ICHTHYOL® PALE

Further and more detailed information is available from

Manufacturer:
ICHTHYOL-GESELLSCHAFT
Cordes, Hermanni & Co. (GmbH & Co.) KG
Sportallee 85 – 22335 Hamburg – Germany
Phone: ++49 40 50714 360
EMail: rawmaterial@ichthyol.com
INTRODUCTION

Personal care formulators world-wide have realized the exciting possibilities ICHTHYOL® PALE offers for new and innovative cosmetics. Due to its broad spectrum of efficacy the well tolerated substance of natural origin can be used in hair care products (e.g. for treatment of dandruff) and for skin care as well (e.g. for treatment of skin blemishes, redness or itching).

ICHTHYOL® PALE (INCI: SODIUM SHALE OIL SULFONATE)

BENEFITS

♦ MULTIFUNCTIONAL - anti-microbial - anti-inflammatory
  - anti-seborrheic - anti-itching
♦ WELL TOLERATED
♦ NATURAL ORIGIN
♦ CLEAR SOLUTIONS

In the following some detailed technical and scientific information on origin, properties, efficacy and tolerance of ICHTHYOL® PALE is given for all those involved in development work with this unique substance.

Hamburg, September 2010
A. Technical Data Sheet

(physico-chemical and microbiological specifications)

Definition:

ICHTHYOL® PALE is the sodium salt of a pale sulfonated shale oil in aqueous solution.

Chemical name: Ichthyolic acid, sodium salt
INCI: Sodium Shale Oil Sulfonate
CAS No. 1340-06-3
EINECS No. 215-671-7
# Product Specifications

**ICHTHYOL® PALE**

(INCI: Sodium Shale Oil Sulfonate)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>in thin layer light-brown, in thick layer dark-brown</td>
</tr>
<tr>
<td>Odour</td>
<td>weak and characteristic</td>
</tr>
<tr>
<td>Consistency</td>
<td>liquid</td>
</tr>
<tr>
<td>Solubility</td>
<td>- soluble in water, miscible with glycerol, partly soluble in alcohol and ether</td>
</tr>
<tr>
<td>Identity</td>
<td>- formation of a precipitate by addition of hydrochloric acid</td>
</tr>
<tr>
<td></td>
<td>- detection of sodium</td>
</tr>
<tr>
<td></td>
<td>- detection of sulfur</td>
</tr>
<tr>
<td>Purity</td>
<td>- pH-value (1% aq. Solution): 6.0 - 7.5</td>
</tr>
<tr>
<td></td>
<td>- sulfated ash: max. 15 %</td>
</tr>
<tr>
<td></td>
<td>- refractive index: 1.44 - 1.46</td>
</tr>
<tr>
<td></td>
<td>- relative density: 1.15 - 1.25</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Assay</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Active content</td>
<td>min. 50 %</td>
</tr>
<tr>
<td>Water</td>
<td>max. 50%</td>
</tr>
<tr>
<td>Content of sulfur</td>
<td>11.0 - 13.5 %</td>
</tr>
<tr>
<td>Sulfur in the form of sulfate</td>
<td>max. 0.25%</td>
</tr>
</tbody>
</table>
Microbiological Specifications of ICHTHYOL® PALE:

Aqueous solutions containing 2% of ICHTHYOL® PALE as well as the pure (undiluted) substance were subjected to a microbiological analysis according to methods given in the European Pharmacopoeia (Ph.Eur. 2.6.12/13).

According to this impurity test the number of germs (microbial count of fungi and bacteria) present in the watery solution of ICHTHYOL® PALE as well as the pure substance is below the detection limit (< 10/g). No aerobic bacteria could be detected.

2% ICHTHYOL® PALE was checked according to paragraph 5.1.3 of Ph.Eur. (preservation properties). For this examination the aqueous solution of ICHTHYOL® PALE was contaminated with certain micro-organisms (10^6/g Pseudomonas aeruginosa, St. aureus, Candida albicans, Aspergillus niger) according to a prescribed method.

The decrease in the number of micro-organisms (microbial count/g) in 2% ICHTHYOL® PALE was assessed to meet the requirements for topical preparations according to the European Pharmacopoeia (category 2 according to chapter 5.1.4 “microbiological quality of pharmaceutical preparations”).

Certificates confirming the above mentioned statements are available on request by ICHTHYOL-GESSELLSCHAFT.

Additional information on the Minimum Inhibition Concentration (MIC) of ICHTHYOL® PALE with regard to different fungi and bacteria are available as well. The antimicrobial effect of ICHTHYOL® PALE helps maintain the microbiological stability of formulations and lessens the amount of preservatives required.
Product Development with ICHTHYOL PALE – What to consider

ICHTHYOL® PALE is a sulfonate salt in an aqueous solution. It is not an oil. The formula can be symbolized as R-SO₃Na.

In the sulfonation process a sulfonyl group (-SO₃H) is introduced into an organic molecule R. R stands for thiophene derivatives (see chapter B.).

As a sulfonate salt ICHTHYOL® PALE has tensio-active properties (lipophilic (R) and hydrophilic part (SO₃Na) in the molecule). As a consequence, there may be possible inference with emulsions and creams.

In general, hydrophilic creams are favoured compared to lipophilic creams.

