
Scientific Committee on Consumer Safety

SCCS

# OPINION ON

# Trimethylbenzoyl diphenylphosphine oxide (TPO)

The SCCS adopted this opinion at its 5th plenary meeting

of 27 March 2014

About the Scientific Committees

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

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

SCCS

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

Scientific Committee members



Contact
European Commission
Health & Consumers
Directorate C: Public Health
Unit C2 – Health Information (Scientific Committees' Secretariat)
Office: HTC 03/073
L-2920 Luxembourg
SANCO-C2-SCCS@ec.europa.eu



ISSN 1831-4767 ISBN 978-92-79-35650-6
Doi 10.2772/45370 ND-AQ-14-003-EN-N

## ACKNOWLEDGMENTS

SCCS Members

Dr. U. Bernauer
Prof. P.J. Coenraads
Prof. G. Degen
Dr. M. Dusinska
Prof. D. Gawkrodger
Dr. W. Lilienblum (rapporteur)
Prof. A. Luch
Dr. E. Nielsen
Prof. Th. Platzek
Dr. Ch. Rousselle
Dr. S. Ch. Rastogi (chairman)
Dr. J. van Benthem

External experts

Prof. V. Rogiers
Prof. T. Sanner
Dr. I.R. White

Keywords: SCCS, scientific opinion, cosmetic ingredients, Trimethylbenzoyl diphenylphosphine oxide (TPO), Regulation 1223/2009, CAS 75980-60-8 , EC 278-355-8

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of Trimethylbenzoyl diphenylphosphine oxide (TPO) in cosmetic products, submission I, 27 March 2014, SCCS/1528/14.

## TABLE OF CONTENTS

## 1. BACKGROUND

Trimethylbenzoyl diphenylphosphine oxide (TPO) CAS 75980-60-8 is used in limited concentration for nail modelling products. This ingredient is not regulated under Cosmetic Regulation n. 1223/2009.

Trimethylbenzoyl diphenylphosphine oxide (TPO, CAS 75980-60-8) was classified as CMR2 (fertility) according to the Commission Regulation (EU) No. 618/2012 of 10 July 2012 amending for the purposes of its adaptation to technical and scientific progress the Regulation (EC) No. 1272/2008. As a consequence, TPO would be prohibited as a cosmetic ingredient by December 2013 according to Regulation (EC) No. 1223/2009 of the European parliament and the Council of 30 November 2009 on cosmetic products.

However, Article 15 of the Regulation (EC) No. 1223/2009 (recast of the so-called Cosmetics Directive) states that '*The use in cosmetic products of substances classified as C MR substances, of category 2, under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited. However, a substance classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products. To these ends the Commission shall adopt the necessary measures in accordance with the regulatory procedure with scrutiny referred to in Article 32(3) of this Regulation*'.

In September 2013 the Commission received a dossier by Cosmetics Europe on the safety assessment of Trimethylbenzoyl diphenylphosphine oxide (TPO). This submission is intended to demonstrate the safety of the ingredient when used as a key processing aid in topically applied artificial nail systems.

## 2. TERMS OF REFERENCE

*1. In view of the above, and taking into account the scientific data provided, SCCS is requested to give its opinion on the safety of Trimethylbenzoyl diphenylphosphine oxide (TPO) when used as a nail modelling product at a concentration of at maximum 5.0%.*

*2. SCCS is requested to address any further scientific concerns with regard to the use of TrimethylbenzoyI diphenylphosphine oxide (TPO) in cosmetic products.*

# 3. OPINION

## 3.1. Chemical and Physical Specifications

### 3.1.1. Chemical identity

#### 3.1.1.1. Primary name and/or INCI name

INCI name: Trimethylbenzoyl diphenylphosphine oxide

#### 3.1.1.2. Chemical names / Synonyms

Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide

#### 3.1.1.3. Trade names and abbreviations

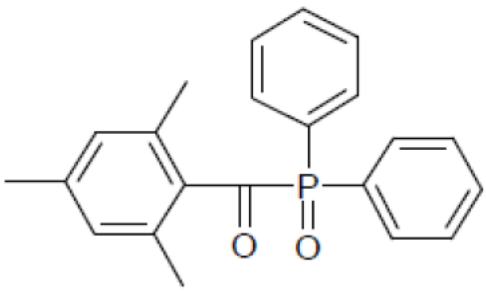
TPO
Irgacure® TPO (old Lucirin® TPO),
Chivacure TPO
Darocur TPO
Darocure TPO
Genocure TPO
Initiator 554
Irgacure TPO
Lucirin LR 8953
Lucirin LR 8728
Lucirin TPO solid
Photocure TPO
Speedcure TPO

Ref.: 15, 20

#### 3.1.1.4. CAS / EC number

CAS: 75980-60-8
EC: 278-355-8

#### 3.1.1.5. Structural formula




Ref 20

#### 3.1.1.6. Empirical formula

$C_{22}H_{21}O_2P$

Ref 20

### 3.1.2 Physical form

Solid (yellow powder)

Ref. 15, 20

### 3.1.3 Molecular weight

348.4 g/mol

### 3.1.4 Purity, composition and substance codes

Purity according to specification: ≥97% (UV-VIS, DIN 55 978, predominantly ≥99%)
Purity in the ECHA documentation: 99.3%.
Purity of batch 110053 investigated in toxicological studies: 99.5 g/100 g (NMR).

Ref. 11, 15, 20

### 3.1.5 Impurities / accompanying contaminants

Impurities: no data available

### 3.1.6 Solubility

Water solubility: 3.3 mg/L at 20 °C (OECD Guideline 105, Flask method)
11.9 mg/L at 20 °C (OECD Guideline 105, Column elution)

Ref. 11

Readily soluble in dichloromethane, acetone, n-butylacetate; soluble in styrene and in other monomers

Ref. 8, 9

### 3.1.7. Partition coefficient (Log $P_{ow}$)

Log Pow: 3.1 at 23 °C and pH = 6.4 (OECD Guideline 117)
Calculated: 3.87 (EPIWIN)

Ref. 11

### 3.1.8. Additional physical and chemical specifications

| | |
|---|---|
| Melting point: | 93 °C (OECD Guideline 102) |
| Boiling point: | >300 °C Ref. 14 |
| Flash point: | / |
| Vapour pressure: | 3.045 x $10^{-6}$ Pa at 25 °C |
| Density: | D4=1.218 g/cm³ at 20 °C (OECD 109) |
| Viscosity: | / |
| Particle size distribution: | <100 µm: 40.2 %; <10 µm: 0.2 % <4 µm: 0.0 % (ISO 13320-1, laser diffraction (Mastersizer 2000)) |
| pKa: | / |
| Refractive index: | / |
| pH: | 4-5 (Ref. 24) |
| UV_Vis spectrum : | λmax 235, 290, 385 nm (Ref. 8, 22, 23) |

Other references: 11, 20

### 3.1.9. Homogeneity and Stability

Shelf life: at least 2 years (cool and dry; light sensitive, to be stored in the dark)

Ref. 8, 9 11

## 3.2. Function and uses

Irgacure® TPO is a photo-initiator for the UV curing of unsaturated polyesters and resins containing acrylic ester groups. Because of its absorption behavior in the long-wave range of the UV spectrum, it is used to cure pigmented UV curable coatings, as well as UV stabilized coatings.
Irgacure® TPO is also widely used because it can photo-bleach resulting in low yellowing coatings, which is particularly needed for white pigmented coatings and printing inks, such as silk-screen printing (Ref. 16).

Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (TPO) is used as a key processing aid in form of a chemical photo-initiator for polymerisation in artificial nail systems, primarily in UV-curable one-component gel systems. TPO is a Norrish Type 1 or alpha-cleavage photo-initiator. This means that TPO splits into two free radical fragments, which subsequently become incorporated into the polymer as chain ends. Therefore, TPO will be consumed rapidly during the polymerisation process and even in the unlikely event that very minor residual amounts remain, they will be trapped in the rapidly hardened polymer matrix of the formed nail coating.
The current and anticipated use concentrations in the gels are in the range between 0.5% - 5.0%.
In principle, two major artificial nail systems are used for fingernails and toenails.
One use is for nail polishes (base, middle and top coats). For polish usage, the nails are cleaned thoroughly and the surface is processed to be sufficiently prepared for the application of the gel. A brush is wetted, dipped into the gel with the initiator and then applied to the centre of the nail plate and shaped. The polymerisation is completed in about 2 – 3 minutes. This procedure can be repeated up to 2 – 3 times. After each application, the nails are cured under a UV lamp. Contact is to the keratin of the nail plate. Careful application to the nail plate should not allow contact with skin. However, without due care, there could be accidental skin contact at the cuticle and the side of the nails. Frequency of use is one complete application every 2-3 weeks. Old gel is removed and the nail cleaned before new application.
The other use is for nail enhancement products. For this use, the artificial nail systems are applied to the nail plate every 2 – 3 weeks with a refill application after 1 - 2 weeks. Full application is in the range of between 2 – 4 g of gel and 1 g of gel for the refill, corresponding to a maximum of 200 mg TPO in total for all nail plates.

Ref. 22, 25

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

#### 3.3.1.1. Acute oral toxicity

**1)**

| | |
|---|---|
| Guideline/method: | OECD 401 |
| Species/strain: | Rat/Sprague-Dawley CFY (5 to 8 weeks old) |
| Group size: | 5 rats/sex |
| Test substance: | Lucirin LR 8728 (TPO) |
| Batch: | 010213 (purity: 99%) |
| Dose levels: | 5000 mg/kg bw |
| Vehicle: | Arachis oil (B. P.) |
| Application volume: | 10 mL/kg bw |

| | |
|---|---|
| Route: | Oral (gavage) |
| Exposure: | Single application |
| Observation period: | 14 days |
| GLP: | Yes |
| Study period: | Feb - March 1989 |
| Date of report: | July 1989 |
| Published: | No |

The test substance was administered by oral administration to each of 5 fastened Sprague-Dawley rats per sex at a single dose level of 5000 mg/kg bw. The test substance was applied in arachis oil (BP) at an application volume of 10 mL/kg bw. The animals were observed for treatment-related effects for a 14-day observation period. The body weight was determined prior to dosing, weekly thereafter and at termination. After the observation period had been completed, animals were sacrificed and gross pathological examination was conducted.

