted]

7.1.2. Product 2 (RID® Shampoo)

Sponsor: OYSTERSHELL

Name of main investigator: Elisabeth Rivera
Phone number in case of emergency: 754 304 9462
Clinical trial ref.: OYS007-0017
Subject n#: XXX
Use / zone: Topical use on hair and scalp
Posology: First treatment: D0; second treatment: D7
Storage: Store at room temperature, keep out of sunlight, close bottle tightly, shake well before use.
For personal use

7.2. Product identity

The test product X92001666 and the lice comb will be supplied by Oystershell Laboratories. The reference product will be purchased by the study center. All investigational products must be stored away from sunlight in a locked area at room temperature. Maintenance of a temperature log (manual or automated) is expected. The following samples of the investigational products have been chosen for the study:

	Investigational Product	Comparative Product
Product Name:	Water-soluble head lice suffocation product (X92001666) - Elimax Ultra®	RID® Shampoo
Dosage form:	Two-phase, water-based lotion	Shampoo
Active Ingredient **Concentration** **Bottle size**	[redacted] 3.4 oz. bottle	Pyrethrum 2 or 4 oz. bottle
Route of Administration:	Topical – hair & scalp	Topical – hair & scalp

Formula preparation and dosing instructions	• [redacted]
	• Apply on dry hair and spread until hair and scalp are fully saturated.
	• After 15 (+2) min exposure, wash the hair with Johnson Baby shampoo.
	• Dry the hair thoroughly with a towel (no hair dryer).
		• Saturate on dry hair with Rid shampoo (see below) and treat for 10 (+2 min); rinse with warm water and then towel dry (no hair dryer).
		  • Short hair: 2 oz.
		  • Shoulder-length: up to 3 oz.
		  • Mid-back hair: 4 oz.

Manufacturer	Oystershell NV	Bayer Group

7.3. Product administration / documentation

Subjects will have the test product applied on the initial visit by the investigative site personnel after it has been determined they are eligible for the study. The date and start time of the application of the test product will be recorded as well as the time the hair is considered dry. The Investigator, or their delegate, is responsible for study treatment accountability, reconciliation, and record maintenance.

The Investigator or their delegate must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of test product received from and returned to the sponsor and the amount supplied and/or administered to subjects. The required accountability unit for this study will be by weight by bottle (weight is to be recorded pre-application and post-application to the subject). Discrepancies are to be reconciled or resolved.

The use and fate of investigational products must be documented. After completion of the study, the containers including any remaining investigational product will be orderly destroyed by the study center. A written explanation regarding the disposition of missing products or their containers is required.

7.4. Assessment of Compliance

Subjects are to be dosed at the study site; they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered/applied in the clinic

will be recorded in the source documents. The dose of test product and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering/applying the test product.

7.5. Product – Instructions for Use

In this study, the individual dose is chosen, according to the leaflet or the instruction of use.

7.5.1. Test product (X92001666; Elimax Ultra®)

The test product will be evenly dispensed (massage) in the hair over its full length, with special diligence on the base of the hair near the scalp (full saturation is essential). After 15 minutes without covering the hair, the hair is washed with Johnson's Baby. A deviation up to plus 2 minutes due to organizational reasons, particularly to deal with children's ability to follow strict procedures, will be accepted. Consequently, the shampoo is rinsed thoroughly with a sufficient amount of water. Afterwards, the hair will be towel-dried (no hair dryer), detangled with a normal comb and finally, combed with the lice comb. Hair is parted into three sections and one section at a time is combed, starting at top of the head and placing teeth to scalp as close as possible. The hair is combed 6x from the scalp to the end of the hair. Following combing, hair and scalp are checked for head lice and nits.

WARNING: Do not smoke during product use. Keep away treated hair from fire, flames and ignition sources.

7.5.2. Reference product (RID® shampoo)

Subjects with short hair will receive approximately 60 mL (2 oz.), while subjects with shoulder length-hair will receive up to 90 mL (3 oz.). Subjects with shoulder length hair from the subject's shoulder until the mid-back hair will be treated with about 120 mL (4 oz.) of the product. No more of the investigational products will be used, as necessary to properly cover the hair and scalp.

The reference product will be evenly dispensed (massage) in the hair over its full length, with special diligence on the base of the hair near the scalp (full saturation is essential). After 10 (+ 2) minutes, warm water is applied to form a lather. Consequently, the hair is rinsed thoroughly to remove foam and residual product. Afterwards, the hair will be towel-dried (no hair dryer), detangled with a normal comb and finally, combed with the lice comb 6x. Place comb as close to scalp as possible and comb with a firm, even motion away from scalp, starting from the roots of the hair.

The applied amount of both investigational products will be documented for each subject in the CRF.

WARNING: Do not smoke during product use. Keep away treated hair from fire, flames and ignition sources.

8. STUDY POPULATION

8.1. Number of Subjects

In total, 70 subjects ages 2+ years and older will be enrolled in the study. Based on previous clinical data with comparable head lice products, a power analysis has been performed. Results indicated that a sample size of 35 subjects in each treatment group results in a power of 80%, considering a cure rate of 95% for

the test product and 55% for the reference product and a superiority margin of 15%. In case of dropouts, subjects will not be replaced.

8.2. Method of assigning volunteers to treatment groups / randomisation

Subjects who meet the inclusion criteria will be randomized to receive test product or reference product. Randomisation will be performed during the screening visit V1 on day 0. A block randomisation (block sizes of 2) will be performed by the external statistician using statistical software (R version 3.4.2; R development core team, 2017). The randomization schedule will be send to the sites prior to study conduct. All subjects will be enrolled into the study, sequentially beginning with Subject Number 1-70.