In case of liquefaction hydrophilic creams containing ICHTHYOL® PALE can be stabilized by addition of hydroxyethylcellulose, for instance.

Lipophilic creams are only formed with very potent water-in-oil emulsifying agents and a small content of water only.

Generally there is no miscibility with oils without help of an emulsifier. Miscibility with water and glycerine, however, normally is problem-free.

In solutions, precipitation may result from addition of other salts. Cations of higher valency and salting out must be avoided.

ICHTHYOL® PALE is stable in a wide range of pH. Only with strong acids precipitation may result.
B. Qualitative and quantitative composition

ICHTHYOL® PALE is the sodium salt of a sulfonated low-boiling shale oil fraction in aqueous solution. The general designation is ‘pale sulfonated shale oil’. It consists of polar sulfonate salts with a high content of organically bound sulfur.

Shale Oil as a product of the dry distillation of kerogen-containing rock is a complex mixture of different molecules. Therefore, a structural formula for shale oil as well as for ICHTHYOL® PALE made thereof is not available.

To understand more about the chemical composition of ICHTHYOL® PALE its origin and manufacture have to be considered.

The origin of ICHTHYOL® PALE goes back to deposits of phytoplankton in a special lagoonal setting in the region of the alps in the Mesozoic era. In the marine environment large amounts of organically combined sulfur were incorporated in the biomass in the course of the biodegradation process.

Oil shale rock (bituminous schist) with fossil plant as source of shale oil as starting material for manufacture of ICHTHYOL® PALE.

The process taking place on the bottom of the lagoon was superimposed by deposition of minerals (stratification). By diagentic processes a sedimentary rock with shale-like appearance developed, which contains the marine biomass in a solidified form today.
By dry distillation of the former lagoon ground the solidified phytoplanktonic biomass can be released from the rock as a sulfur-rich shale oil.

The crude shale oil is subjected to a vacuum distillation where a low boiling shale oil fraction is produced.

After further refining the volatile oil fraction is sulfonated by means of sulfuric acid at low temperatures. The resulting sulfinic acids (containing the SO₃H-group) are subsequently neutralized by addition of sodium hydroxide solution.

\[
\text{low boiling fraction} + \text{H}_2\text{SO}_4 \rightarrow \text{low boiling fraction} + \text{H}_2\text{O}
\]

\[
\text{low boiling fraction} + \text{NaOH} \rightarrow \text{low boiling fraction} + \text{H}_2\text{O}
\]

Finished ICHTHYOL® PALE contains about 44 - 50% of water and 50 - 56% dry substance (sodium salts of sulfonated shale oil). Only a small amount of Na₂SO₄ (from reaction of NaOH with unreacted H₂SO₄) is left in the product. 2-Propanol is used to salt out Sodium Sulfate and is virtually quantitatively removed afterwards by distillation.

The amount of sulfur in form of sulfates is below 0.25%. The main part of sulfur appears in heterocyclic thiophene-derivates and organic sulfides (the amount of sulfur in total is between 11.0 and 13.5%).
IDENTITY

Resin formation

Dissolve 1.5 g in 15 ml of water. To 2 ml of this solution add 2 ml of hydrochloric acid. A resinous precipitate is formed. Decant the supernatant liquid. The precipitate is partly soluble in ether.

Identification of sodium (reference: Ph.Eur.\(^1\) 2.3.1):

2 ml of an aqueous solution containing 15% of potassium carbonate (m/v) are added to 2 ml of a solution of 1.5 g of substance in 15 ml of water. The mixture is shortly heated to boiling. No precipitate is formed. Subsequently, 4 ml of potassium hexahydroxoantimonate(V) solution R is added and again heated to boiling. By subsequent cooling with ice/water and rubbing with a glass bar a thick white precipitate is formed.

\(^1\) Ph.Eur. = European Pharmacopoeia
PURITY

pH (reference: Ph.Eur. 2.2.3)
The pH is measured potentiometrically in an aqueous solution containing 1% of substance (m/m).
Limits: 6.0 - 7.5

Relative density (reference: Ph.Eur. 2.2.5)
The relative density is the ratio of the density of the substance at 20°C to that of the density of water at the same temperature. For determination of the relative density a suitable pycnometer is used.
Limits: 1.15 - 1.25

Refractive index (reference: Ph.Eur. 2.2.6)
The refractive index is determined using white light at a temperature of 20°C.
Limits: 1.44 - 1.46

Sulfated ash (reference: Ph.Eur. 2.4.14)
Weigh accurately 1 to 2 g of the substance in a suitable crucible that previously has been ignited, cooled, and weighed. Heat, gently at first, until the substance is thoroughly charred, cool, then moisten the residue with 1 ml of sulfuric acid, heat gently until white fumes no longer are evolved, and ignite at about 600°C until the carbon is consumed. Cool in a desiccator, weigh, and calculate the percentage of residue. If the amount of the residue so obtained exceeds the limit, again moisten the residue with 1 ml of sulfuric acid, heat and ignite as before, and again calculate the percentage of residue. Continue the ignition until constant weight is attained.
Limit: max. 15%
ASSAY

Water

0.5 - 1 g of substance put in a glass with sufficient surface is dried for 4 hours at 105°C in a drying chamber.