Results
No mortality occurred. Clinical signs recorded during the day of dosing consisted of hunched posture, lethargy, pilo-erection and decreased respiratory rate. All animals appeared normal one day after treatment. All animals showed expected gain in bodyweight over the study period. No abnormalities were noted at necropsy.

Conclusion
Based on this study, the acute oral toxicity (LD50) was >5000 mg/kg bw for male and female Wistar rats.

Ref. 31

**2)**
The acute oral toxicity was investigated in yet another acute oral toxicity study in rats. Five male and five female Sprague-Dawley rats were fasted for 15 – 20 h prior to administration. The animals received the test substance (Initiator 554, as a 0.5% aqueous carboxymethyl cellulose (CMC) suspension) at dose levels of 1000 and 5000 mg/kg bw as a single application by oral gavage. The post treatment observation period was 14 days. There was no mortality, no clinical and no necropsy findings. Based on this study, the acute oral toxicity (LD50) was >5000 mg/kg bw for male and female Sprague-Dawley rats.

Ref. 2

**SCCS comment**
The report of 1984 is a translation from German to English of the original study report (study conducted June 1979) which is not available. The characterization of the test substance is described in the raw data of the original study report and not available.

### 3.3.1.2. Acute dermal toxicity

| | |
|---|---|
| Guideline/method: | OECD 402 |
| Species/strain: | Rat/Wistar |
| Group size: | 5 rats/sex |
| Test substance: | Lucirin TPO |
| Batch: | 110053 (purity: 99.5 g/100 g ([1H]-NMR)) |
| Dose levels: | 2000 mg/kg bw |
| Application area: | ≥10% of total body surface |
| Vehicle: | Olive oil (Ph. Eur.) |
| Application volume: | 5 mL/kg bw |
| Route: | Dermal (semi-occlusive) |
| Exposure: | Single application |
| Observation | period: 14 days |

| | |
|---|---|
| GLP: | Yes |
| Study period: | September 2011 |
| Date of report: | October 2011 |
| Published: | No |

The test substance was tested for its acute dermal toxicity in a limit test in 5 male and 5 female Wistar rats. The animals were dermally exposed to a single dose of 2000 mg/kg bw of the test substance suspended in olive oil (Ph. Eur.) to the clipped skin (dorsal and dorso-lateral parts of the trunk) and covered by semi-occlusive dressing for 24 hours. The application area comprised about 40 $cm^2$, i.e. at least 10% of the total body surface area. Body weights were determined shortly before administration (day 0), weekly thereafter and on the last day of observation. Clinical signs were recorded on the day of administration, and at least once daily thereafter. The skin was examined and the findings were scored according to Draize (1959), 30 – 60 minutes after removal of the semi-occlusive dressing (day 1) and at regular intervals thereafter until the last day of observation. After the observation period had been completed, animals were sacrificed and gross pathological examination was conducted.
Samples of the test substance were sent to the sponsor in order to confirm identity, stability, homogeneity and concentration of the test substance in the test suspension. The analytical report is not a part of the GLP study.

Results
No mortality occurred and no signs of systemic toxicity were observed. The dermal application led to slight to moderate erythema (grade 1 – 3), incrustations and scaling in individual animals. The body weight gain was not affected. No abnormalities were noted at necropsy.

Conclusion
The acute dermal toxicity (LD50) was >2000 mg/kg bw for male and female Wistar rats.

Ref. 10

#### 3.3.1.3. Acute inhalation toxicity

No data available.

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1. Skin irritation

| | |
|---|---|
| Guideline/method: | Federal Register 38, No 187, § 1500.41, p. 27029, 27 Sept 1973 |
| Species/strain: | Rabbit/White Vienna (Gaulker) |
| Group size: | 6 animals (2 males, 4 females) |
| Test substance: | Initiator 554 |
| Batch: | / |
| Purity: | / |
| Dose level: | 0.5 g of test substance as a 50% aqueous formulation |
| Route: | intact shaved and abraded skin (2.5 x 2.5 cm) |
| Duration: | / |
| Skin readings: | 24, 48 hours, day 8 |
| Observation period: | 8 days |
| GLP: | / |
| Study period: | May 1979 |
| Date of report: | 1979 / 1984 (see comment) |
| Published: | No |

The skin irritation potential of Initiator 554 was investigated in White Vienna rabbits. An amount of 0.5 g of the test substance as a 50% aqueous formulation was applied to the

shaved skin or to the shaved and abraded skin of 3 rabbits each on an area of 2.5 x 2.5 cm. It is not stated whether there was occlusion and the duration of application is not explicitly stated but assumed to be 24 hours as this was the first observation point.
The animals were observed for signs of skin irritation (erythema, oedema) at 24 and 48 h and on day 8.
Results
On the intact skin, erythema was observed in all rabbits at 24 hours together with oedema in 2 rabbits; all reactions had resolved by the 38-hour reading.
On the abraded skin, erythema was observed in all animals at 24 hours together with oedema in 4 rabbits; the erythema persisted to the 48-hour reading in 2 rabbits.

Conclusion
Under the conditions of the study, Initiator 554 was evaluated as slightly irritating to the skin of rabbits.

Ref. 3

**SCCS comment**
The above report is a 1984 translation of an original study report in German. Experimental detail is lacking.
Initiator 554 has irritant potential to rabbit skin under the conditions of the experiment.

### 3.3.2.2. Mucous membrane irritation

| | |
|---|---|
| Guideline/method: | Federal Register 38, No 187, §1500.42, p27019, 27 Sept 1973 |
| Species/strain: | Rabbit/White Vienna (Gaulker) |
| Group size: | 6 animals (2 males, 4 females) |
| Test substance: | Initiator 554 |
| Batch: | / |
| Purity: | / |
| Dose level: | 0.1 mL (56 mg) neat substance |
| Route: | instillation into the conjunctival sac |
| Readings: | 24, 48, 72 hours |
| Observation period: | 72 hours |
| GLP: | / |
| Study period: | May 1979 |
| Date of report: | 1979 / 1984 (see comment) |
| Published: | No |

The potential irritant effect of Initiator 554 to the eyes was investigated by instillation of 0.1 mL (56 mg) of the neat test substance into the conjunctival sac of one eye of each animal. The eyes were examined at 24, 48 and 72 h and the effects on the cornea, iris and conjunctivae (reddening, swelling, discharge) were scored.

Results
No effects on the iris were noted. At 24 hours, conjunctival redness was observed in all animals and this persisted to the 48-hour reading in 2 animals. In 2 animals there was some corneal opacity by the 72-hour reading.

Conclusion
Under the conditions of the study, Initiator 554 showed irritant effects to the eyes of the rabbits.

Ref. 4

**SCCS comment**
The above report is a 1984 translation of an original study report in German. Experimental detail is lacking.

Initiator 554 showed irritant effects to the eyes of all animals. There was a conjunctival erythema present in all animals and some corneal opacity present at the 72-hour reading in 2 animals. The latter is not referred to in the report or the sponsor's submission.

### 3.3.3. Skin sensitisation

**Local Lymph Node Assay (LLNA)**

| | |
|---|---|
| Guideline/method: | OECD 429 |
| Species/strain: | Mouse/CBA/CaOlaHsd |
| Group size: | 5 females per group (20 in total) |
| Test substance: | Lucirin TPO |
| Batch: | 110053 |
| Purity: | 99.5% |
| Vehicle: | Acetone:olive oil (AOO, 4:1) |
| Concentrations: | 0, 10, 25, 50% (highest achievable) |
| Route: | Epidermal (topical) application on the surface of the dorsal ear lobe |
| Positive control: | alpha hexyl cinnamaldehyde (historical control, August 2011) |
| GLP: | Yes |
| Study period: | October 2011 |
| Date of report: | 2012 |
| Published: | No |

The sensitizing potential of Lucirin TPO was tested in the murine local lymph node assay. Groups of 5 female CBA/ CBA/CaOlaHsd mice received daily topical applications on the dorsal surface of both ears with 10%, 25% and 50% (w/v) TPO in 4:1 acetone:olive oil for three consecutive days. Control groups were treated with the vehicle alone. On day 6, the mice were intravenously injected in the tail vein with 20.1 µCi of radiolabelled [3H]-thymidine in phosphate-buffered saline (PBS). The mice were killed by i.p. injection of pentobarbital sodium 5 hours later, and the draining auricular lymph nodes were removed, weighed and pooled for each animal. A single cell suspension was prepared by pooling the lymph nodes from each group. After washing twice with PBS, cells were re-suspended and precipitated overnight at 4 °C with 5% trichloroacetic acid. A β-scintillation counter was used to count radioactivity in the pellets, and increases in [3H]-thymidine incorporation relative to control groups. The β-scintillation counter expressed [3H]-thymidine incorporation as the number of radioactive disintegrations per minute (DPM). The Stimulation Index (SI) values were calculated for each dose level based on pooled lymph nodes, and an SI of 3 or more was considered a positive response. Linear interpolation of the dose response data from each LLNA was used to derive an estimated concentration (EC3) required to elicit a SI value of 3. The EC3 value was then taken as a measure of relative potency.