8.3. Blinding

As both products are substantially different from each other in terms of packaging, smell and application method, it is not possible to do a double-blinded trial. Both, the subjects and the clinic personnel that will apply the investigational products, will be able to recognize the products. Therefore, an investigator-blinded study will be performed, and an independent assessor (investigator or study staff performing the assessments of hair and scalp, eyes, as well the efficacy and safety evaluations) will be blinded to the treatment. Appropriate measures will be taken to keep the assessor blind during the whole study. The assessor will not be involved in any procedure of the trial involving the handling, storage, and use of the products.

8.4. Removal of volunteers from treatment or assessment

8.4.1. Withdrawal criteria

- Serious Adverse Events (SAE's)
- Diseases requiring treatment that occur during the study, which do not constitute SAE's but which, in the opinion of the Principal Investigator, would probably prevent achievement of the study objectives.
- Non-adherence to the study conditions or relevant deviations from procedures as established in the study protocol.
- Withdrawal of consent.
- Duration of treatment < 15 minutes for test product or < 10 minutes for reference product.
- Severe irritation of the skin or the eyes on pre-treatment assessment on day 7.
- Use of any other anti-head lice therapy.

All details and reasons for removal of trial subjects from the study will be recorded in the study termination section of the Case Report Form (CRF). CRFs of all trial subjects entered into the study must be kept in the documentation of the study. Reasons for withdrawal of trial subjects will be entered into the final report of the study. Every effort must be made to follow-up trial subjects who terminate with drug-related AE's to determine the final outcome.

8.4.2. Follow-up of withdrawn subjects, drop outs

A drop out is a subject, who prematurely discontinues participation after being enrolled. If a subject drops out, possible AE's will be recorded as long as necessary. A post-study examination should be performed if possible.

All AE's documented during the study for the drop out will be considered in the assessment. Other data recorded for the drop out are contained in the individual subject listing in an appendix to the study report.

8.4.3. Premature Termination of the study

The sponsor has the right to close this study, and the Principal Investigator has the right to close the centre, at any time, although this can occur only after consultation between involved parties. The Independent Ethics Committee (IEC) and the Institutional Review Board (IRB) must be informed. If the study/centre had to be closed prematurely, the study centre will provide all essential documents necessary for the sponsor's trial master file (TMF), as defined in the GCP Note for Guidance.

The trial will be terminated prematurely in the following cases:

- If AE's occur which are so serious that the risk-benefit ratio is not acceptable.
- If the trial conduct (e.g. recruitment rate, drop-out rate, data quality, protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

8.4.4. Replacements

Subjects who prematurely discontinue participation will not be replaced.

8.4.5. Removal of volunteers from assessment Screening failures

Screening failures are volunteers who were screened but not enrolled. Screening failures including reason will be listed in the screening list. All data obtained within the screening will be documented in the subject's record. In case that entries were made to a CRF it will be archived, but not entered in the database. Screening failures will not be listed, as no CRF data is included in the database.

8.5. Intention-to-treat (ITT) and Safety population

The safety set/population consists of all subjects who were randomised and exposed to study medication.

The ITT population contains any subject included and randomized in the study, with available baseline value of the primary endpoint and at least one follow-up visit for the efficacy parameters.

8.6. Eligibility Criteria

8.6.1. Inclusion Criteria

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

A subject will be eligible for inclusion in this study only if all the following criteria apply:

1. Gender: male / female.
2. Women of childbearing potential is a premenopausal female that is anatomically and physiologically capable of becoming pregnant following menarche.

   Female subjects: are women of childbearing potential who test negative for pregnancy and agree to use a reliable method of birth control or remain abstinent during the study. Methods of contraception

considered acceptable include oral contraceptives, contraceptive patch, intrauterine device, vaginal ring, diaphragm with contraceptive gel, or condom with contraceptive gel

-or

are women of non–childbearing potential, defined as: women who have had surgical sterilization (hysterectomy, bilateral oophorectomy, or tubal ligation),

-or women who are ≥60 years of age.

3. Age: ≥ 2 year of age at the time of enrollment.
4. Subject must have an active head lice infestation defined as at least 5 live lice (adults and/or nymphs) and 5 apparently live nits, present on the scalp and/or hair, as determined by a trained evaluator.
5. Subject is in good general health based on medical history.
6. The subject or his/her parent/legal guardian must give written informed consent, after having been oral and written informed about benefits and potential risks of the trial, as well as information regarding the insurance, taken out to cover the subjects participating in the study. A caregiver must sign an informed consent agreement for children not old enough to do so. Children ages 6-18 years of age will be administered a child's assent form. Subject or his/her parent/legal guardian must be capable of understanding and providing written informed consent.
7. Following application and rinsing of the test products, subject agrees not to shampoo, wash, or rinse their hair or scalp until the 24-hour post treatment evaluation has been completed.
8. The subject agrees not to cut or chemically treat their hair while participating in the study.
9. No more than one working male per household may be excluded from evaluation if he is assessed as being lice free by himself or caregiver.
10. Subject agrees to follow all study instructions, including attending all follow-up appointments.
11. Agree to not use any other pediculicides or medicated hair grooming products for the duration of the study (through Day 10 visit).
12. The parent or legal guardian of a child must be willing to have other family members screened for head lice. If other household members are found to have head lice and are eligible, they must be either enrolled in the study OR receive the standard of care at the site and in the same manner as study participants.
13. Have a single place of residence.
14. The subject or his/her parent or legal guardian must give written informed consent, after having been oral and written informed about benefits and potential risks of the trial, as well as details of the insurance taken out to cover the subjects participating in the study
15. Subjects must agree to not use any other ant-lice treatment for the duration of the study