Limits: 44% - 50%

Total sulfur (reference: Ph.Eur. 2.5.10):

The Schöniger method is carried out with 10 to 13 mg of substance weighed accurately in a cotton swab that is jammed in an appropriate (platinum) carrier. A small stripe of ashfree paper used for ignition is connected to the cotton swab. 30 ml of an aqueous solution containing 3% of H$_2$O$_2$ is used for absorption of combustion products. After combustion and cooling the flask is shaken to solve the combustion products in the solution. Subsequently, the content of the flask is transferred quantitatively to a beaker by means of 60 ml of 2-propanol. 0.5 ml of a solution containing 0.2% Thorin-indicator are added and the beaker is put into an ultrasonic bath for degasing. The titration is carried out with 0.01 n Ba(ClO$_4$)$_2$-solution using photometric indication at a wavelength of 490 nm. 1 ml of 0.01 n Ba(ClO$_4$)$_2$-solution is equivalent to 0.1603 mg total sulfur.

Limit: min. 11.0 %

As an alternative total sulfur can also be determined as follows:

Mix 0.5 g of substance, accurately weighed, with 4 g of anhydrous sodium carbonate and 3 ml of methylene chloride in a porcelain crucible of about 50 ml capacity, warm and stir until all the methylene chloride has evaporated. Add 10 g of coarsely powdered copper nitrate, mix thoroughly and heat the mixture very gentle using a small flame. When the initial reaction has subsided, increase the temperature slightly until most of the material has blackened. Cool, place the crucible in a large beaker, add 20 ml of hydrochloric acid and, when the reaction has ceased, add 100 ml of water and boil until all the copper oxide has dissolved. Filter the solution, add 400 ml of water, heat to boiling and add 20 ml of barium chloride solution (61 g/l). Allow to stand overnight, filter, wash with water, dry and ignite at about 600°C for 2 hours. 1 g of residue is equivalent to 0.1374 g of total sulfur.
Sulfur in the form of sulfate:

Dissolve 2 g, accurately weighed, in 100 ml of water, add 2 g of cupric chloride dissolved in 80 ml of water and dilute to 200.0 ml with water. Shake and filter. Heat 100.0 ml of the filtrate almost to boiling, add 1 ml of hydrochloric acid and 5 ml of barium chloride solution (61g/l) dropwise and heat on a water-bath. Filter, wash the precipitate with water, dry and ignite at about 600°C until two successive weighings do not differ by more than 0.2% of the mass of the residue. 1 g of residue is equivalent to 0.1374 g of sulfur.

Limit: max. 0.25%
D.

DECLARATION
concerning
ORIGIN, MANUFACTURE and PURITY
of ICHTHYOL® PALE
(INCI: SODIUM SHALE OIL SULFONATE)

ORIGIN: The starting material for the production of ICHTHYOL® PALE (pale sulfonated shale oil) is sulfur-rich shale oil resulting from a dry distillation process of a kerogen-containing sedimentary rock.

PRODUCTION: A purified and refined volatile distillate of crude shale oil is mixed with sulfuric acid and neutralised afterwards.

No materials from animal or plant origin and no genetically modified organisms (GMO) or ingredients produced from GMO are used in the manufacture of ICHTHYOL® PALE.

For this reason, ICHTHYOL® PALE does not pose the risk of transmitting Transmissible Spongiform Encephalopathies (TSE) or Bovine Spongiform Encephalopathy (BSE).

In the manufacture of ICHTHYOL® PALE no substance is added the use of which is restricted or subject to specific labelling under current cosmetic EU regulations.

This includes the 26 substances (identified as an important cause of contact-allergy reactions in fragrance-sensitive consumers) listed in Annex III (Part I) of the 7th amendment of Directive 76/768/EEC starting with reference number 67.

ICHTHYOL® PALE as ingredient of finished cosmetics conforms with article 2 & 4 of the European Cosmetic Directive 76/768/EEC in its current version.

ICHTHYOL® PALE is a product manufactured in the European Union.

Hamburg, May 2006
E. On the Efficacy of ICHTHYOL® PALE in Treatment of Dandruff and Skin Blemishes – a Summary

ICHTHYOL® PALE has a lot to offer cosmetic formulations due to multi-functionality combined with natural origin and good tolerance.

E1. ICHTHYOL® PALE in Treatment of Dandruff

E1.1 OUTLINE OF DANDRUFF

Dandruff is a phenomenon related to the renewal of the skin. In skin regeneration cells formed at the inner side of the epidermis move to its outer side. On their way these keratinocytes are subjected to a transformation process in the end of which they mostly consist of lifeless keratin (corneocytes) that is cast off the skin in nearly invisible particles. However, this natural process can be impaired in such a way that it becomes a cosmetic problem with large agglomerates of corneocytes (scales) on scalp and hair.

The origin of dandruff is still disputed. Often it is referred to as a vicious cycle in which among others genetic predisposition, the level of sebaceous activity and excessive colonisation with micro-organisms are determining factors.