Results

Table: Local lymph node assay results

| Concentration (%) | Mean lymph node weight (mg) | Mean lymph node cell count x10E06 | DPM/animal | Stimulation index | $EC_3$ |
|---|---|---|---|---|---|
| AOO (4:1) control | 4.81 | 7.44 | 792.1 | 1.00 | |
| 10 | 6.13* | 9.85* | 1757.5* | 2.22 | |
| 25 | 7.23* | 14.19* | 2347.1* | 2.96 | |
| 50 | 7.33* | 13.58* | 2742.3* | 3.46 | 27.0% |
| * statistically significant increase vs. control group ($p<0.05$, ANOVA and Dunnett test) | | | | | |

Conclusion
Under the conditions of this local lymph node assay, Lucirin TPO showed a potential to

induce dermal sensitization in mice with an EC3 value of 27.0%

Ref. 12

**SCCS comment**
Lucirin TPO has an EC3 of 27% indicating a moderate sensitising potential (see ref. AR 2.).

### 3.3.4. Dermal / percutaneous absorption

No data available. See 3.3.12, Special Investigations.

### 3.3.5. Repeated dose toxicity

#### 3.3.5.1. Repeated Dose (28 days) oral toxicity

**1)**

| | |
|---|---|
| Guideline/method: | Japanese MHW Guidelines 1986 for Testing of Chemicals, |
| Species/strain: | Rat/Sprague-Dawley (CFY) |
| Group size: | Main group: 5 male and 5 female rats per group |
| Recovery group: | 5 animals per sex for high dose and control group |
| Test substance: | Lucirin LR 8728 (TPO) |
| Batch: | 010213 (purity: 99%) |
| Dose levels: | 0, 50, 250, 750 mg/kg bw/day |
| Vehicle: | Arachis oil (B.P.) |
| Dose volume: | 4 mL/kg bw |
| Route: | Oral (gavage) |
| Exposure period: | 28 days |
| Exposure frequency: | once daily for 7 consecutive days/week |
| Recovery period: | 14 day (control and high dose) |
| GLP: | Yes |
| Study period: | April-May 1989 |
| Date of report: | September 1989 |
| Published: | No |

The subacute toxicity of TPO (batch: 010213) was examined in male and female Sprague-Dawley rats after repeated oral (gavage) administration for 28 consecutive days.
Prior to the main study, a 14-day range-finding study was conducted to determine the dose levels for the definitive 28-day repeated dose toxicity study. The test substance was administered by gavage to Sprague-Dawley rats (3/sex/dose) for 14 consecutive days, at dose levels of 0, 50, 250, 500, 750 and 1000 mg/kg bw/day in arachis oil (B. P.). The highest dose was lethal and adverse effects in form of clinical symptoms and reduced body weight gains were noted at 500 and 750 mg/kg bw/day. Minor clinical signs were observed at 250 mg/kg bw/day. The low dose of 50 mg/kg bw/day was the No observed adverse effect level in this range finding study. Consequently, dose levels of 0, 50, 250 and 750 mg/kg bw/day were selected for the main study.
In the main study, dose levels of 50, 250 and 750 mg/kg bw/day were administered orally by gavage to groups each made up of five male and five female Sprague-Dawley rats for twenty-eight consecutive days. A control group of five males and five females was dosed with the vehicle arachis oil (B. P.) alone. Recovery groups, each made up of five males and five females, were treated with the high dose (750 mg/kg bw/day) or the vehicle alone for 28 consecutive days and then were maintained without treatment for a further 14-day period. Analytical verification of concentrations and stability of the test substance in the formulation was performed by UV/vis spectroscopy during the study. Clinical signs, body weight/body weight gain, food and water consumption were monitored during the study. Haematology, blood chemistry and urinalysis were evaluated for all non-recovery animals at the end of the treatment period and for all recovery group animals at the end of the

treatment-free period. At termination, the animals were sacrificed and necropsy was performed. The weights of adrenals, brain, gonads (testes, epididymides, ovaries), heart, kidneys, liver, pituitary and spleen were measured. A comprehensive histopathological examination was done in these organs. Statistical analysis was performed by means of Kruskal Wallis for non-parametric analysis and by Mann Whitney U-test for parametric analysis.

Results
One female from the satellite high dose group was found dead on day four and one female from the satellite control group died during blood sampling on day forty-two. A dose-related impaired health status was noted in animals at 250 and 750 mg/kg bw/day including hunched posture, increased salivation, lethargy and piloerection.
Towards the end of treatment, the body weight gains were reduced in animals treated with 250 and 750 mg/kg bw/day, body weight losses were noted in several high dose females. During the treatment-free period the body weight gain recovered.
Food and water consumption in test animals was not affected, but food efficiency was reduced in animals treated with 250 and 750 mg/kg bw/day.
The liver and kidney weights were increased at 250 and 750 mg/kg bw/day associated with macroscopically enlarged livers at the high dose level. The male animals revealed small testes by gross pathology.
Blood chemistry and urinalysis showed a number of abnormalities, which can be considered as indicative for kidney and liver impairment.
In particular, there were increases in bilirubin, triglycerides, cholesterol, gamma glutamyl transpeptidase and alkaline phosphatase, creatinine and urea in the plasma and presence of ketones in the urine.
Histopathology showed periportal vacuolation in the liver and basophilia and dilatation of tubules in the kidneys in both sexes and testicular atrophy in males at the high dose level. Most observed treatment-related findings recovered during the treatment free period but testicular atrophy and hepatomegaly were persistent.

Conclusion
The subacute oral (gavage) administration of TPO to male and female Sprague-Dawley rats on 7 consecutive days per week for 28 days at dose levels of 0, 50, 250 and 750 mg/kg bw/day led to toxicologically relevant effects in both sexes at 250 and 750 mg/kg bw/day in the form of impaired general state of health, reduced body weight parameters and functional as well as morphological findings indicative for kidney and liver damage. In addition, the males at 750 mg/kg bw/day showed testicular atrophy. With the exception of the testes and liver finding, most of the findings improved or disappeared during the recovery period.
Thus, the No Observed Adverse Effect Level (NOAEL) for male and female Sprague-Dawley rats was 50 mg/kg bw/day.

Ref. 32

**SCCS comment**
No analytical evidence for the purity of the test substance was provided in the study report.

**2)**
A subsequently performed oral (gavage) 28-day repeated dose toxicity study (non-GLP, conducted in March-April 2000, study report Jan 2001) in another strain of male rats, designed specifically to investigate testicular effects, did not confirm the above findings. Three male Wistar rats received another batch of TPO with higher purity (batch: 99-0029, purity: 99.3%) suspended in 0.5% aqueous carboxy methyl cellulose (CMC) orally by gavage for 28 consecutive days at a dose level of 1000 mg/kg bw/day A group of 3 males was treated with the solvent as control. The animals were observed for mortality and clinical findings and at termination the animals were necropsied, body weight and testes weight were determined and the testes were examined histopathologically. The male animals

treated at the dose of 1000 mg/kg bw/day did not show any clinical findings and especially not testicular atrophy.

Ref. 7

**SCCS comment**
No analytical evidence of the purity of the test substance, its content and stability in test formulations was provided in the study report.

### 3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

**1)**
A 90-day repeated dose toxicity study (non-GLP, conducted in March-June 2000, study report Jan 2001) was carried out with male Wistar rats to investigate the effects on the testes with another batch of Lucirin LR 8728 (TPO) with higher purity (batch: 99-0029, purity: 99.3%). Each of 10 male animals received dose levels of 0 and 1000 mg/kg bw/day of the test substance in carboxymethyl cellulose (0.5% aqueous solution) once daily by oral gavage. The rats were regularly observed for mortality and clinical signs of toxicity. At the end of the test period, the rats were sacrificed and subjected to necropsy. Weights were determined for the whole body and testes. Gross lesions as well as the testes were processed further for histological examination.

Results
The body weights were reduced by 10% compared to controls. Also, the relative and absolute testes weights were reduced. A reduced size of the testes in 8 out of 10 rats was noted at necropsy (mean testes weight 72% of controls). Histopathology at 1000 mg/kg bw/day revealed a slight to severe diffuse atrophy of the seminiferous tubules of the testes with gradings of 2 (slight) up to 4 (severe) in all animals. In 4 test animals, oedemas and minimal to slight hyperplasia of the Leydig cells were noted. All 8 epididymides with a reduced organ size were examined histopathologically and correlated with an oligozoospermia up to grade 5 (azoospermia).

Conclusion
The testicular effects noted in the 28-day oral study performed in Sprague-Dawley rats (ref. 32) were confirmed in this additional study.

Ref. 7

**SCCS comment**
No analytical evidence of the purity of the test substance, its content and stability in test formulations was provided in the study report.

**2)**

| | |
|---|---|
| Guideline/method: | EPA-TSCA Test Guideline "Functional Observational Battery" and EPATSCA Guideline "Neuropathology" comparable to OECD guideline 408 |
| Species/strain: | Rat/Wistar |
| Group size: | 10 male and 10 female rats per group |
| Test substance: | Lucirin LR 8728(TPO) |
| Batch: | 49/0193 (purity: 94.8%) |
| Dose levels: | 0, 100, 300, 1000 mg/kg bw/day |
| Vehicle: | 0.5% aqueous carboxy methyl cellulose (CMC) |
| Application volume: | 10 mL/kg bw |
| Route: | Oral (gavage) |
| Exposure period: | 90/91 days |
| Exposure frequency: | once daily |
| GLP: | Yes |
| Study period: | Oct 1987 - Jan 1988 |
| Date of report: | 1991 |

Published: No

The subchronic toxicity of TPO (batch: 49/0193), suspended in 0.5% CMC, was examined in male and female Wistar rats after repeated oral (gavage) administration for 90/91 consecutive days at dose levels of 0, 100, 300 and 1000 mg/kg bw/day.
Clinical signs, body weight/body weight gain and food consumption were monitored during the study. Additionally, specific neurofunctional tests were performed on day 3 prior to start, on 1, 6 and 24 hours following the first dose and on days 7, 14, 42, 63 and 91. Blood sampling for haematology and clinical chemistry was performed on days 33 and 87. At termination, 3 animals per dose group and sex were taken for perfusion fixation and further processed to perform a sensitive neuro-histopathology, while the remaining surviving animals were taken for routine immersion fixation. All animals were assessed by gross pathology and in the group for immersion fixation, the weight of the anesthetized animals as well as of liver, kidneys, adrenals, testes and fixed brain was determined. Based on the clinical results, the scope of histopathological and neuropathological examinations was changed and the skin of the auricles of all animals and of the paw region as well as liver, kidneys, spleen and testes were examined histopathologically. Due to the complete absence of neurotoxic signs in concomitance with life threatening toxicity, specific neuropathological examinations were not carried out but routine sections of brain, medulla oblongata, spinal cord, and gastrocnemius muscle were examined histopathologically in the high dose and control animals.
Statistical analysis was performed by means of the analysis of variance (ANOVA) and Dunnett's test.