8.6.2. Exclusion Criteria

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. Application of any form of head lice treatment, whether prescription or over-the-counter (OTC), or home remedy for 30 days prior to their screening visit (Day 1).
2. Application of any topical medication of any kind on the hair for a period of 48 hours prior to the screening visit.
3. Use of systemic or topical drugs or medications, including systemic antibiotics, which in the opinion of the investigative personnel may interfere with the study results.
4. Known skin allergies, multiple drug allergies or multiple allergies to cosmetic products.
5. History of allergy or hypersensitivity to ragweed, active ingredients or constituents of the test products.
6. Subject with any visible skin/scalp condition at the treatment site which, in the opinion of the investigative personnel, will interfere with the evaluation of the test product.
7. Subjects with chronic scalp disorder.
8. Subject or his/her legal guardian who, in the opinion of the investigative personnel, do not understand the subject requirements for study participations and/or may be likely to exhibit poor compliance with the required visits.
9. Females who are pregnant or nursing.
10. Hair longer than mid-back.
11. Subject suspected or known not to follow instructions
12. Previous participation in this study or participation in any other investigational trial within the preceding 30 days
13. The subject is directly affiliated to the investigator site personnel and/or their immediate families. Immediate family is defined as a spouse, parent, child, or sibling, whether biological or legally adopted
14. The subject is an Oystershell employee or is an employee of a third-party organizations involved in the study.

9. STUDY ASSESSMENTS AND PROCEDURES

This section lists the parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table (Section 6.7).

Assessments for skin irritation and eyes irritation prior to administration (baseline as well as assessments on day 7) will be performed within 1 hour before administration.

Assessments for local tolerability, global tolerability (V4), skin irritation and eyes irritation after administration will be performed directly after start of the treatment plus 5 minutes as well as after the end of treatment (the contact time is over and the investigational product is rinsed) within 15 minutes. For all other assessments, a deviation up to ± 20 min from scheduled time will be accepted.

9.1. Study Visit and Assessment Timing

During the study, the evaluation and follow-up visits must be performed 7 (+ 1) and 10 (+2) days after the initial application of the test product, respectively.

9.2. Demographic/Medical History Assessments

The following demographic parameters will be captured: date of birth, gender, race, size of household, and hair characteristics (length, texture, and curliness). Medical history and current medication usage will be assessed as related to the eligibility criteria listed in chapter 8.6.

9.3. Efficacy

9.3.1. Assessment of Hair and Scalp

An assessment of the hair and scalp will be performed with nit comb (dry combing) to determine the number of live lice observed pre- and post-treatment (Visits 1 to 4). An estimate of the number of apparently live nits will be made prior to treatment only. Each assessment will count the number of live lice and nits observed in the left, middle, and right side of the head.

9.4. Safety

9.4.1. Global tolerability assessment

Global tolerability (general well-being and comfort of the subject) will be assessed by blinded study staff and by the subject or his/her guardian at day 10. The study staff will perform the rating in each case prior to the assessment by the subject to avoid bias. Afterwards, the subject will self-assess the global tolerability. The global tolerability will be rated on a 4-category scale with:

- Score 1 = very good
- Score 2 = good
- Score 3 = moderate
- Score 4 = poor

9.4.2. Local tolerability assessment

Subjective symptoms (burning, paraesthesia, pruritus) after administration of the investigational products will be rated by the subjects on a 4-category scale at day 0 (0h, 1h p.a.) and day 7 (0h, 1h p.a.) directly after study drug application, day 1 (24h), and day 10 p.a.:

- Score 1 = none
- Score 2 = mild
- Score 3 = moderate
- Score 4 = severe

It will be investigated, whether the treatments have any influence on the above mentioned subjective symptoms.

9.4.3. Skin irritation assessment

Skin irritation (secondary infection, erythema, pruritus, excoriation) will be assessed by blinded and trained study staff within 1 hours before first treatment (baseline), as well as directly after study drug

application and after the end of treatment on study day 0, day 1, day 7 (pre-treatment, 0h and 1h p.a.) and on day 10. For the assessment of skin irritation, the following 4-category scale will be used:

- Score 1 = no
- Score 2 = mild
- Score 3 = moderate
- Score 4 = severe

It will be investigated, whether the investigational products may cause symptoms of skin irritation.

9.4.4. Eye irritation assessment

To investigate a potential irritation of the eyes due to the treatment, the trained and blinded study staff will be assessed the severity of redness on both eyes (left, right) within 1 hours before first treatment (baseline), as well as directly after study drug application and after the end of treatment on study day 0, day 1, day 7 (pre-treatment, 0h and 1h p.a.) and on day 10. For the assessment of eyes, the following 4-category scale as mentioned above will be used.

- Score 1 = no
- Score 2 = mild
- Score 3 = moderate
- Score 4 = severe

It will be investigated, whether the investigational products may irritate the eyes.

9.4.5. Vital Signs

Vital sign measurements will include systolic and diastolic blood pressure and pulse rate after the subject has rested in a seated position for at least 5 minutes. These will be recorded at each visit.

9.4.6. Urine Pregnancy Test

A urine pregnancy test will be performed at the screening visit (Day 1) and day (10) to ensure all females of child-bearing potential are NOT pregnant.

9.4.7. Assessment of esthetical properties

The esthetical properties of the investigational products will be evaluated to determine the satisfaction by the subjects with the products after application using a questionnaire about hair and scalp feeling, greasiness, hair look, shininess and volume. The questioning will be performed on day 0 and day 7 after treatment and drying the hair.