An enhanced proliferation rate in the epidermis causes an accelerated process of cell movement to the outer skin. In this case the adhesion between different cells cannot be resolved completely and scales appear. This process may appear with concomitant over-secretion of oil glands. The skin sebum and the oil soaked scales are like a nutrient substratum for micro-organisms. Bacteria and fungi are found in the stratum corneum and in the hair follicles. Staphylococcus epidermis, different Propionibacteria and Candida belong to the leading cutaneous micro-organisms. (Pityrosporum spec. were given a major role by some researchers.) Germs like these increase in number and their lipolytic activity enhances the amount of free fatty acids on the scalp. These acids on their part may lead to irritations on the skin that are connected with itching and reddening. In principle, they are able to cause small inflammations on the skin. As a result a vicious circle may appear since inflammations again can be the cause of an enhanced proliferation rate of epidermal cells (see above).

E1.2 BENEFITS OF ICHTHYOL® PALE IN DANDRUFF TREATMENT

From the outline of dandruff described above different approaches to treat this problem and its consequences are possible. Ichthyol® Pale offers different starting points. As could be shown by Gloor, to begin with, ICHTHYOL® PALE slows desquamation of the scalp effectively. In fact, an equivalent reduction of enhanced cell growth could be observed for coal tar and ICHTHYOL® PALE [2,7]. ICHTHYOL® PALE reduces the enhanced cell growth to the normal degree.
The control of micro-organisms by application of mild antibiotics results in a reduction of dandruff. As could be shown recently ICHTHYOL® PALE exhibits antibacterial as well as anti-fungal action (including Pityrosporum spec.). [3,4,22].

At the same time, ICHTHYOL® PALE has a soothing effect on the scalp. Itching is relieved. The consumer benefits here from the anti-oxidative and anti-inflammatory properties of ICHTHYOL® PALE [e.g. 5,6]. Anti-inflammatory properties of anti-dandruff agents are also important with respect to the fact that small localized inflammations may cause parakeratosis.

In a UVB erythema test it could be demonstrated that 4% of ICHTHYOL® PALE is as effective in reducing skin redness as 0.5% of hydrocortisone [6,7]. Skin redness may be a problem of the skin of the scalp appearing together with dandruff.

Concerning oily hair and scalp a reduction in the oversecretion of oil glands of the scalp is important. The anti-seborrheic action of ICHTHYOL® PALE was pointed out by skin lipid researcher Gloor [8]. By therapy with sulfonated shale oils the amount of skin surface lipids can be reduced in oral and topical administration [23,9]. In a study on the efficacy of a combination of chloramphenicol and ICHTHYOL® PALE used in the treatment of very severe acne (acne papulopustulosa) the quantitative determination of squalene, a lipid formed in the oil glands, served as a measure for the anti-seborrheic efficacy [9]. With respect to the reduction of squalene the combination of chloramphenicol and ICHTHYOL® PALE was far superior to the base as well as Chloramphenicol alone. The content of squalene could be decreased by more than 35% in presence of ICHTHYOL® PALE. However, no decrease could be observed with chloramphenicol and base alone.

E1.3 CLINICAL/COMPARISON STUDIES

The efficacy of ICHTHYOL® PALE with respect to treatment of scalp and hair was tested in different clinical studies [10-12,15,19]. Concerning tolerance and safety all of the studies show that preparations (e.g. shampoos) containing ICHTHYOL® PALE (INCI: Sodium Shale Oil Sulfonate) can be used over long periods of time without side effects.

E1.3.1 Rinseable Cream (ICHTHODERM®)

The efficacy and tolerance of a cream containing 2% Ichthyol® Pale has been recently confirmed in a study on persons suffering from psoriasis of the scalp [15].

The study was performed on 24 subjects aged between 10 and 68 years. According to the advice of the dermatologists, the cream was mainly used once a day. It was applied to the wet scalp, rubbed into it and rinsed off after 2 or 3 minutes.

The results of the study indicate that a 4 week application leads to a distinct improvement of the symptoms „redness“, „infiltration“ and „dandruff“. The average redness-score was reduced by 48%, the infiltration score by 63% and the dandruff-score by 56%. Besides one exception, the dermatologists noted in each subject a clinical relevant improvement for at least one of the individual symptoms. The best results were seen for the dandruff symptom - with great improvement in 23 of 24 cases.

2 for a detailed review see Gayko, 2003 [21]
Besides efficacy, tolerance of the formulation was evaluated as well. With two exceptions („moderate“) the dermatologists rated it „very good“ or „good“. No adverse drug effects occurred during the trial.

**E1.3.2 Shampoo**

The clinical efficacy of a shampoo containing 0.5% ICHTHYOL® PALE was substantiated in a study involving 40 persons suffering from severe dandruff [11]. During the six weeks’ treatment period a continuous decrease in the amount of dandruff to about 55% of the starting score was observed.

In the beginning of the treatment period 21 of 40 subjects suffered from moderate, severe or very severe itching. On the average itching was reduced by about 50% after the first week. During the following time period it was further decreased to about 20% of the starting score. In more than 50% (11/21) of the cases, itching was completely eliminated after six weeks.

While other antidandruff substances such as Piroctone Olamine, Zinc Pyrithione and Selenium Sulphide often lead to an objectively or subjectively felt unpleasant increase of hair and scalp oiliness, ICHTHYOL® PALE exhibits anti-seborrheic properties. 7 out of 13 test persons (54%) with moderate or severe seborrhoea at the beginning of the study showed a decrease in the degree of seborrhoea after 6 weeks, whereas in only one case a slight increase was observed. For the remaining five persons no change was observed.