Results
The chemical analyses confirmed the stability, the homogeneity and the correctness of TPO concentrations in the vehicle. However, concentrations for all doses were reduced by about 10% at the end of the study but still in the range of the analytical variability (UV/vis spectroscopy).
Two females of the 1000 mg/kg bw/day group died during the experimental period. An increase in feed consumption (max .21%) was found in the female rats of the 1000 mg/kg group throughout the conduct of the study, while feed consumption of the male rats remained uninfluenced. The feed consumption of the animals of both sexes of the 300- and 100 mg/kg group corresponded to the control values.
The test substance administration caused a significant reduction of the body weight in the male rats of the 1000 and 300 mg/kg group by 26 and 12% compared with the control group, respectively. The body weight of the female rats of the high dose was significantly reduced (about 12%) in comparison to the control values at the end of the study. The body weights of the animals of both sexes of the 100 mg/kg group and of the females of the 300 mg/kg group were not changed compared to the untreated controls.
The females at 1000 mg/kg bw/day showed a reduced general state of health and lesions (rhagades) on the hairless skin of the extremities and reddening and scale formation on the ears were reported for males and females.
In the females at 1000 mg/kg bw/day, haematology and clinical chemistry showed that erythrocytes, haemoglobin, haematocrit and thromboplastin time were decreased and leucocytes, platelets, eosinophilic granulocytes and neutrophilic polymorphonuclears were increased. In the females only, alkaline phosphatase, gamma-glutamyltransferase, total protein, globulins and cholesterol were elevated and triglycerides were decreased. At 300 mg/kg bw/day, haemoglobin and haematocrit of the females were decreased and leucocytes, eosinophilic granulocytes, neutrophilic polymorphonuclears and calcium content of the blood were increased.
In the males at 1000 mg/kg bw/day, alkaline phosphatase, gamma-glutamyltransferase and alanine aminotransferase were increased and triglycerides were decreased.

Neither functional neurotoxic impairment nor any sign of neurotoxicity was observed at any dose level.

Necropsy revealed significantly increased absolute kidney and liver weights in females at 1000 mg/kg bw/day. Relative organ weights of kidney and liver were markedly increased at the highest dose (40-60% above the control values). At the mid dose, relative liver weight was also significantly increased. In males, similar increases of absolute and relative kidney and liver weights were observed at the highest and the mid dose. Relative brain weights were significantly increased at the highest and the mid dose (27% and 10% above the control values, respectively) and the relative weight of the adrenal glands was increased by 35% compared to the controls. Relative weights of brain and adrenal glands of the females were not significantly changed.
Notably, the absolute and relative testes weights were markedly decreased at 300 and 1000 mg/kg bw/day with marked diffuse atrophy of the testicular parenchyma and slight to moderate interstitial oedema in all animals noted at histopathology. At 100 mg/kg bw/day, one animal was found with a moderate reduced spermiogenesis. All animals of this group showed minimal up to moderate vacuolar degeneration of spermatogonias in some seminiferous tubules. These lesions were also seen in the control group up to the same gradings and were considered not to be substance-related. Also considered not to be substance-related were the focal atrophy findings which were found in one animal of dose group 1 and two control animals. The moderately reduced spermiogenesis of one animal in the low dose group with no convincing transitional steps up to a diffuse atrophy seen in the mid and high dose groups is interpreted, also because of its single appearance in this dose group, as an incidental, spontaneously occurring event concerning this single animal and not as being possibly substance-induced.

Table: Body and testes weight development; results of testes pathology (ref. 20)

| Dose group/finding | 0 mg/kg bw/day | 100 mg/kg bw/day | 300 mg/kg bw/day | 1000 mg/kg bw/day |
|---|---|---|---|---|
| **Body weight** | | | | |
| day 0 | 190.4 ± 7.9 | 190.1 ± 7.4 | 190.9 ± 5.1 | 192.4 ± 5.5 |
| day 91 | 498.8 ± 44 | 493.5 ± 29.8 | 451.3 ± 37 | 384.0 ± 27.6 |
| **Testes** | 10 | 10 | 10 | 10 |
| absolute weights | 3.563 ± 0.193 g | 3.68 ± 0.353 g | 1.691 ± 0.328 g | 1.693 ± 0.369 g |
| relative weights | 0.78 ± 0.062 g | 0.818 ± 0.094 g | 0.421 ± 0.1 g | 0.477 ± 0.1 g |
| diffuse atrophy | -- | -- | 10/10 | 10/10 |
| edema | -- | -- | 10/10 | 10/10 |
| focal atrophy | 2/10 | 1/10 | -- | -- |
| vacuolar degeneration | 7/10 | 10/10 | -- | -- |
| reduced spermiogenesis | -- | 1/10 | -- | -- |

-- , not detectable

Table: Incidence and grading of microscopic findings in testes (ref. 20)

| Dose group | **Grading** | **0 mg/kg bw/day** | **100 mg/kg bw/day** | **300 mg/kg bw/day** | **1000 mg/kg bw/day** |
|---|---|---|---|---|---|
| Testes | | 10 | 10 | 10 | 10 |
| diffuse atrophy | | -- | -- | 10 | 10 |
| | 1 (minimal) | -- | -- | -- | -- |
| | 2 (slight) | -- | -- | -- | -- |
| | 3 (moderate) | -- | -- | 1 | -- |
| | 4 (marked) | -- | -- | 9 | 10 |
| | 5 (severe) | -- | -- | -- | -- |
| edema | | -- | -- | 10 | 10 |
| | 1 (minimal) | -- | -- | -- | -- |
| | 2 (slight) | -- | -- | 3 | 7 |
| | 3 (moderate) | -- | -- | 7 | 3 |
| | 4 (marked) | -- | -- | -- | -- |
| | 5 (severe) | -- | -- | -- | -- |
| focal atrophy | | 2 | 1 | -- | -- |
| | 1 (minimal) | -- | -- | -- | -- |
| | 2 (slight) | -- | -- | -- | -- |
| | 3 (moderate) | 1 | -- | -- | -- |
| | 4 (marked) | 1 | 1 | -- | -- |
| | 5 (severe) | -- | -- | -- | -- |
| vacuolar degeneration | | 7 | 10 | -- | -- |
| | 1 (minimal) | 3 | 3 | -- | -- |
| | 2 (slight) | 1 | 6 | -- | -- |
| | 3 (moderate) | 3 | 1 | -- | -- |
| | 4 (marked) | -- | -- | -- | -- |
| | 5 (severe) | -- | -- | -- | -- |
| reduced spermiogenesis | -- | -- | 1 | -- | -- |
| | 1 (minimal) | -- | -- | -- | -- |
| | 2 (slight) | -- | -- | -- | -- |
| | 3 (moderate) | -- | 1 | -- | -- |
| | 4 (marked) | -- | -- | -- | -- |
| | 5 (severe) | -- | -- | -- | -- |

-- , not detectable

Conclusion

The subchronic administration of TPO to male and female Wistar rats for 90/91 days at dose levels of 0, 100, 300 and 1000 mg/kg bw/day led to substance related findings at 300 and 1000 mg/kg bw/day in form of reduced body weights, impaired clinical chemistry and indications for inflammation in females, while the males showed testicular atrophy.

In any case and in contrast to the distinct general toxicity at doses at ≥300 mg/kg bw/day, the testing for neurotoxic effects according to the "Functional Observational Battery" revealed no functional defects of any kind or any other signs of neurotoxicity during the study. The NOAEL for male and female Wistar rats derived from this study was 100 mg/kg bw/day.

Ref. 6

**SCCS comment**
No analytical evidence of the purity of the test substance was provided in the study report.

### 3.3.5.3. Chronic (> 12 months) toxicity

No data

### 3.3.6. Mutagenicity / Genotoxicity

### 3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

**Bacterial reverse mutation tests (Ames)**

**1)**

| | |
|---|---|
| Guideline: | Japanese MITI, MHW, MOL and MAFF Guidelines for Screening Mutagenicity Testing of Chemicals, equivalent to OECD Guideline 471 |
| Test system: | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* strain WP2 |
| Replicates: | Triplicate plates |
| Test substance: | LUCIRIN LR 8728 (TPO) |
| Batch: | 010213 (purity: 99%) |
| Concentrations: | Experiment I:<br>±S9 mix: 0, 8, 40, 200, 1000, and 5000 µg/plate<br>Experiment II:<br>±S9 mix: 0, 312.5, 625, 1250, 2500 and 5000 µg/plate |
| Vehicle: | Ethanol |
| Positive Controls: | -S9 mix:<br>TA 100, TA1535: Methyl-N-nitro-N-nitrosoguanidine (MNNG), 2 µg/plate;<br>TA98: 4-Nitro-o-phenylenediamine (4-NOPD), 10 µg/plate;<br>TA1537: 9-Aminoacridine (9-AA), 50 µg/plate;<br>WPA2: 4-Nitroquinoline N-oxide (4-NQO) 3.3µg/plate.<br>+S9 mix (Aroclor 1254):<br>For all strains: 2-aminoanthracene (2-AA), 3.3 or 10 µg/plate<br>Negative controls: untreated and vehicle control |
| Study period: | 1989 |
| Date of report: | 1989 |
| GLP: | Yes |
| Published: | No |

**Material and methods:**
TPO (batch: 010213) was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9 mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver) according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) method. The *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli strain* WP2 were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 8 – 5000 µg/plate.
For control purposes, the negative, vehicle (purified water) and positive controls (MNNG , 4-NOPD, 9-AA, 4-NQO, 2-AA) were also investigated.

