Annex XV

Proposal for Harmonised Classification and Labelling of a Chemical Substance

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance name: Diphenyl(2,4,6-trimethylbenzol)phosphine oxide

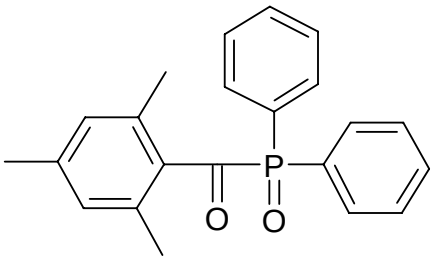
EC number: 278-355-8

CAS number: 75980-60-8

Registration number(s):

Molecular formula: $C_{22}H_{21}O_2P$

Structural formula:



Purity: 99.3 %

Impurities:

Proposed classification under Directive 67/548/EEC: Repr. Cat. 3; R62

Proposed classification under GHS: Repr. 2; H361f

Proposed labelling under Directive 67/548/EEC:

Xn, R62, S37

Proposed labelling under GHS:

Symbol: HEALTH HAZARD

Signal Word: WARNING

suspected of damaging fertility by causing atrophy of the testes.

Proposed specific concentration limits (if any): None.

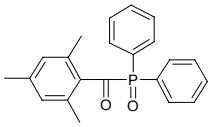
Proposed notes (if any): None.

This dossier reviewed the mutagenicity, and reprotoxicity endpoints only. Classification for carcinogenicity, or respiratory sensitisation was not considered due to lacking data. The classification is based on the properties of the substance itself.

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

Name: Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide
EC Number: 278-355-8
CAS Number: 75980-60-8
IUPAC Name: Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide
Molecular Formula: $C_{22}H_{21}O_2P$
Structural Formula:



Molecular Weight: 348.4 g/mol
Synonyms: Lucirin TPO

1.1 Purity/Impurities/Additives

Degree of purity: 99.3%

1.2 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

REACH ref Annex, §	Property	Value	Reference
V, 5.1	Physical state at 20°C and 101.3 KPa	solid	
V, 5.2	Melting / freezing point	75°C (calculated)	[1]
V, 5.3	Boiling point	474.2°C (calculated)	[1]
V, 5.5	Vapour pressure	3.9×10^{-8} hPa (calculated)	[1]
V, 5.7	Water solubility	3.13 mg/L at 25 °C (calculated)	[2]
V, 5.8	Partition coefficient n-octanol/water (log value)	3.87 at 25°C (calculated)	[2]

2 MANUFACTURE AND USES

Not relevant for this dossier.

3 CLASSIFICATION AND LABELLING

The substance is not currently classified in Annex I of Directive 67/548/EEC.

4 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No data available.

5.2 Acute toxicity

Not relevant for this dossier.

5.3 Irritation

Not relevant for this dossier.

5.4 Corrosivity

Not relevant for this dossier.

5.5 Sensitisation

Respiratory sensitisation has not been considered here due to lacking data.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

5.6.1.1 Initial 28-day study

In a 28-day repeated dose study, the test substance (purity 99%) was administered to Sprague-Dawley rats by oral gavage once daily. The dose groups 50, 250 and 750 mg/kg bw/day consisted of five male and five female animals each; a control group of five males and five females was dosed with vehicle alone (arachis oil B.P.). Two satellite groups, each of five males and five females were treated with the high dose (750 mg/kg bw/day) or the vehicle alone throughout the twenty-eight day study period and then maintained without treatment for a further fourteen days.

Clinical signs, bodyweight, food and water consumptions were monitored during the study. Hematology, blood chemistry and urinalysis were evaluated for all main group animals during the final week of dosing. Parameters showing abnormalities were examined in satellite group animals at the end of the treatment-free period (14 days).

All animals were subjected to a gross necropsy examination with histopathological evaluation of selected tissues.

At the start of the treatment the males weighed 152 to 192g, and the females weighed 155 to 189 g, and were approximately six to seven weeks old.

Mortality: One female of the 750 mg/kg bw /day satellite group died on day 4 of treatment. One female of the control satellite group died during blood sampling on day 42 (post treatment period).

Clinical signs of toxicity: whereas no signs of toxicity were seen at 50 mg/kg bw/day, the rats of the 250 mg/kg bw displayed from day 4 of treatment a series of signs including salivation, red-brownish staining around the snout and the mouth as well as of the fur, which also was wet, hair loss, piloerection, hunched posture, lethargy, ptosis and diuresis. At 750 mg/kg bw, the same symptoms were seen and were more severe than at 250 mg/kg; additionally, diarrhea, abdominal distension and an isolated case of vocalization were reported. In the satellite 750 mg/kg group, these symptoms disappeared immediately following cessation of dosing.