**E1.3.3 Comparison with Zinc Pyrithione [12]**

In another monitor-controlled study the efficacies of shampoos containing 1% ICHTHYOL® PALE and 1% micronized Zinc Pyrithione, respectively, were compared.

After the treatment phase a significant decrease in dandruff score could be observed in both groups. The results indicate that ICHTHYOL® PALE is slightly superior to the world-wide established anti-dandruff agent Zinc Pyrithione in reducing the amount of scales visible on scalp and hair. The average remaining dandruff score is 40.3% for the ICHTHYOL® PALE group and 43.6% for the Zinc Pyrithione group.

A clear superiority of the ICHTHYOL® PALE-containing shampoo could be demonstrated for the effectiveness in reducing itching.

The average itching was reduced to 31.4% of the initial value for the ICHTHYOL® PALE group, but only to 40.0% for the Zinc Pyrithione group.

The additional anti-seborrheic action that is missing with Zinc Pyrithione makes ICHTHYOL® PALE to a superior anti-dandruff agent: All concomitant symptoms appearing together with dandruff are influenced positively.

**E1.3.4 Comparison with Piroctone Olamine**

Due to its multifunctionality ICHTHYOL® PALE is also superior to Piroctone Olamine in dandruff treatment as could be shown by another comparison study [10]. If an optimal shampoo is defined as a treatment which completely eliminates dandruff and itching, in this study the ICHTHYOL® PALE shampoo achieved a total improvement rate of about 66%. By contrast, the Piroctone Olamine shampoo achieved a considerably lower value of about 57%. And ICHTHYOL® PALE offers the advantage of being antiseborrhoic.
E1.3.5 Comparison with Coal Tar

The ideal anti-dandruff agent should be a safe and well-tolerated substance with a broad action profile offering relief from dandruff and all concomitant symptoms. One anti-dandruff agent that offers a broad action profile as ICHTHYOL® PALE is Coal Tar. However, despite of undisputed curative potential, Coal Tar possesses carcinogenic properties without doubt. PAHs as main components of Coal Tar penetrate the skin even after short time exposure by means of a shampoo.

Therefore, allowing an uncontrolled use of Coal Tar on the skin as it is possible with OTC shampoos over long time periods does not seem to be reasonable. Therefore, in many countries Coal Tar has been forbidden.

A comparison study unequivocally demonstrates that Coal Tar can be replaced by ICHTHYOL® PALE in anti-dandruff shampoos [19]. According to the results, ICHTHYOL® PALE is as effective as Coal Tar but offers the advantage of being safe in long term treatment.

In this study a reduction of the average dandruff score by 86 and 83% could be observed in a treatment phase of 8 weeks for the ICHTHYOL® PALE and Coal Tar group, respectively. In the beginning, the average dandruff score could be evaluated as “severe” in both groups. After the treatment phase 14 out of 20 subjects were totally free from scales in the ICHTHYOL® PALE group, and 11 out of 20 subjects in the Coal Tar group.

In course of treatment the degree of itching was reduced continuously and nearly eliminated in both groups. In the end, 80% of subjects in the ICHTHYOL® PALE group and 75% of subjects in the Coal Tar group were free from itching symptoms.

E1.3.6 Combination with Ketoconazole

Recent studies have shown that ICHTHYOL® PALE is superior to well-established anti-dandruff agents as Zinc Pyrithione, Piroctone Olamine and Coal Tar because of its multifunctionality and good tolerance. Now it could be demonstrated that ICHTHYOL® PALE is able to add most valuable effects to the performance of highly potent ketoconazole [24].

In a study on 24 subjects suffering from moderate to severe dandruff a shampoo containing a combination 0.5% of ICHTHYOL® PALE and 0.5% ketoconazole was found to be more effective than one containing 1.0% of ketoconazole alone. With 71% improvement in itching and 75% improvement in skin redness after a treatment phase of just two weeks the combination product outpaced the ketoconazole shampoo considerably. The latter resulted in only 56% improvement in itching and no (0%) improvement in skin redness at all. Effects on scaling were similar.

The results are remarkable all the more as by addition of ICHTHYOL® PALE the concentration of ketoconazole could be decreased, i.e. better results were obtained with a lower concentration of the anti-fungal azole derivative.

As irritation and itching are reported as side effects for topical administration of ketoconazole and ICHTHYOL® PALE is known for its anti-irritating as well as anti-itching effects, the superiority of the combination product with reduced content of ketoconazole becomes clear.
Once again ICHTHYOL® PALE demonstrates a considerable advantage concerning the concomitant symptoms dandruff most often is accompanied by. If an optimal shampoo is defined as a treatment which completely eliminates dandruff, itching and skin redness, the ICHTHYOL® PALE shampoo achieved an impressive total improvement rate of 68% already after two short weeks of application.

In course of treatment scaling and concomitant symptoms are reduced further achieving an excellent total improvement rate of 82% after four weeks. On the other hand, ketoconazole alone does not exhibit this fast broad spectrum effect. Although its anti-scaling effect is comparable to the combination product, its influence on the concomitant symptoms is rather weak (total improvement rate of between 30 and 40% only in the first 4 weeks).