1. Your hair does not feel too dry after treatment.

- Score 1 = strongly agree
- Score 2 = agree
- Score 3 = disagree
- Score 4 = strongly disagree

2. Your scalp feels pleasant after treatment.

   - Score 1 = strongly agree
   - Score 2 = agree
   - Score 3 = disagree
   - Score 4 = strongly disagree

3. The greasiness of your hair after treatment is normal.

   - Score 1 = strongly agree
   - Score 2 = agree
   - Score 3 = disagree
   - Score 4 = strongly disagree

4. Your hair looks good after the treatment.
   - Score 1 = strongly agree
   - Score 2 = agree
   - Score 3 = disagree
   - Score 4 = strongly disagree

5. The shininess of your hair after treatment is fine.

   - Score 1 = strongly agree
   - Score 2 = agree
   - Score 3 = disagree
   - Score 4 = strongly disagree

6. The volume of your hair after treatment is fine.

   - Score 1 = strongly agree
   - Score 2 = agree
   - Score 3 = disagree
   - Score 4 = strongly disagree

9.4.8. Assessment of the hair and scalp (diagnostic combing)

An assessment of the hair and scalp will be performed to determine the number of live lice in the hair with a special lice comb (dry combing). The lice comb will be provided by Oystershell (OYS internal code: 07080019).

For this assessment, the hair will be divided into 3 sections: left, middle, and right side of the head. Each section will be combed 6x with a lice comb and the number of live lice encountered per section is recorded. The hair will be assessed for lice on day 0 to determine if the subject complies with the inclusion criterion: ≥ 5 live lice. This procedure will be repeated for all visits before and after treatment.

The life stage of lice found during assessments on all visits must be recorded as this is necessary for determination of re-infestation (see chapter 9.5). In case that the examination on day 1, 2 , 7 or day 10 will show active head lice infestation, the lice caught by the comb will be discarded. The subjects with active head lice infestation on day 10 will be considered a treatment failure and will receive an appropriate treatment.

9.5. Efficacy measurements

The endpoints for efficacy assessment will be based on the cure rate on day 10 p.a. The cure rate is defined as proportion of subjects without any live lice, corrected for the rate of reinfestation. A re-infestation [16] is defined as:

a) no adult lice or third stage nymphs present following the first treatment AND
b) no more than two adult lice or third stage nymphs found by combing on day 10.
c) Safety measurements

9.5.1. Specific safety measurements

Due to the short treatment duration, the topical administration (associated with a low risk of systemic availability of pyrethrum) and comprehensive data on the safety profile of the active substance, special safety investigations (e.g. laboratory safety tests) will not be performed. No further special safety investigations will be carried out during the study as sufficient information about the investigational products is already available.

9.5.2. Checks for general well-being

Checks for general well-being will be performed in a non-leading manner. In addition to the checks for general well-being at the first visit (0h, 1h p.a.), checks will also be performed, on study day 1 as well as on study day 7 (0h, 1h p.a.) and at the final visit on study day 10.

A registered physician will be available throughout the entire study and all subjects will be observed for signs of clinical toxicity during the entire study.

9.5.3. Pre-treatment signs and symptoms and Adverse Events

9.5.3.1. Definition of pre-treatment signs and symptoms and Adverse Events

An **Adverse Event (AE)** is any unintended or unfavorable sign (including an abnormal finding), symptom or disease occurring in a subject after signing the informed consent until the last study visit, whether or not the event is believed to be causally related to study medication (IMP) or comparative compound. This definition includes any worsening of conditions that were present at the time of entry into the study (signing of the informed consent) (see International Conference on Harmonization (ICH-E2A). AEs occurring after signing the informed consent but before administration of study medication are defined as non-treatment-emergent events. Those events are evaluated separately because in these cases a causal relationship with the study medication can be excluded.

Adverse Drug Reaction: In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered Adverse Drug Reactions. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an Adverse Event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

Unexpected Adverse Drug Reaction: An Adverse Drug Reaction, the nature or severity of which is not consistent with the applicable product information e.g. Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an unapproved product or package insert/summary of product characteristics for an approved product (see: ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

A Serious Adverse Event (SAE) / Serious Adverse Drug Reaction (Serious ADR) is defined as any untoward medical occurrence that at any dose results in death, or is life-threatening, or requires subject hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity, or results in a congenital anomaly/birth defect.

Important medical reactions that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Examples of such events are intensive treatment in an emergency unit or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

The term "life threatening" in the definition refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

Hospital admission is usually interpreted as requiring at least one overnight stay. A requirement for out-subject treatment in an emergency room is not, by itself, an SAE although the event requiring treatment may be. Elective surgery or other elective procedures which require hospitalization are not SAEs if the condition being treated or investigated was preexisting at the time of entry into the study and did not worsen during the study. However, any untoward outcome of any such procedure should be reported as a non-serious AE or an SAE, as appropriate.

Persistent or significant disability or incapacity means that symptoms which disable the subject, defined as a substantial disruption in a person's ability to conduct normal life functions, do not resolve when study drug is discontinued. To ensure that there is no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following should be considered: The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on subject / event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.
Scientific and medical judgment should be used when deciding whether an event is serious. However, in situations where there is any uncertainty or ambiguity, the event should be managed and reported as if it were serious.

Suspected Unexpected Adverse Drug Reaction – SUSAR is an unexpected Serious Adverse Event causally at least possibly related to the investigational product. If a SAE will be classified as a SUSAR will be decided by sponsor's drug safety department.