Body weight development: a substantial reduction in the body weight gain of rats treated with 250 and 750 mg/kg test substance became evident during the last treatment week. Four females of the 750 mg/kg group showed weight losses during this period. Cessation of dosing resulted in a rapid recovery and normalization of the body weight gain as demonstrated in the satellite 750 mg/kg group.

Food and water consumption: whereas the food consumption was similar in all groups, a marked reduction in food efficiency during the last week of dosing was reported for the 250 and the 750 mg/kg groups, which was related to the adverse body weight effects (see above). Food efficiency turned back to normal in the 750 mg/kg satellite group following cessation of dosing. Water consumption was similar and inconspicuous in all groups.

Hematology, blood chemistry and urinalysis: a statistically significant increase in leukocyte counts, mainly related to lymphocyte fraction, was reported for the males of the 750 mg/kg group and at a lesser extent for those of the 250 mg/kg group. A slight increase in lymphocytes also was reported for the females of both the 250 and the 750 mg/kg groups. The hemoglobin levels were reduced in the males and females of the 750 mg/kg group as well as in the females of the 250 mg/kg group. The erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin also were reduced. Platelets also were slightly reduced in the females of the 750 mg/kg group, but the clotting potential was unaffected.

In the males of the 750 mg/kg satellite group, a slight reduction in hemoglobin persisted until the end of the post exposure period but was within the normal range.

Blood chemistry: The investigated blood parameters (cholesterol, gamma glutamyl transpeptidase, alkaline phosphatase, bilirubin, triglycerides, glucose, creatinine, calcium, aspartate aminotransferase) appeared to be significantly influenced by the treatment with 250 and 750 mg/kg test substance. These changes taken together were indicative of hepatic and renal abnormalities. E.g. the increased bilirubin levels were indicative for

cholestatic hepatic injury whereas the elevated creatinine levels associated to the increased blood urea levels were likely indicating renal obstruction.

The investigation of the blood parameters in the rats of the 750 mg/kg satellite group at the end of the post exposure period revealed a slight increase in cholesterol and in calcium in respectively females and males; the levels however were within the normal range and indicated reversibility.

Urinalysis: The incidence of ketones in the urine was increased in both males and females of the 750 mg/kg group, and in the males of the 250 mg/kg group. Furthermore, males and females of the 750 mg/kg group showed increased urine volume and reduced specific gravity. The urinalysis of the rats of the 750 mg/kg satellite group particularly at the end of the post exposure period demonstrated the reversibility of these findings.

Necropsy: The necropsy of the control animals, which were sacrificed on day 29 revealed no macroscopic abnormalities.

In the 50 mg/kg group, excepted for two cases (1 male and 1 female) of multiple dark foci in lungs, no abnormalities were seen.

In the 250 mg/kg group one case (male) of isolated dark foci in lungs as well as one case (female) of pale adrenals were reported.

In the 750 mg/kg group, all males showed enlarged liver, very pale adrenals and small testes. Three females displayed ventral fur loss and brown staining of the ano-genital area. One of them had a distended abdomen whereas another had liquid feces. All females had enlarged, sometimes dark liver and the adrenals were pale.

In the satellite control group, necropsy of the males revealed no abnormalities whereas two females showed lesions within the lungs such as congestion.

In the satellite 750 mg/kg group, one male showed multiple dark foci in the lungs and small testes. One female displayed general fur staining, patchy pallor of the liver and dilated blood vessels in the stomach with extensive hemorrhage and ulceration within the glandular part of the stomach and white thickening of the forestomach. The small intestine of this female also displayed dilated blood vessels and contained blood stained fluid contents. The ileum and caecum of this animal showed compacted contents and the large intestine was empty.

Organ weighing revealed a marked increase in relative liver weights for rats of both sex and of both, the 250 and 750 mg/kg groups. The relative kidney weights also were increased in males and females of the 750 mg/kg group, and in males of the 250 mg/kg group. In the satellite 750 mg/kg group, the liver still were increased in weight after two weeks without dosing; in contrast, kidney weights turned back to normal.