It can be concluded that by addition of ICHTHYOL® PALE to ketoconazole an exceptional anti-dandruff performance comes forward. Better and faster treatment results can be achieved at a reduced concentration of the azole derivative. Symptoms concomitant to dandruff are successfully countered by broad spectrum efficacy of ICHTHYOL® PALE. The overall tolerance is enhanced.

**E1.3.6.1 Experiences with a commercially available shampoo [25]**

In a prospective study 25 patients with seborrheic dermatitis of the scalp were treated for 28 days solely with a commercially available shampoo for treatment of oily dandruff containing ICHTHYOL® PALE in combination with Ketoconazole and Piroctone Olamine. There was no control group. Symptoms erythema, desquamation and pruritus (among others) were assessed by means of 4 point scales (0 = no symptoms, 3 = severe symptoms).

In the first two weeks erythema scores decreased from 1.76 to 0.20 (by 89%), desquamation from 2.24 to 0.44 (by 80%) and pruritus from 1.68 to 0.8 (by 52%). After 4 weeks all patients were virtually free from symptoms (final scores “0” for erythema and pruritus and “0.04” for desquamation. These results lasted during the entire follow-up period.

The effectiveness of the shampoo was valued by the dermatologist as excellent in 72% of the cases (24% good). Its tolerability was assessed to be “very well tolerated” in 92% of the cases (8% good).

The benefits in a combination shampoo are summarized in the following.

- Provision of valuable additional actions
- Exceptional anti-dandruff performance
- Faster and broader effects
- Symptoms concomitant to dandruff are successfully countered
- Better tolerance
- Reduction of original active ingredient concentration

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3 finished product from Spanish manufacturer
E2 ICHTHYOL® PALE in Treatment of Skin Blemishes

E2.1 OUTLINE OF SKIN BLEMISHES
Acne as well as dandruff may be accompanied with or caused by inflammations. However, the reasons for the development of acne or dandruff can be manifold.

As an example, in the following the characteristics of acne are given.

- Increase of sebum deposits (seborrhea) in people with sex hormones being out of balance in the puberty or suffering from mental stress
- Disturbed hornification in the hair follicles and excretory ducts of the oil glands leading to an accumulation of sebum and subsequently to the formation of comedones
- Microbial colonisation of oil glands and hair follicles

A plug of sebum in the opening of the sebaceous glands leads to formation of comedones. Open comedones are enlarged oil glands the opening of which is ‘sealed’ by a plug of scalings and sebum. Inflammatory efflorescences are formed when comedones are invaded by bacteria (propionibacterium acnes). Bacterial lipases split sebum triglycerides to form free fatty acids (R-COOH) with strong inflammatory potential. These fatty acids penetrate the surrounding tissue of the follicle and cause an inflammation which appears in shape of pimples and skin blemishes. Besides bacteria (staphylococcus aureus and propioni-bacterium acnes) also fungi (malassezia furfur) are found among the microorganisms present in persons suffering from acne.

E2.2 BENEFITS OF ICHTHYOL® PALE IN TREATMENT OF SKIN BLEMISHES
The complex profile of action needed for treatment of acne is offered by ICHTHYOL® PALE.

E2.2.1 Anti-inflammatory action
The anti-inflammatory action of ICHTHYOL® PALE could be demonstrated using a modified UVB erythema test [6]. 4% ICHTHYOL® PALE, 0.5% hydrocortisone and the base without active ingredient were compared. The test persons were irradiated with UVB light and afterwards they were treated with the test preparations. After 24 hours the skin colour of irradiated test areas was normalized by 31% using hydrocortisone, by 26% using ICHTHYOL® PALE and by only 8% using the Placebo. Since no significant difference between 0.5% hydrocortisone cream and the test preparation with 4% ICHTHYOL® PALE could be demonstrated, a pronounced anti-inflammatory effect can be confirmed for ICHTHYOL® PALE.

Substances with anti-inflammatory properties can suppress inflammations in different ways. This is done by suppressing the formation of enzymes that in turn catalyze the formation of inflammation mediators (e.g. Leukotriene B4, Prostaglandins, etc.).

By investigating the metabolism of 14C-arachidonic acid in vitro ICHTHYOL® substances were shown to inhibit 5-lipoxygenase activity in human polymorphonuclear neutrophils [16]. This results in a decreased release of the inflammation mediator Leukotriene B4 (LTB4).
Similar in vitro results were obtained in other studies. Inhibition of the release of chemotactic arachidonic acid metabolites (LTB₄) from human leukocytes, and inhibition of neutrophil chemotactic migration were both noted [17,18]. The inhibition is dose-dependent and occurs at non-cytotoxic concentrations of the substance. Furthermore, when applied to the ear skin of mice pre-treated with croton oil, ICHTHYOL® substances reduce the inflammatory reaction (edema). Since the edema is caused by the action of the inflammation mediator LTB₄ this result shows that ICHTHYOL® is able to inhibit the formation of LTB₄ in vivo as well. A summary of the available studies regarding anti-inflammatory properties of ICHTHYOL® PALE investigated on the cellular level is given in the following table.