**Results:**
No bacteriotoxicity was observed at any concentration. The number of revertant colonies did not differ between plates containing the test substance and those containing the negative controls either with or without metabolic activation. The positive controls induced large

numbers of revertant colonies in each bacterial strain demonstrating the sensitivity and suitability of the test system.

**Conclusion:**
TPO did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial strains used in this Ames test either in the presence or absence of S9-mix. Thus, TPO was shown to be non-mutagenic in this bacterial gene mutation test.

Ref. 33

**2)**
This result is supported by the result of a second Ames test earlier conducted and using the plate incorporation method and exposing five Salmonella strains (TA 1535, 100, 1537, 1538, and 98) in the range between 4 - 2500 µg/plate of Initiator 554 (TPO) for 48 hours in the presence or absence of Aroclor 1254 induced rat liver S9 mix. Cytotoxicity was observed at 2500 µg/plates in the presence of metabolic activation in all strains and without S9 in TA1535, but no increase in the number of his-positive revertants could be detected under all conditions tested.

Ref. 5

**SCCS Comment**
The available report from 1984 of the second Ames test was a translation into English of the original German report from 1979.

***In vitro* mammalian cell gene mutation test (*hprt* locus)**

| | |
|---|---|
| Guideline/Method: | OECD 476 |
| Test system: | Chinese hamster V 79 cells |
| Replicates: | Duplicate cultures, two independent experiments |
| Test substance: | Lucirin OP (TPO) |
| Batch: | 110053 (purity: 99.5 g/100 g ([1H]-NMR)) |
| Concentrations: | Experiment I:<br>4 h: -S9 mix: 1.3, 2.5, 5.0, 10, 20, 30*, 40*µg/mL<br>4 h: +S9 mix: 3.4, 6.8, 13.5, 27, 40.5, 54 µg/mL<br>Experiment II:<br>24 h: -S9 mix: 5.0, 10, 15, 20, 25, 30, 35, 40* µg/mL<br>4 h: +S9 mix: 10, 20, 30, 40*, 45*, 50*, 55*, 60* µg/mL (* = precipitation) |
| Vehicle: | a) TPO: Ethanol<br>b) positive controls: nutrient medium (EMS) or DMSO (DMBA) |
| Positive Controls: | -S9 mix: ethyl methanesulfonate (EMS)<br>+S9 mix: 7,12-dimethylbenz(a)anthracene (DMBA) |
| Study period: | Oct-Dec 2011 |
| Date of report: | 2012 |
| GLP: | Yes |
| Published: | No |

**Material and methods:**
In an *in vitro* gene mutation assay, the potential of TPO to induce gene mutations at the *hprt* locus in V79 cells of the Chinese hamster was investigated. The assay was performed in two independent experiments, using two parallel cultures each. In the first experiment, TPO dissolved in ethanol was added to cultures for 4 hours at dose levels of 1.3 - 40 µg/ml (without metabolic activation) or at 3.4 – 54 µg/ml (with metabolic activation). In the 2nd experiment, the test substance preparation was added to cultures at dose levels of 5.0 – 40 µg/ml (24 hours: -S9 mix) or at 10 - 60 µg/ml (4 hours: +S9 mix). The metabolic activation system was obtained from phenobarbital/β-naphthoflavone induced liver of male Wistar rats. The highest concentration of the pre-experiment (3480 µg/mL) was equal to a molar

concentration of about 10 mM and the concentrations for the main experiments were selected on the observed cytotoxicity in the pre-experiment.
The colonies used to determine the cloning efficiency (survival) were fixed and stained approx. 7 days after treatment with 10 % methylene blue in 0.01 % KOH solution. The stained colonies with more than 50 cells were counted. Negative controls (ethanol) and positive controls (EMS without S9 mix, DMBA with S9 mix) were tested in parallel.

**Results:**
In the first experiment precipitation was observed at 30.0 and 40.0 µg/mL in the absence of metabolic activation. In experiment II in the absence of metabolic activation, precipitation occurred after 24 hours continuous treatment at the maximum concentration of 40.0 µg/mL. In the presence of S9 mix, precipitation occurred solely in experiment II at 40.0 µg/mL and above. Cytotoxic effects defined by a relative cloning efficiency or a relative cell density of below 50 % were observed in all experimental parts at higher concentrations of ≥10 µg/mL (-S9 mix) and ≥40 µg/mL (+S9 mix).
No relevant and reproducible increase in mutant frequency was observed in the main experiments up to the maximum concentrations of ≥30 µg/mL (-S9 mix) and ≥40.5 µg/mL (+S9 mix). The mutant frequency remained well within the historical range of solvent controls. Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies indicating that the tests were sensitive and valid and confirmed the activity of the metabolic activation system.

**Conclusion:**
Under the conditions of the study, TPO did not induce gene mutations at the *hprt* locus in Chinese hamster V79 cells in the absence and presence of metabolic activation and was therefore considered to be not mutagenic.

Ref. 13

***In vitro* mammalian cell chromosomal aberration tests**

| | |
|---|---|
| Guideline/Method: | Japanese MITI, MHW, MOL and MAFF Guidelines for Screening Mutagenicity Testing of Chemicals, equivalent to OECD Guideline 473 |
| Test system: | Chinese hamster lung cells (CHL) |
| Replicates: | Duplicate cultures |
| Test substance: | LUCIRIN LR 8728 (TPO) |
| Batch: | 010213 (purity: 99%) |
| Concentrations: | 6 + 18 h: -S9 mix: 0, 15, 20, 23.3 and 25 µg/mL<br>24 h: -S9 mix: 0, 5, 10, 15, 20 µg/mL<br>48 h: -S9 mix: 0, 2.5, 5, 10, 20 µg/mL<br>6 + 18 h: +S9 mix: 0, 20, 23.3, 26.6, 30 µg/mL |
| Vehicle: | DMSO |
| Positive Controls: | -S9 mix: Mitomycin C (MMC), 0.075 µg/mL<br>±S9 mix: Cyclophosphamide (CP), 10 µg/mL (exp. I) / 5 µg/mL (exp. II) |
| Negative control: | Vehicle |
| Study period: | 1989 |
| Date of report: | 1989 |
| GLP: | Yes |
| Published: | No |

**Material and methods:**
TPO was tested for its potential to induce structural chromosome aberrations in Chinese hamster lung (CHL) cells *in vitro*. The test article was dissolved in DMSO and was tested in the presence and absence of metabolic activation (S9 mix prepared from Aroclor 1254 induced male Sprague-Dawley rat liver). Without S9 mix, the cells were incubated for 6 hours, followed by an 18-hour recovery time, or they were exposed for 24 or for 48 h. With

metabolic activation, the cells were exposed for 6 h followed by a 18 h recovery period. Due to a very steep toxicity dose-response curve in a preliminary cytotoxicity study, a narrow test concentration range in all four treatment groups of the main study was selected. The concentration range without metabolic activation was 15 - 25 µg/mL for 6 h treatment; 5 - 20 µg/mL for 24 h treatment and 2.5 - 20 µg/mL for 48 h treatment. The concentration range with metabolic activation was 20 - 30 µg/mL. Thereafter, the CHL cells were treated with demecolcine to arrest mitosis, trypsinized to detach from the tissue culture flask and suspended prior to fixing, staining with Giemsa and air drying. A sample of each cell suspension from each harvest was counted to measure growth inhibition at each concentration. One hundred metaphases from each culture were analyzed for structural and numerical aberrations. Mitomycin C (0.075 µg/mL) in the absence and Cyclophosphamide (10 µg/mL) in the absence and presence of S9 mix served as positive controls. A negative control (DMSO) was also included in the test.

**Results:**
Precipitation was observed at 80 µg/ml. Sufficient cytotoxicity to the CHL cells at the highest concentrations in the range between 50 – 70% was achieved, still allowing evaluation as required according to the guideline. No significant, concentration-related increase in the frequency of cells with aberrations was found in any of the treatment cases up to the cytotoxic dose levels of ≥20 µg/mL (-S9 mix) and 30 µg/mL (+S9 mix).
The sensitivity of the test system was demonstrated as the positive controls, MMC in the assay, without S9 mix and CP in the presence of S9 assay showed significant structural increases in chromosomal aberrations.

**Conclusion:**
Under the experimental conditions of this chromosomal aberration test, TPO did not induce any toxicologically significant or biologically relevant increase in the frequency of cells with chromosomal aberrations, and thus showed no clastogenic potential in the absence or presence of metabolic activation in Chinese hamster lung cells when tested up to cytotoxic concentrations.

Ref. 34

**Overall conclusion on mutagenicity/genotoxicity**

TPO was tested for mutagenicity/genotoxicity covering all relevant endpoints in *in vitro* studies according to test guidelines under GLP conditions.
No genotoxic/mutagenic potential was noted in two bacterial gene mutation assays with *Salmonella typhimurium* or *Escherichia coli* in the presence or absence of metabolic activation.
TPO did not induce gene mutations at the *hprt* locus in Chinese hamster V79 cells in the absence and presence of metabolic activation using a rat liver metabolic activation system.
TPO revealed no clastogenic potential in Chinese hamster lung cells tested with/without metabolic activation up to pronounced cytotoxic concentrations.
In conclusion, based on the results of the complete test battery for mutagenic/genotoxic effects *in vitro*, TPO is not expected to cause mutagenic effects *in vivo*. Consequently, TPO is not expected to provide a risk to humans with regard to these endpoints.