9.5.3.2. AE-Assessment

Each AE will be assessed by an investigator according to the following categories:

Category	Characteristics	Explanations
Serious	Non-serious	
	Serious	• Results in death • Life threatening • Persistent or significant disability / incapacity • Hospitalization/Prolongation of existing hospitalization • Congenital anomaly/birth defect
Expected	Not applicable	• There is no causal relationship to investigational product assumed.
	No	• Nature, severity, intensity or outcome of the adverse reaction is not consistent with SPC/Investigator's Brochure.
	Yes	• Nature, severity, intensity or outcome of the adverse reaction is consistent with SPC/Investigator's Brochure.
Intensity	Mild	• Subject perceives signs or symptoms, but is easily able to tolerate those, his activity is not reduced.
	Moderate	• Signs and symptoms limit subject's normal activities, but don't stop them.
	Severe	• Subject is not able to pursue his normal activities.
Causal relationship to investigational product	No relation	Components of assessments of causal relationship • Exposition: Was the investigational product really applied? • Time course: Was the administration of the investigational product followed by the AE in a reasonable time interval? • Probability: Are there other possible reasons for the AE, e.g. concomitant diseases, concomitant medication, environmental or individual factors? • Was the AE terminated or improved after reduction or the stop of medication? • Did the AE reoccur or worsen after reapplication of study drug?
	Possible relationship	
	Probable relationship	
	Certain relationship	
Action taken regarding study drug	None	• No change of the planned further application.
Category	**Characteristics**	**Explanations**
	Interrupted	• Application was interrupted for a short time.
	Disrupted	• Application was terminated.
Category	**Characteristics**	**Explanations**
	Increased	• Dose was increased.
	Reduced	• Dose was reduced.

	Unknown	• No details known.
	Not applicable	• Change of dose was not possible, because for instance application was terminated.
Outcome	Recovered/resolved	• The AE is completely subsided. There are no effects felt. Previous status is restored.
	Recovered with sequelae	• The AE is subsided, but subject suffers from symptoms, for instance persistent cough.
	Recovering	• The AE is improved, but subject still feels effects. Recovery is foreseeable.
	Ongoing	• The AE is not resolved, symptoms persist unchanged.
	Fatal	• Subject dies due to the AE.
	Follow-up impossible	• Outcome is unknown because subject didn't come to the follow-up visit. Efforts to get follow-up information failed.

9.5.3.3. Documentation and Reporting

Documentation of AE

Any AE, reported spontaneously by a volunteer or observed by the study personnel must be documented in the CRF regardless if causally related to the investigational product or not.

The following characteristics of an AE must be documented:

- Description of AE
- Time of onset and stop time
- Seriousness
- Expectedness
- Relationship to study drug
- Action taken regarding study drug
- Action taken regarding subject
- Outcome

Reporting of SAE (Serious Adverse Event)

All SAE causally related to study drug or not have to be documented using an additional SAE reporting form. The investigator must fax SAE report forms within 24 hours after receiving information about the occurrence of an SAE or changed information about a previously reported SAE to sponsor's drug safety.

Pharmacovigilance **Joke Naessens** **Fax: + 32-(9) 377 24 85**

If the SAE results in death, the investigator is obliged to provide further information on request of the ethics committee, the authorities or the sponsor. An event only classified as a SAE due to hospitalization starts at that time, when the subject arrives at hospital.

The initial SAE report should contain all available information at least:

- Study code
- Subject number
- Date of first dose
- Date of last dose
- Start time
- Description of SAE (diagnosis, symptoms)
- Individual subject data like age, gender
- Concomitant therapy
- Assessment of causality
- Name of investigator

Any effort must be made to get further information. If new information is received a follow-up SAE reporting form must be sent within 24 hours to the drug safety. The case report may be seen as complete if sufficient information regarding causal relationship and outcome is available and no further information is to be expected.

Follow-up of subjects after Adverse Events

The study staff of the clinical facility must monitor the trial subject's safety from the occurrence of an AE until recovery, return to baseline or a stable state will be achieved. In single cases follow up of an AE/SAE after last visit can be omitted if there is no causal relationship to study drug and subject's state is improved or stabilized.

Sponsor's responsibility

The sponsor should expedite the reporting to all concerned investigator(s) / institution(s) and to the regulatory authority(ies) of all AE's that are both serious and related. Such expedited reports should comply with the applicable regulatory requirement(s) and with the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

9.6. Prior and concomitant therapy

Topical use of medication on scalp and hair, prior to the first intended administration of the investigational products, which is listed in the exclusion criteria (see section 8.6.1), will prevent inclusion into the study.

Treatment of a previous anti-lice treatment with exception of combing the hair using a lice comb within 30 days will prevent inclusion into the study. Treatment with an anti-lice treatment during the study will lead to exclusion of the subject.

Any pre-existing chronic medication taken for treatment of existing disorders and not considered to interfere with the study course or to influence scalp healing will be allowed.

Intake of pre-existing chronic medication used by the subject should be continued throughout the study at a conventional dose and schedule. Their use is to be recorded in the CRF and medical records.

Checks for concomitant medication will be performed at all study visits.

If a subject needs concomitant medication during the study, the responsible investigator should be asked. Concomitant medication is to be documented by brand, type (generic name, if applicable), amount and duration on the CRF. The Principal Investigator / investigator will then decide together with the responsible project manager of the study center, whether the subject must be excluded.

9.6.1. Concomitant treatment in the event of relevant AEs

Depending on the severity and type of the AEs, the following treatments are suggested treatments and may be changed at the discretion of the responsible physician.

Allergic reaction

Treatment with best standard treatment option according to the local investigator's site circumstances (e.g. antihistamines, glucocorticoids). The investigator will provide all the necessary emergency equipment at the study centre and specially trained staff to handle emergency events during this study (anaphylactic shock).