**Table.** Anti-inflammatory action of ICHTHYOL® PALE on the cellular level.

<table>
<thead>
<tr>
<th>Target</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influence on the migration of leukocytes as well as on formation of inflammation mediators</td>
<td>Inhibits the migration of leucocytes and the formation of chemotactic arachidonic acid metabolites (LTB₄)</td>
</tr>
<tr>
<td>Inhibition of the enzyme 5-LOX</td>
<td>Concentration-dependent reduction of the formation of LTB₄</td>
</tr>
<tr>
<td>inhibition of different lipoxygenases and cyclooxygenases</td>
<td>concentration-dependent inhibition of all tested enzymes</td>
</tr>
<tr>
<td>release of the enzyme hexosaminidase from PMN by f-MLP</td>
<td>suppresses completely the action of tripeptide f-MLP</td>
</tr>
<tr>
<td>formation of active oxygen compounds and mobilisation of Ca²⁺ by LTB₄</td>
<td>Inhibition of the release of O₂⁻ and H₂O₂ as well as of Ca²⁺ mobilisation</td>
</tr>
</tbody>
</table>

E.2.2.2 Anti-seborrheic action

The anti-seborrheic action of ICHTHYOL® PALE was pointed out by skin lipid researcher Gloor [8]. By therapy with sulfonated shale oils the amount of skin surface lipids can be reduced in oral and topical administration [23,9]. In a study on the efficacy of a combination of chloramphenicol and ICHTHYOL® PALE used in the treatment of very severe acne (acne papulopustulosa) the anti-seborrheic action of ICHTHYOL® PALE could be elucidated unambiguously [9]. The quantitative determination of squalene, a lipid formed in the oil glands, served as a measure for the antiseborrheic efficacy. With respect to the reduction of squalene the combination of chloramphenicol and ICHTHYOL® PALE was far superior to the base as well as Chloramphenicol alone. The content of squalene could be decreased by more than 35% in presence of ICHTHYOL® PALE. However, no decrease could be observed with chloramphenicol and base alone.

E.2.2.3 Anti-microbial action

The anti-microbial action of ICHTHYOL® PALE against bacteria relevant in skin diseases was determined in studies of the minimum inhibitory concentration (MIC, see table). The most relevant bacterium in acne is Propionibacterium acnes. Here ICHTHYOL® PALE is especially effective with a MIC of only 0.039% [3].
Table: Minimal inhibitory concentration (MIC) of different bacteria following administration of ICHTHYOL® PALE

<table>
<thead>
<tr>
<th>Test specimens</th>
<th>ICHTHYOL® PALE [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td>0.039</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228</td>
<td>0.039</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 12344</td>
<td>0.039</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em> ATCC 11829</td>
<td>0.039</td>
</tr>
</tbody>
</table>

*a* incubation under anaerobic conditions

E2.3 CLINICAL STUDIES
Taking into account the anti-seborrheic, anti-microbial and anti-inflammatory action of ICHTHYOL® PALE (which means that ICHTHYOL® PALE is multifunctional) it is well understood why clinical trials turned out to be successful as well.

In a multicentric study in clinical practices on efficacy of a cream containing 1% of ICHTHYOL® PALE 101 test persons participated who suffered from mild to moderately severe acne. After a treatment period of six weeks the efficacy of the cream was judged as follows (fig. 3). In 76.2% of the cases the efficacy was evaluated as good or very good. No efficacy was found only in 5.9% of the subjects [14].

![Figure](image)

**Figure.** Judgement of efficacy of a cream containing 1% of ICHTHYOL® PALE in the treatment of mild to moderately severe acne (after [14]).

In a monocentric study in a dermatological practice on efficacy and tolerance of a cream containing 1% of ICHTHYOL® PALE and 0.5% of salicylic acid 20 test persons participated suffering from mild to moderately severe acne. In the treatment period of 8 weeks the average number of efflorescences could be reduced from 31.4 to 6.3 (see fig. 4). This is a decrease of about 80% [20].
In addition to the clinical efficacy the tolerance of ICHTHYOL® PALE was judged to be good to very good in both studies.

**Figure.** Reduction in the average number of efflorescences in a study on the efficacy of a cream containing 1% ICHTHYOL® PALE and 0.5% salicylic acid (after [20]).

The benefits of ICHTHYOL® PALE in the treatment of acne therefore can be summarized as follows.

**Benefits**

- anti-seborrhoic
- anti-inflammatory
- anti-microbial
- well tolerated and safe
- natural origin
F. Toxicological information on ICHTHYOL® PALE

ICHTHYOL® PALE is thoroughly investigated with respect to toxicology. The studies carried out were summarized by Cholcha et al. [1]. The application of ICHTHYOL® PALE gives no hint of any toxic effect and is well tolerated both for short-term and long-term administration. The following table gives an overview of currently available studies including most recent ones (indicated by *) that are not considered in the above mentioned article.