### 3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data available.

### 3.3.7. Carcinogenicity

No data available.

### 3.3.8. Reproductive toxicity

No data available. See 3.3.5, repeated dose toxicity, testes weight reductions.

### 3.3.9. Toxicokinetics

No data available.

### 3.3.10. Photo-induced toxicity

No data available.

### 3.3.11. Human data

**Repeated insult patch test**
**1)**

| | |
|---|---|
| Guideline: | / |
| Species: | Human |
| Group size: | 53 enrolled female volunteers (age range: 18 – 65 years)/51 completed |
| Test substance: | Diphenyl-(2,4,6-trimethylbenzoyl) phosphine oxide (TPO, 2.6%) containing nail gel GelColor |
| Batch: | no data on the batch but specification of the composition is available as confidential information (ref. 28) |
| Route: | Topical application to the fingernails |
| Induction phase: | Application of the test material to the fingernails three times per week (e.g., Monday, Wednesday, Friday) for a total of nine applications |
| Challenge phase: | About 2 weeks after final induction application, a challenge application was applied to the nail following the same procedure as for induction |
| Scoring system: | Modified scoring scale with grade 0 – 4 (no visible nail and cuticle reaction – severe erythema, possible oedema, vesiculation, bullae and/or ulceration) |
| Study period: | 2010 |
| Date of report: | 2010 |
| GCP: | Yes |
| Published: | No |

**Material and methods:**
A 2.6% TPO containing nail gel (GelColor®) was tested for its potential to induce primary or cumulative irritation and/or allergic contact sensitization in a repeated insult patch test, when applied prior to polymerisation to the nails of healthy human volunteers. For the study 53 female volunteers (age range: 18 – 65 years) were enrolled and 51 volunteers completed the study. The fingernails were the test area and the test material was applied using a brush. The brush was dipped into the test material and a coat of the test material was applied to the nail. The test material was left on the nail for 10 min. and thereafter, the material was wiped off using a nail wipe and a commercial cleansing solution provided by the sponsor. Within the induction phase, this procedure was performed three times per week (e.g., Monday, Wednesday, Friday) for a total of nine applications. After a rest period of about 2 week after the last induction application, a challenge application was applied to the nail following the same procedure as for induction. The reactions were scored on a scale of grade 0 - grade 4 (no visible nail and cuticle reaction up to severe erythema, possible oedema, vesiculation, bullae and/or ulceration).

**Results:**
During the whole induction period and also after challenge, no findings were observed and the reactions were consistently scored as grade 0 (no visible nail and cuticle reaction).

**Conclusion**
There was no indication for an irritative or sensitising potential of the tested gel containing TPO at a concentration of 2.6%, when applied prior to polymerisation to the nails of healthy human volunteers.

Ref. 26, 28

**Repeated insult patch test**
**2)**

| | |
|---|---|
| Guideline: | / |
| Species: | Human |
| Group size: | 50 enrolled female volunteers (age range: 18 – 65 years)/50 completed |
| Test substance: | Nail product Axxium gel system-thick gel sealer containing diphenyl-(2,4,6-trimethylbenzoyl) phosphine oxide (TPO) |
| Batch: | no data on the batch and no specification of the composition including the concentration of TPO |
| Route: | Topical application to the finger nails |
| Induction phase: | Application of the test material to the finger nails three times per week (e.g., Monday, Wednesday, Friday) for a total of nine applications. |
| Challenge phase: | About 2 weeks after final induction application, a challenge application was applied to the nail following the same procedure as for induction |
| Scoring system: | Modified scoring scale with grade 0 – 4 (no visible nail and cuticle reaction – severe erythema, possible oedema, vesiculation, bullae and/or ulceration) |
| Study period: | 2010 |
| Date of report: | 2010 |
| GCP: | Yes |
| Published: | No |

**Material and methods:**
The study was performed in the same way as the first study.

**Results:**
During the whole induction period and also after challenge, no findings were observed and the reactions were consistently scored as grade 0 (no visible nail and cuticle reaction).

**Conclusion**
There was no indication for an irritative or sensitising potential of the tested gel containing TPO at a concentration of 2.6%, when applied prior to polymerisation to the nails of healthy human volunteers.

**SCCS comment**
These are non-guideline but compatibility studies. Under the conditions of the experiments, local irritation was not observed. The studies do not exclude a sensitising potential of the products.
The SCCS considers HRIPT tests unethical.

### 3.3.12. Special investigations

**Extraction of TPO after simulation of use on artificial nails**

The possible extraction of TPO to simulate potential bioavailability after use was investigated in a non-GLP study using an application of a TPO containing base coat gel, one intermediate colour coat gel and a top coat gel applied to a plastic nail tip made of an ABS copolymer (acrylonitrile-butadiene-styrene). Each step was followed by curing. Thus, only the base coat gel was applied to the nail, while the other applications were to the polymerized base coat. This investigation was performed with gels containing 3% of TPO by weight as photo-initiator before curing. The weight of gels applied to the two ABS plastic nail tips used was 0.072 and 0.078 grams, respectively. Curing was for 3 minutes with 4 X 9 watt bulbs (UV: 380-420 nm). The total weight of the finished coated nail was 0.072 grams for the room temperature sample and 0.078 grams for the other sample envisaged for extraction at 50 °C, corresponding to about 0.0022 – 0.00234 g TPO in the uncured gels. For extraction, the cured polish was soaked with an aqueous 0.1% sodium chloride solution for 16 hours at room temperature (22 °C) and at 50 °C. After extraction the solution was injected on an Agilent 1200 HPLC fitted with a UV detector.
The analytical measurements were performed by means of an Agilent 1200 HPLC fitted with a UV detector. The limit of detection (LOD) was 0.2 ppm for TPO. The results showed that extraction of TPO ($4.4 \times 10^{-6}$ g at 22 °C, $4.68 \times 10^{-6}$ g at 50 °C) was practically not measurable due to the curing process. In consideration of the LOD, an amount below the quantification limit of the equipment was extracted, i.e., less than 0.2% of the total TPO.
Under the assumption that the weight of the coat is equivalent to the weight of the uncured gel, this result means that of the initial amount of 2.16 – 2.34 mg TPO/nail polish gel (3% TPO of 72 - 78 mg/nail) prior to curing only less than 0.14 – 0.16 mg TPO/nail (<0.2% TPO of 72 – 78 mg/nail) could have theoretically been extracted.
Although this would represent a worst case scenario, the value of 0.2% TPO as the theoretically maximum extractable amount is taken as an alternative value for dermal absorption in the exposure assessment performed and calculation of Margin of Safety (MoS).

Ref. 38

**SCCS comment**
Instead of a maximum concentration of 5% TPO in nail hardening gel, a concentration of 3% was used. The areas of the two coated artificial nails were not reported. It is not described in the report whether the extraction procedure was performed under light protection. It is questionable if a UV detection method can be used for a UV-sensitive substance such as TPO. The limit of detection/quantification of TPO is not traceable in the report. However, despite these shortcomings, the study results are consistent with very low amounts of residual TPO to be expected at the end of a radical polymerization reaction under the conditions described. In the light of these shortcomings of the study, a value of 1% residual TPO will be used for the MoS calculation below.

For nail polishes using varnish applications, representative data from France is available (about 450 mg of varnish for 3 coatings; Ficheux *et al*. 2014; see ref. AR1). However, data of this experiment suggest that hardening gel amounts applied for nail polishes may be different from amounts used for varnish applications.

### 3.3.13. Safety evaluation (including calculation of the MoS)

In principle, two major artificial nail systems are used for the fingernails and toenails. One use is for nail polishes (base, middle and top coats). Frequency of use is one complete application every 2-3 weeks. Old gel is removed and the nail cleaned before new application. The other use is for nail enhancement products. For this use, the artificial nail systems are applied to the nail plate every 2 – 3 weeks with a refill application after 1 - 2 weeks. Full application is in the range of between 2 – 4 g of gel and of 1 g of gel for the refill. The use of 4 g gel with a maximum content of 5% TPO will lead to a total nail application of 200 mg TPO/human. (This corresponds to an amount of 10 mg/nail or considering the total fingernail and toenail area of 22 cm² (reference: SCCP, 2006 for the

fingernail area) to an amount of 9.09 mg/cm² nail area.) Under consideration of the default human body weight of 60 kg, the total nail application of 200 mg TPO/human will result in a value of 3.33 mg/kg bw. As worst case it is assumed that 1% of the initial TPO amount remains as residue, of which 100% becomes systemically available via the human nail (see Discussion).

**CALCULATION OF THE MARGIN OF SAFETY**

## Trimethylbenzoyl-diphenylphosphine oxide (TPO)

| | |
|---|---|
| **Amount of the gel applied:** | **4 g** |
| **Concentration of TPO in the gel:** | **5%** |
| **Total amount of TPO applied:** | **200 mg** |
| **Default human body weight:** | **60 kg** |
| **Amount of TPO applied/kg human b.w.:** | **3.33 mg/kg bw** |
| **Assumed residue:** | **1%** |
| **Assumed absorption through the nail plate:** | **100%** |
| **Systemic exposure dose (SED):** | |
| **3.33 mg/kg bw * 0.01 =** | **0.033 mg/kg bw/day** |
| **No Observed Adverse Effect Level (NOAEL)** | **100 mg/kg bw/day** |
| **(90 day repeated dose oral toxicity, rat)** | |
| **Corrected NOAEL for 50% bioavailability*:** | **50 mg/kg bw/day** |

| | |
|---|---|
| **Margin of Safety** | **adjusted NOAEL/SED = 1515** |

* Standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

### 3.3.14. Discussion

*Physico-chemical properties, function and use*

TPO is a yellow powder which is sparingly soluble in water but readily soluble in various organic solvents and typical monomers of use.