9.7. Appropriateness of measurements

All methods used for safety assessments are standard methods for which reliability, accuracy and relevance have been documented (e. g. pregnancy test).

9.7.1. Clinical laboratory parameters

In this study, no clinical laboratory parameters will be routinely determined.

9.7.2. Clinical parameters

Body weight and height will be measured with commercial instruments. Lice assessment will be done according to the instructions given in chapter 7.2. The study staff will be trained for combing and determine the live stages of lice.

9.8. Handling and documentation of clinical data

The Principal Investigator must ensure that all data required according to this protocol and not mentioned as exception in the following will be entered promptly in the CRF.

The following variables will be documented in the subject's records ore in raw data sheets prior to transfer in the CRF. A paper CRF will be used in this study.

Category	Variables	Source	Details
Identification	Subject number, random number, screening number	Screening/enrolment log, subject file, random list	
Demographic data	Ethnic origin, gender, age, height, weight, participation in another clinical trial within 4 weeks,	Subject file or raw data sheet	
	Childbearing potential, pregnancy, breastfeeding, contraceptive method, result pregnancy test	Subject file or raw data sheet	Woman only

Description of study condition	Confirmation of head lice infestation, severity of head lice infestation, hair length, number of living head lice, live stage of lice	Subject file or raw data sheet	
Randomisation	Check inclusion/exclusion criteria	Subject file or raw data sheet	
	Randomisation	Enrolment log, Subject file	
	Reasons for non-inclusion into treatment	Subject file	
Concomitant medication	Trade name, indication, route, frequency, total daily dose, start date, stop date, ongoing	Subject file or raw data sheet	
Medical history	Disease, date of diagnosis, stop date, ongoing	Subject file or raw data sheet	Relevant findings
Safety	Local tolerability	Subject file or raw data sheet	
	Global tolerability by subject	Raw data sheet	
Category	**Variables**	**Source**	**Details**
	Global tolerability by investigator	Subject file or raw data sheet	prior to subject's assessment
	Skin irritation	Subject file or raw data sheet	
Category	**Variables**	**Source**	**Details**
	Eyes irritation	Raw data sheet	
	Satisfaction with the esthetical effect	Raw data sheet	
Administration	Amount of study medication, date of administration, start time, stop time	Drug dispensing log	
Completion	Date of completion, type of termination, reason for premature termination, date of premature termination, health state	Subject file	
AE	Description, start date/time, stop date/time, seriousness, severity, causal relationship, expectedness, action taken regarding study drug, action taken regarding subject	Subject file and raw data sheet	
PTSS	Description, start date/time, stop date/time, severity, action taken regarding subject, deterioration to AE	Subject file and raw data sheet	

In view of quality control, a member of the study team will check data on the CRF's for formal correctness, completeness and legibility of the entries.

Entries on the CRF's must be made with a ball-point pen and must be legible. Pencils and correction fluids are not to be used. If corrections are necessary, they will be entered by a member of the study team in

the following manner: the wrong CRF entry will be crossed out; however, it must remain legible, and the correct entry will be placed in the correction field (if applicable) or next to the wrong entry. Corrections will be initialed and dated.

A sample CRF is provided in the Trial Master File. The originals are to be returned to the Sponsor. One copy will remain at the Investigator's site. The CRFs for any subject leaving the study should be completed at the time when study participation is terminated. CRFs should accurately reflect data contained in subject's records (i.e. source documents).

9.9. Clinical data management

The data management department of the study centre will create a database, which reflects the final version of the CRF. Upon receipt of CRF's in data management, the information from the CRF's will be entered in the validated database according to the corresponding SOP's. Edit checks on completeness, correctness, plausibility (such as range checks, cross-checks) will be performed. All identified discrepancies will be queried by the data manager(s) using data discrepancy forms.

The completed data discrepancy forms will become part of the CRF and, therefore, the original data discrepancy form will be filed with the original CRF; copies must be archived at the centre. All changes to the database will be tracked (audit trail).

Details on data management procedures will be fixed in a detailed data management plan.

10. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Els Adriaens (Adriaens Consulting, Bellem, Belgium) will perform all statistical analyses.

10.1. Sample size determination

Based on previous clinical data with comparable head lice products, a power analysis has been performed. Results indicated that a sample size of 30 subjects in both treatment groups result in a power of 80%.

10.2. Data Analysis Considerations

Based on previous clinical data with comparable head lice products, a power analysis has been performed. In order to detect superiority (superiority margin of 15%) of the test product (cure rate of 95%) over the reference product (cure rate 55%) with a power of 80% at 5% level of significance, 30 subjects are needed per treatment group.

10.3. Description

Continuous variables will be summarized by their mean, standard deviation (SD), median, minimum and maximum. Categorical data will be summarized by frequencies and percentages.

10.4. Efficacy Analyses

Efficacy analyses will be performed on the ITT population, consisting of all subjects who were included and randomized in the study, with available baseline value of the primary endpoint and at least one follow-up visit for the efficacy parameters. All statistical test will be performed at a 5% level of significance.

10.4.1. Analysis of primary objective

The cure rate (p) corresponds to the proportion of subjects who are cured at day 10 among all subjects that received any treatment at day 0. The aim of the study is to show superiority for the cure rate of the test product versus a predefined limit of 70%.

The following null hypothesis will be tested:

$H_{0,\ prim}$: $p_T - p_R = 0$ and H_a: $p_T - p_R > 15\%$

A χ2-test of independence will be used to test the null hypothesis.