All animal studies cited were conducted one decade or more ago when there were no alternative methods available. However, there was request for proof of safety and tolerance from the authorities that had to be complied with. As manufacturer of ICHTHYOL® PALE we have not commissioned or undertaken any animal testing for this raw material relating to development and safety evaluation or any other purpose after September 11th 2004. In accordance with the current Cosmetic Directive 76/768/EEC (7th Amendment) it is not intended to commission or perform any animal testing in future.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Doses</th>
<th>Application</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Toxicity</td>
<td>Rat</td>
<td>17,800; 21,500 mg/kg BW</td>
<td>Topical</td>
<td>No signs of poisoning</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>7,900-10,000 mg/kg BW</td>
<td>Oral</td>
<td>No signs of poisoning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LD₅₀ &gt;10,000 mg/kg BW</td>
</tr>
<tr>
<td>Chronic toxicity</td>
<td>Rat</td>
<td>330; 1000; 3000 mg/kg BW daily for 6 months</td>
<td>Oral</td>
<td>No substance-related changes</td>
</tr>
<tr>
<td>Fertility</td>
<td>Rat</td>
<td>100; 500; 2500 mg/kg BW daily, 10 weeks before breeding tests</td>
<td>Oral</td>
<td>No-substance related changes</td>
</tr>
<tr>
<td>Teratogenicity</td>
<td>Rat</td>
<td>1000 mg/kg BW daily 6-15 day of pregnancy</td>
<td>Oral</td>
<td>No teratogenic changes</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>100; 400; 1,600 mg/kg BW daily, 6-18 day of pregnancy</td>
<td>Oral</td>
<td>Not teratogenic changes</td>
</tr>
<tr>
<td>Cancerogenicity</td>
<td>Rat</td>
<td>330; 1000; 3000 mg/kg BW daily for 24 months</td>
<td>Oral</td>
<td>No substance-related changes</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>330; 1000; 3000 mg/kg BW daily for 21 months</td>
<td>Oral</td>
<td>No substance-related changes</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- AMES test</td>
<td>Salmonella typhimurium</td>
<td>0.316 – 10,000 µg/plate</td>
<td>Plate</td>
<td>No indication of mutagenic characteristics</td>
</tr>
<tr>
<td>- HGPRT test</td>
<td>V79-cells (hamster)</td>
<td>0.3-3,000 µg/ml medium</td>
<td>In vitro</td>
<td>No indication of mutagenic characteristics</td>
</tr>
<tr>
<td>- Chromosome analysis</td>
<td>Hamster</td>
<td>2,500; 5,000; 10,000 mg/kg BW (single application)</td>
<td>Oral</td>
<td>No indication of mutagenic characteristics</td>
</tr>
</tbody>
</table>
### Study, Species, Doses, Application, Results

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Doses</th>
<th>Application</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin compatibility</td>
<td>Rabbit</td>
<td>10; 100; 1000 mg/kg BW daily for 21 days</td>
<td>Topical</td>
<td>10; 100 mg/kg BW well tolerated; 1000 mg/kg BW DRAIZE grade 1-2</td>
</tr>
<tr>
<td>Skin sensitization</td>
<td>Guinea pig (Buehler test)*</td>
<td>25-100%</td>
<td>Topical</td>
<td>No delayed contact hypersensitivity</td>
</tr>
<tr>
<td>Mucosal Compatibility</td>
<td>Rabbit*</td>
<td>Aqueous solution of 5% (single application)</td>
<td>Eye</td>
<td>No changes</td>
</tr>
<tr>
<td>Phototoxicity</td>
<td>Human Skin Model Test (EpiDerm)*</td>
<td>0.0316-3.16%</td>
<td>In vitro</td>
<td>Non-phototoxic</td>
</tr>
<tr>
<td></td>
<td>Human patch test*</td>
<td>5; 10%</td>
<td>Topical</td>
<td>Non-phototoxic</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>3%; 5% in petrolatum, 96h, UVA and UVB irradiation</td>
<td>Topical</td>
<td>Non-phototoxic</td>
</tr>
<tr>
<td>Photosensitization</td>
<td>Guinea pig</td>
<td>1% aqueous solution daily for 5 days UV irradiation</td>
<td>Topical</td>
<td>No photosensitive characteristics</td>
</tr>
</tbody>
</table>

The safety of the raw material is best illustrated by calculating the safety factor. A safety factor of at least 100 is generally recognized for substances which are subject to the German Federal Food Law, meaning that there is an interval of a factor of 100 between the no-effect-level (NOEL) found in animal experiments, and the respective quantity of substance which, when applied as intended and applied correctly, under the most unfavourable conditions can have an effect on humans or will be absorbed by the body. For application in a shampoo a safety factor of 230769 (required > 100) is calculated. ICHTHYOL® PALE, therefore, can be classified as a safe active substance in accordance with the new EC-Directive for cosmetics [13].
G. Literature


[22] Grimm V., Engst R., Seidl P., Ring J., Untersuchungen zur antimikrobiellen Wirksamkeit von Natriumbituminosulfonat (hell), Poster No. 201, Congress of the German dermatological society (DDG), Berlin, 1 – 5 May; 2001


[24] Gayko, G., Warnecke, J., Zelenkova, H., The use of a pale type of Ichthyol® in cosmetic dermatology - Cosmetic, single-centre, controlled and randomized double blind study for proof of efficacy and tolerance of a combination of Ichthyol Pale 0.5% and Ketoconazole 0.5% vs. Ketoconazole 1.0% shampoo in treatment of moderate to severe dandruff, Clinical Dermatology 8 (4), (2006) 243 - 247


Literature available on request from ICHTHYOL-Gesellschaft