TPO is used as a key processing aid in form of a chemical photo-initiator for polymerisation in artificial nail systems, primarily in UV-curable one-component gel systems. This means that TPO splits into two free radical fragments, which subsequently become incorporated into the polymer as chain ends. Therefore, TPO will be rapidly consumed during the polymerisation process. Even if minor residual amounts remain, they will be trapped in the rapidly hardened polymer matrix of the formed nail coating. The current and anticipated use concentrations in the gels are in the range between 0.5% - 5.0%.

Careful application to the nail plate should not allow contact with skin. However, without due care, there could be accidental skin contact at the cuticle and the side of the nails. It is important to distinguish between professional and home-made applications, as the latter may cause more accidental skin contact than the former.

*General toxicity*

The acute oral LD50 in rats, obtained independently in two studies, was >5000 mg/kg bw.

The dermal LD50 in rats was >2000 mg/kg bw. Data on acute inhalation toxicity is not available.

The toxicological profile of TPO after repeated oral application was characterized in 28-day and 90-day studies in rats.

The subacute oral (gavage) administration of TPO to Sprague-Dawley rats for 28 days at dose levels of 0, 50, 250 and 750 mg/kg bw/day led to toxicologically relevant effects in both sexes at 250 and 750 mg/kg bw/day in the form of impaired general state of health,

reduced body weight parameters and functional as well as morphological findings indicative for kidney and liver damage. In addition, the males at 750 mg/kg bw/day showed testicular atrophy. With the exception of the testes and liver findings, the findings disappeared during the recovery period.
The NOAEL obtained in this study was 50 mg/kg bw/day.
A subsequently performed oral (gavage) 28-day repeated dose toxicity study in male Wistar rats (non-GLP), designed specifically to investigate testicular effects, did not confirm the above findings as the animals treated at the single dose of 1000 mg/kg bw/day did not show any clinical findings and especially no testicular atrophy.
An additional 90-day repeated dose toxicity study in male Wistar rats (non-GLP) with a dose level of 1000 mg/kg bw/day confirmed the findings in the 28-day study in Sprague-Dawley rats and led to reductions of body weight and relative and absolute testes weights as well. Histopathology revealed a slight to severe diffuse atrophy of the seminiferous tubules of the testes, oedemas and hyperplasia of the Leydig cells and oligo- to azoospermia in the epididymes with reduced size.
The subchronic administration of TPO to Wistar rats for 90/91 days at dose levels of 0, 100, 300 and 1000 mg/kg bw/day led to substance-related findings at 300 and 1000 mg/kg bw/day in the form of reduced body weights, increased absolute and relative kidney and liver weights, impaired clinical chemistry and indications for inflammation in females, while the males showed testicular atrophy. The testing for neurotoxic effects revealed no functional defects of any kind or any other signs of neurotoxicity during the study. The NOAEL obtained in this study was 100 mg/kg bw/day.
Finally, it can be concluded that in general, comparable effects are noted in the 28-day and 90-day studies. Especially no increase in severity of the organ damages at almost identical dose levels occurred with increased exposure time (28- versus 90-day). The most susceptible organs were the testes. A clear NOAEL was obtained for testicular atrophy in both studies. The NOAEL in the 28-day study was 50 mg/kg bw/day and the NOAEL in the 90-day study was 100 mg/kg bw/day. As the apparently lower NOAEL may be due to the respective dose selection and as there was no significant increase in severity of the observed effects over time, an overall NOAEL of 100 mg/kg bw/day for repeated dose oral toxicity will be used for the calculation of the Margin of Safety (MoS).

*Irritation / sensitisation*
TPO has irritant potential to rabbit skin and rabbit eyes.
TPO has been positively tested in an LLNA test in mice. An EC3 of 27% was calculated indicating a moderate sensitising potential.

*Dermal absorption via human nail plate*
There is no dermal absorption study or penetration study through the nail plate available for TPO. Whereas dermal penetration through the skin can be tested according to the respective OECD guidelines and the SCCS guidance, currently no standardized or validated test system exists to investigate penetration through the nail plate. Human nail forms a formidable barrier for substances, for instance drug formulations topically applied for medical purposes (ref. AR 3, AR 4). Current knowledge indicates that the nail plate behaves more like a hydrogel than a lipophilic membrane and the aqueous pathway plays the dominant role in drug penetration through the nail. Furthermore, water is the principle nail plasticizer. Once hydrated, the nail becomes more elastic and possibly more permeable to topically applied substances (ref. AR 3).
TPO is a lipophilic substance and sparingly soluble in water and is therefore considered not a good candidate for penetrating the nail plate. Furthermore, it has to be considered that TPO is used as a chemical photo-initiator for polymerisation of monomeric mixtures applied on the nail plate and most of it is consumed and chemically bound to polymer chains. After application of TPO containing gel to the nail plate, the UV-initiated polymerisation process can be considered as complete within about 2 – 3 minutes. TPO will be consumed rapidly during the polymerisation process. In the event that a minor residual amount will remain, it will be trapped in the rapidly hardened polymer matrix. This process reduces the chance of

possible penetration through the nail plate or the accidentally exposed surrounding skin and thus, the systemic bioavailability can be considered as very low, if any.
A poorly reported experimental study was conducted that simulated release of TPO from cured coatings after UV-initiated polymerisation of gels applied to an artificial nail (see section 3.3.12). The data suggest that residual TPO was extracted into the water phase only in traces if at all.
For the skin, the SCCS recommends in case of no dermal penetration data to use 100% absorption. Alternatively, a value of 10% dermal absorption can be considered in case of a molecular weight (MW) of >500 g/mol and a Log Pow smaller than -1 or higher than 4. Consequently, for TPO with a MW of 348.4 g/mol and a Log Pow of 3.1, a dermal penetration of 100% normally has to be considered. However, this worst case scenario for the skin by far exceeds the practical exposure conditions as primarily it is the nail plate and not the skin that is exposed to the artificial nail system containing TPO.
In principle, two major artificial nail systems are used for the fingernails and toenails. In both cases, a residual amount not higher than 1% TPO in the cured coatings and a penetration of residual TPO via the nail of not more than 1% can be assumed. Nevertheless, although it represents a worst case assumption, a value of 1% is applied for systemic bioavailability (i.e., 100% bioavailability across the nail plate) in the safety assessment and the calculation of the Margin of Safety, bearing in mind that the penetration via the nail may be even two orders of magnitude lower.

Home-made UV-initiated coatings by use of gels containing TPO:
Careful application to the nail plate does not allow contact with skin. However, without due care, there could be minimal skin contact at the cuticle and the side of the nails. Considering the large Margin of Safety, it can be concluded that minor skin contact does not raise a concern apart from potential skin sensitization.

*Mutagenicity / Genotoxicity*
TPO was tested for mutagenicity/genotoxicity covering all relevant endpoints in *in vitro* studies according to test guidelines under GLP conditions.
No genotoxic/mutagenic potential was noted in two bacterial gene mutation assays with *Salmonella typhimurium* or *Escherichia coli* in the presence or absence of metabolic activation.
TPO did not induce gene mutations at the *hprt* locus in Chinese hamster V79 cells in the absence and presence of metabolic activation of a rat liver metabolic activation system.
TPO revealed no clastogenic potential in Chinese hamster lung cells tested with/without metabolic activation up to pronounced cytotoxic concentrations.
Based on the results of the complete test battery for mutagenic/genotoxic effects *in vitro*, TPO is not expected to cause mutagenic effects *in vivo*. Consequently, TPO is not expected to provide a risk to humans with regard to these endpoints.

*Carcinogenicity*
No data available.

*Reproductive toxicity*
In several repeated dose toxicity studies in rats, TPO induced marked testicular atrophy.
TPO is classified as toxic to reproduction (classification as Repr. 2; H361f according to CLP Regulation).

*Toxicokinetics and metabolism*
No data available.

## 4. CONCLUSION

The SCCS is of the opinion that Trimethylbenzoyl diphenylphosphine oxide (TPO) is safe when used as a nail modelling product at a concentration of at maximum 5.0%.

However, TPO is considered a moderate skin sensitizer.

Other potential uses of Trimethylbenzoyl diphenylphosphine oxide in cosmetic products cannot be evaluated without further documentation.