In case superiority could not be proven, non-inferiority will be tested. According to ICH guideline "Points to consider on switching between superiority and non-inferiority" [17], it is allowed to assess non–inferiority in to the absence of superiority. Prerequisites include:

- predefinition of a non-inferiority margin
- no correction in terms of multiplicity

As non-inferiority margin (δ), a 7.5% worse cure rate is regarded as clinically not relevant. The following null hypothesis will be used: $H_{0,\ NI}$: $p_T - p_R < \delta$, whereby $\delta = -7.5\%$. The lower, 95% confidence interval (CI) of the difference p_T-p_R will be used for the test. If $H_{0,\ NI}$ will be rejected, non-inferiority cannot be shown.

10.5. Safety Analyses

Safety analyses will be performed in the Safety Population, including all enrolled subjects who administered the investigational product, independently of the duration of treatment.
Safety data will be presented in tabular and/or graphical format and summarized descriptively. A comparison of the secondary characteristics of the lice infestation (see chapter 9.4) will be performed along with tabulation of the reported adverse events.

10.6. Statistical software

All statistical analyses will be performed with R-version 3.4.2 (R development core team, 2017).

10.7. Quality control for statistical procedures

The statistical analyses will be conducted in compliance with the ICH E9 Note for Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96, 1998). Quality control will include data check, calculations check, check of tables, listings, and figures. All steps of quality control will be documented with signature of the reviewer and the review date.

11. QUALITY ASSURANCE

Quality assurance includes all activities undertaken during and after a clinical study to verify and control quality. It embraces internal quality control by the staff itself and by independent second persons as well as monitoring and separate auditing activities. All activities will be performed according to written procedures of the CROs and the facilities involved.

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such

11.2. Study and Site Closure

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and the sponsor procedures.

In addition, the sponsor reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. If the sponsor determines such action is needed, the sponsor will discuss this with the investigator or the head of the medical institution (where applicable), including the reasons for taking such action. When feasible, the sponsor will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action prior to it taking effect.

If the study is suspended or prematurely discontinued for safety reasons, the sponsor will promptly inform investigators or the head of the medical institution (where applicable) and the regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action. If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

11.3. Data Management

The site or a data management partner will provide a file containing all the needed data to the statistician so the appropriate statistical analyses can be performed. Subject data will be entered into case report forms (CRFs). Subject initials will not be transmitted to the sponsor or data management partner. Case Report Forms will be given to the sponsor, while the sites will retain a copy.

12. ETHICS

12.1. Independent Ethics Committee (IEC)

The documents required by the ICH-GCP regulation, including study protocol, informed consent form as well as any subsequent amendment will be submitted to the relevant Independent Ethics Committee for positive vote.

12.2. Ethical conduct of the study

The study will be conducted in accordance with the study protocol, the ethical principles of the Declaration of Helsinki, ICH-GCP guidelines (International Conference on Harmonisation of Technical Requirements for registration of Pharmaceutical for Human Use – Good Clinical Practice), and the requirements of the US regulations.

12.3. Volunteer information and consent

12.3.1. Informed consent and assent

The subject will receive a consent form. The subject information document contains all relevant information about the study in accordance with the GCP requirements. Every subject must be informed verbally and in writing by receiving the subject information document. The written information for subjects must be approved by Ethics Committee. The consent form must be signed and dated by the

subject prior to the start of the study. The informed consent form must be signed and dated by the physician who conducted the informed consent discussion. The subjects must be given a copy of the document.

The Principal Investigator must ensure that subjects are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical studies in which they volunteer to participate. The principles of Informed Consent, according to FDA Regulations and ICH GCP, will be followed. A copy of the proposed consent form must be submitted to the IRB, together with the protocol, for approval. Prior to beginning of the study, the Principal Investigator must have the IRB's written approval of the written informed consent form, assent form, and any other information to be provided to Subjects.

Every subject has the right to refuse further participation in the study at any time and without giving reasons. Confidentiality and pseudonymity of the subjects are assured. Children from 2-11 years and adolescents from 12 to less than 18 years can be included in this study. However, special requirements for this subject group are described below.

As the minor is unable to provide legally binding consent, the parent/s or legal guardian has/have to give informed consent on his/her behalf prior to enrolling a minor in the trial. In case both parents are the legal guardian, it may be sufficient that at least one parent gives the informed consent.

The parent(s)/legal guardian will be given sufficient time and necessary information to consider the benefits and risks of involving the minor in the clinical trial. Information will be given by experienced personnel to parent(s), or the legal guardian on the purpose of the trial and its nature, the potential benefits and risks, and the name of investigators(s) who are responsible for conducting the trial with background professional information (such as education, work experience) and direct contact details (telephone, address, e-mail).

There will not be financial inducement to enroll the minor in the trial; no financial incentive will be offered. Parent(s)/legal guardian will be informed of the possibility to revoke informed consent even though it was made in writing.

Parent(s)/legal guardian will be reassured that the child's treatment will not be prejudiced by withdrawal from the trial.

Consent will be obtained from the parent(s)/legal guardian before assent is sought from the child.

Parent(s)/legal guardian will be made aware of the rights to refuse participation in a clinical trial and are entitled to withdraw informed consent, without giving reasons.

Parent(s)/legal guardian will be reassured that the withdrawal from the trial will not prejudice the child. Legal representatives who gave informed consent for a child to participate in clinical trials will have the opportunity to follow research as it proceeds (unless clinically inappropriate, e.g., during an operation under general anesthesia), to be able to withdraw the child from the research at any time.

Minors will be involved in discussions and decision-making process only after obtaining consent from the parent(s) or the legal guardian as, where appropriate, the central role of parents should be recognized. An assent (age appropriate) in addition to informed consent of the legal guardian is required. If the child's assent is not collected, this will be recorded in the consent form signed by the parents/legal representative and investigator, with the reasons.