## 5. MINORITY OPINION

/

## 6. REFERENCES

*Submission I*

1. Baden, HP (1970) The physical properties of nail, J. Investigative Dermatology, 55, 115-122.
2. BASF SE (1979a) Report on the study of acute oral toxicity of "Initiator 554" in the rat (English translation: 1884-09-20), Study No. 79/85, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 08-Oct-1979.
3. BASF SE (1979b) Report on the study of the primary irritation of "Initiator 554" to the dorsal skin of white rabbits based on Federal Register 38, No 187, § 1500.41, p 27029, Sept 27 1973 (English translation: 1984-09-20), Report No.: 79/85, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 05-Sep-1979.
4. BASF SE (1979c) Report on the study of the primary irritation of "Initiator 554 " to the eye of white rabbits based on Federal Register 38, No 187, §1500.42, p27019, 27 Sept 1973 (English translation 1984-09-20), Report No.: 79/85, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 05-Sep-1979.
5. BASF SE (1979d) Report on the Study of 2,4,6-Trimethylbenzoyldiphenylphosphinoxid (Initiator 554) in the AMES TEST (Standard Plate Test with Salmonella typhimurium; English translation: 1984-09-20), Report No.: 79/85, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 12-Jun-1979.
6. BASF SE (1991) Report on the study of the oral toxicity of LUCIRIN LR 8728 in conjunction with the "Functional Observational Battery" in rats - Administration by gavage over 13 weeks, Report No.: 99S0085/86062, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 04-Oct-1991.
7. BASF SE (2001a) Diphenyl (2,4,6-trimethylbenzoyl) phosphinoxid (new batch) - Study to evaluate testes toxicity in Wistar rats exposed via gavage for up to 3 months, Report No.: 51C0293/99096, Department of Toxicology, BASF SE, Ludwigshafen, Germany, confidential/unpublished data (Original report in German), 19-Jan-2001.
8. BASF SE (2001b) Technical Information, Coating Raw materials, Lucirin® TPO, June 2001.
9. BASF SE (2007) Technical information, EVP 003405 e – Lucirin® Grades, BASF SE, Ludwigshafen, Germany, April 2007.
10. BASF SE (2011) Lucirin TPO - Acute dermal toxicity study in rats, Report No.: 11-BFDT106, Study No. 11A0490/03X010, Bioassay, Heidelberg, Germany for BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 02-Nov-2011.
11. BASF SE (2012a) Final Report – Physico-chemical properties of "Lucirin TPO", Study No. 11LOO409 (confidential), Competence Center Analytics, BASF SE, Ludwigshafen, Germany, confidential/confidential/unpublished data, 03-Aug-2012
12. BASF SE (2012b) Lucirin TPO - Local Lymph Node Assay (LLNA) in Mice (CBA/CaOlaHsd), Report No.: 1443004, BASF SE study No. 58V0490/03X009, Harlan

Cytotest Cell Research GmbH, Rossdorf, Germany for BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 23-Feb-2012.
13. BASF SE (2012c) Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT) with Lucirin OP, Report no.: 1443101, BASF SE Study No. 50M0490/03X013, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany for BASF SE, Ludwigshafen, Germany, confidential/unpublished data, 31-Jan-2012.
14. BASF SE (2012d) Material Safety Data Sheet, Lucirin® TPO, 12 March 2012.
15. BASF SE (2013a) Product specification, Irgacure® TPO (old Lucirin® TPO), 27-Aug-2013.
16. BASF SE (2013b) Printing & Packaging, Industrial Coatings, Technical Data Sheet, Irgacure® TPO (old Lucirin® TPO), March 2013.
17. CND (2013) Brochure - BRISA® UV gel enhancements sculpted on a form, Step-by-Step Guide, 13/03, #0673, Creative Nail Design Inc. (CND), Vista, CA, USA, 2013.
18. Commission Regulation (EU) No. 618/2012 of 10 July 2012, Official Journal of the European Union, L 179/3, 11-Jul-2012.
19. ECHA (2010a) European Chemicals Agency (ECHA), Committee for Risk Assessment (RAC), Opinion proposing harmonised classification and labelling at Community level of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, ECHA/RAC/CLH-O-0000001405-81- 01/F, ECHA, Helsinki, Finland, 27-Oct-2010.
20. ECHA (2010b) European Chemicals Agency (ECHA), Committee for Risk Assessment (RAC), Annex 1. Background document to the to the Opinion proposing harmonised classification and labelling at Community level of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, ECHA/RAC/CLH-O-0000001405-81-01/A1, EC number: 278-355-8, CAS number: 75980-60-8, submitted by Federal Institute for Occupational Safety and Health (BAuA), Dortmund, Germany, 27-Oct-2010.
21. Fleckman P, Allan C (2001) Surgical anatomy of the nail unit, Dematol. Surg., 27, 257-260.
22. Ikemura K, Ichizawa K, Yoshida M, Ito S, Endo T (2008) UV-VIS spectra and photoinitiation behaviors of acylphosphine oxide and bisacylphosphine oxide derivatives in unfilled, light-cured dental resins, Dental Materials J., 27, 765—774.
23. Lambson (2011) Technical Data Sheet, Speedcure TPO, Lambson Ltd., Wetherby, West Yorkshire, UK, 09-Sep-2011.
24. Lambson (2012) German: Sicherheitsdatenblatt, Speedcure TPO, Lambson Ltd., Wetherby, West Yorkshire, UK, 10-Jan-2012.
25. NBM (2013) Brochure – German: Neumodellage mit Tip – Step by Step, Nails Beauty & More (NBM), Akzent-direct GmbH, 31-Jan-2013.
26. OPI Products (2010a) Final Report – Human insult repeat patch evaluation of the nail product Gel Color®, Sponsor code GCI-3149A/GCI-3150, ETC No. 1090-6410-77-004, Biometrix, San Francisco, CA, USA for OPI Products Inc., N. Hollywood, CA, USA, confidential/unpublished data, 03-Sep-2013.
27. OPI Products (2010b) Final Report – Human insult repeat patch evaluation of the nail product Axxium Gel System-Thick Gel Sealer, Sponsor code: Bondex, ETC No. 1090-6410-77-0015, Biometrix, San Francisco, CA, USA for OPI Products Inc., N. Hollywood, CA, USA, confidential/unpublished data, 12-Feb-2010.
28. OPI Products (2013) Ingredient Declaration Gel Color by OPI, Base Gel (GC 010), content of TPA, content of other ingredients anonymized, OPI Products Inc., N. Hollywood, CA, USA, confidential/unpublished data, 02-Aug-2013.
29. Rhein LD (2001) Nails - Review of Structure, Function and Strategies to Treat Disorders, GlaxoSmithKline, November 2001, http://www.nyscc.org/techarchives.html.
30. Runne U, Orfanos CE (1981) The human nail: structure, growth and pathological changes, Curr. Probl. Dermatol., 9, 102-149.
31. Safepharm Laboratories Ltd (1989a) Lucirin LR 8728 [Chemical name diphenyl (2,4,6- trimethylbenzoyl) phosphineoxide], acute oral toxicity (limit test) in the rat - Project No. 214/8, Safepharm Laboratories Ltd PO Box 45 DERBY, DE1 2BT, UK Japan Synthetic Rubber Co Ltd, 2-11-24 Tsukiji, Chuo-ku, Tokyo 104; BASF Japan Ltd, 3-3 Kioicho, Chiyoda-ku, Tokyo 102; Japan, confidential/unpublished data, 21-

Jul-1989.
32. Safepharm Laboratories Ltd (1989b) Lucirin LR 8728 [Chemical name diphenyl (2,4,6-trimethylbenzoyl) phosphineoxide], twenty-eight day oral (gavage) toxicity study in the rat, Report No.: 214/7, Safepharm Laboratories Ltd PO Box 45 DERBY, DE1 2BT, UK Japan Synthetic Rubber Co Ltd, 2-11-24 Tsukiji, Chuo-ku, Tokyo 104; BASF Japan Ltd, 3-3 Kioicho, Chiyoda-ku, Tokyo 102; Japan, confidential/unpublished data, 15-Sep-1989.
33. Safepharm Laboratories Ltd. (1989c) Lucirin LR 8728: Japanese MITI/MHW/MOL/MAFF "Ames Test" using Salmonella typhimurium and Escherichia coli - Projetct number 214/10, Report No.: 1546-214/10, Safepharm Laboratories Ltd PO Box 45 DERBY, DE1 2BT, UK Japan Synthetic Rubber Co Ltd, 2-11-24 Tsukiji, Chuo-ku, Tokyo 104; BASF Japan Ltd, 3-3 Kioicho, Chiyoda-ku, Tokyo 102; Japan, confidential/unpublished data, 11-Jul-1989.
34. Safepharm Laboratories Ltd. (1989d) Lucirin LR 8728: Japanese MOL/MHW/MITI Metaphase analysis in CHL cells *in vitro*, Projetct No. 214/9, Safepharm Laboratories Ltd PO Box 45 DERBY, DE1 2BT, UK Japan Synthetic Rubber Co Ltd, 2-11-24 Tsukiji, Chuoku, Tokyo 104; BASF Japan Ltd, 3-3 Kioicho, Chiyoda-ku, Tokyo 102; Japan, confidential/unpublished data, 10-Jul-1989.
35. SCCNFP (2002) Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP), The use of benzyl peroxide (BPO), hydroquinone (HQ), hydroquinone methylether (MEHQ) in artificial nail systems, adopted at 20th plenary meeting, 04-Jun-2002, SCCNFP/048/01, final.
36. SCCP (2006) Scientific Committee on Consumer Products (SCCP) - The SCCP's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, 6th Revision, adopted by the SCCP during the 10th plenary meeting of 19 December 2006.
37. SCCS (2012) Scientific Committee on Consumer Safety (SCCS), SCCS'S Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation, 8th Revision, adopted at 17th plenary meeting, 11-Dec-2012, SCCS/1501/12.
38. Steffier L (2013) Report on the Analysis of TPO in Light Cured Nail Gels, Keystone Industries, Cherry Hill, NJ, USA, unpublished/confidential information, 15-Aug-2013.
39. Dossier on the Safety of TPO as key processing aid in artificial nail systems.

*Data Base Literature Search*

*List of references not included in the safety assessment dossier*

AR 1. Ficheux, AS, et al. (2014) Probabilistic assessment of exposure to nail cosmetics in French consumers. Food Chem Toxicol 66, 36-43
AR 2. SCCP/0919/05: Memorandum on the classification and categorization of skin sensitisers and grading of test reactions, 20 September 2005. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_01.pdf
AR 3. Elkeeb R, AliKhan A, Elkeeb L, Hui X, Maibach HI (2010) Transungual drug delivery: Current status. Internat J Pharmceut 384, 1-8
AR 4. Walters KA, Abdalghafor HM, Lane ME (2012) The human nail – barrier characterisation and permeation enhancement. Internat J Pharmceut 435, 10-21