Consent and assent forms will be used to provide age appropriate information. Objections raised by a minor at any time during a trial will be considered. The child's will is respected any time, provided it is not considered detrimental to his/her health. The child will not be forced to provide reasons. The legal guardian's consent should be checked. The child will be informed of the possibility to withdraw from the trial.

12.3.2. Infants and pre-school children

In this age group, it is not possible to obtain assent and the understanding of research is not expected. Where the child has some capacity of understanding (pre-school children), age-appropriate information will be provided even though it will not be possible to obtain assent.

12.3.3. Children of school age (from about 6 years old)

In any case assent, preferably in writing will be obtained when the child is able to read and write, and keeping track of such assent will be performed.

12.3.4. Consent and assent in adolescents

Additional informed consent to participate in clinical trial is required for enrolment of minors of this age group.

12.4. Informed consent (and assent for children) of families with different cultural background

Where appropriate, a cultural mediator independent from the sponsor and investigator, experienced in the language, social habits, culture, traditions, religion and particular ethnic problems will assist in the process of obtaining informed consent and assent.

12.5. Insurance

All subjects participating in the study will have insurance coverage by the sponsor, which is in line with applicable laws and/or regulations. A copy of the insurance certificate must be available at the study site.

13. PROTOCOL AMENDMENTS

Neither the Investigator nor the Sponsor may modify the protocol without prior written agreement with the other party. Changes in any part of the protocol must be documented in the Study Protocol Amendment. All amendments that would increase the risk to the subject or may alter the results of the study, i.e. increase of the number of subjects, a day dose of study drugs, the age range of study subjects, addition of blood sample(s) or another procedure(s), must be re-submitted to the Institutional Ethics Committee and to regulatory authorities and must be approved before their implementation.

If an amendment to the Study Protocol substantially alters the study design or the potential risks to the subjects, a new subject's consent to continued participation will be needed and approved as stated above.

If the changes in Study Protocol involve only logistical or administrative aspects of the trial (e.g. change of monitor, telephone number), written approvals are necessary from Sponsor, but not from the Institutional Ethics Committee and the Regulatory Authorities before their implementation.

14. REPORTING

A summarizing report according to ICH standard will be presented to the sponsor including a complete and detailed description of the performance of the study, the biometric planning, the methods and materials, all raw data and calculations for each volunteer, the statistical analysis, summarizing calculations, a biometric evaluation and a conclusive assessment of the results.

15. RECORD KEEPING

Any documents related to the study must be retained for at least 7 years after the termination of the project or at least two years after the last approval in an ICH region or at least two years after the formal discontinuation of the clinical development. The sponsor will inform the investigator about developments which affect the storage period.

16. PUBLICATION POLICY

It is anticipated the sponsor will publish the results of this study.

17. CONCOMITANT MEDICATIONS AND HOME REMEDIES

All medications, prescription or over-the-counter (OTC), used by the subject during the study will be recorded in the source documents.

17.1. Prohibited Medications

Subjects must abstain from taking prescription or non-prescription medications for the treatment of lice other than the test product beginning 30 days prior to the screening visit through the end of the study.

17.2. Home Remedies

Subjects must abstain using any home remedies for the treatment of lice other than the test product beginning 4 weeks prior to the screening visit through the end of the study.

18. SUBJECT COMPLETION

A completed subject is one who has completed all study visits. The end of the study is defined as the last subject's last visit (or in case the last subject withdraws from the study, this date will be the day of study end).

19. SUBJECT WITHDRAWAL CRITERIA

A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral or administrative reasons.

19.1. Subject Withdrawal Procedures

If a subject is prematurely withdrawn from study participation for any reason, the Investigator must make every effort have the subject return to the clinic for a final evaluation. Subjects that withdraw early from

the study for any reason may be replaced with another subject to meet the original target of 50 subjects to complete the study.

20. TREATMENT AFTER THE END OF THE STUDY

Subjects will not receive any additional treatment from the sponsor after completion of the study; however, the investigative site can appropriately treat the subject based on any remaining lice infestation.

21. SCREEN FAILURES

Data for screen failures will be collected in source documentation at the site but will not be transmitted to the sponsor.

22. STUDY CONDUCT CONSIDERATIONS

22.1. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements. The study will also be conducted in accordance with all applicable subject privacy requirements, and, the guiding principles of the 2008 Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB/IEC review and favourable opinion/approval to conduct the study and of any subsequent relevant amended documents.
- Written informed consent (and any amendments)/assent (for minor subjects) to be obtained for each subject before participation in the study.
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC).

The following documents will be received by the Sponsor prior to the initiation of the study:

- Current curricula vitae, signed and dated for the Principal Investigator and each Sub-Investigator (current within 2 years)
- Current medical licenses
- Documentation of IRB approval of this study protocol, Principal Investigator and informed consent forms
- Current IRB membership list or roster
- A copy of the protocol agreement page signed by the Principal Investigator
- Financial Disclosure Statement for the Principal Investigator
- Statement of Non-Debarment is required for all sites

22.2. Study Monitoring

In accordance with applicable regulations including GCP, and sponsor procedures, sponsor monitor's will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and sponsor requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the CRF will serve as the source document.

The sponsor will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements.
- GCP, and all applicable regulatory requirements.

23. SUBJECT CONFIDENTIALITY

The monitor(s), the auditor(s), IRB/IEC, will be granted direct access to the Subject's original medical records for verification of the clinical trial procedures and/or data, without violating the confidentiality, to the extent permitted by the applicable laws and regulations and that by signing a written informed consent form, the Subject is authorizing such access.

The identifying the Subject will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available. If the results of the trial are published, the Subject's identity will remain confidential.

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