Histopathology revealed treatment-related changes in liver, kidney and testes. In the liver of two females of the 750 mg/kg group, periportal hepatocyte vacuolization was reported. The kidney of males and females of the 750 mg/kg groups displayed basophilia, sometimes accompanied by dilatation of distal tubules. The males of the 750 mg/kg group displayed a reduction in testicular size (mostly bilateral) that was identified

microscopically as testicular atrophy (for details see Table 10 below). The testicular atrophy was also seen in males of the 750 mg/kg satellite group.

The study revealed a LOAEL of 250 mg/kg bw/day and a NOAEL of 50 mg/kg bw/day. [3]

Table 1: Body and testis weight development

Dose group/parameter	0 mg/kg	0 mg/kg satellite	50 mg/kg	250 mg/kg	750 mg/kg	750 mg/kg satellite
Body weight						
-day 0 [g]	161 ± 8	175 ± 12	168 ± 8	170 ± 8	175 ± 9	161 ± 6
-at end of study [g]	378 ± 15	468 ± 35	372 ± 27	360 ± 30	332 ±13	378 ± 15
Testis weight						
Testis weight (absolute) [g]	3.39 ± 0.27	4.64 ± 0.56	3.92 ± 0.30	3.91 ± 0.30	3.09 ± 0.41	3.61 ± 0.87
Testis weight (% body weight)	1.04 ± 0.10	0.97 ± 0.06	1.05 ± 0.08	1.06 ± 0.07	0.91 ± 0.12	0.85 ± 0.23

Table 2: Incidences and grading of testicular atrophy

Dose group/ finding	0 mg/kg	0 mg/kg satellite	50 mg/kg	250 mg/kg	750 mg/kg	750 mg/kg satellite
Number of animals	5	5	5	5	5	5
Atrophy testis 1						
Grade 1	0	0	4	4	1	0
Grade 2	0	0	1	1	2	2
Grade 3	0	0	0	0	1	0
Grade 4	0	0	0	0	1	0
Grade 5	0	0	0	0	0	1
Atrophy testis 2						
Grade 1	0	0	1	0	0	1
Grade 2	0	0	0	0	2	1
Grade 4	0	0	0	0	1	0
Grade 5	0	0	0	0	0	1

Legend: grade 1: minimal; grade 2: slight; grade 3: moderate; grade 4: marked; grade 5: severe

5.6.1.2 90-day study with emphasis on neuropathology

In a 90-day study in accordance with the EPA-TSCA guideline “functional observational battery” and “neuropathology”, Wistar rats were exposed to the test substance by oral gavage. At the beginning of the experiment, the animals were 42 days old and had a mean body weight of 191 g (males) and 156 g (females). The rats were randomly distributed into 4 test groups of 20 animals each (10 per group). The test substance was administrated as a 0.5% aqueous carboxymethyl cellulose solution as 0, 100, 300 and 1000 mg/kg bw/day. During the whole experimental period, the rats were observed for mortality, clinical signs of toxicity, body weight development, and food consumption. Additionally, specific neurofunctional tests were performed at the following time points:

1. On day 3 prior to start of the experiment
2. 1, 6 and 24 hours following the first dose
3. On days 7, 14, 42, 63 and 91 of the study

These tests considered different symptoms, e.g. tremors, convulsions, lacrimation, salivation, piloerection, vocalization, paresis, paralysis and ataxia. They also considered impairments referring to, e.g., posture, locomotor activity, respiration, winking and righting reflexes, behavior, grip strength, olfaction/audition, pain perception, tail pinch and toe pinch.

Blood sampling was performed on day 33 and day 87 and served for the investigation of: (1) Hematological parameters (e.g. leucocytes and erythrocytes count, mean corpuscular volume, and hemoglobin concentration), and (2) Biochemical parameters (e.g., enzymes such as alanine aminotransferase, alkaline phosphatase and serum gamma-glutamyltransferase, and further blood biochemical parameters such as sodium, potassium. creatinine, urea, glucose, bilirubin and cholesterol). A clotting analysis (Hepato Quick´s test) also was done.

At the end of the experimental period, the (surviving) rats were sacrificed for the purpose of necropsy.

The results of the study can be summarized as follows:

Mortality: Two females of the 1000 mg/kg group died during the experimental period.

Clinical signs of toxicity: the females of the 1000 mg/kg group showed a reduced general state of health. Lesions on the hairless skin of the extremities and reddening and scale formation on the ears were reported for both males and the females of the 1000 mg/kg group. In the males of both the 300 and the 1000 mg/kg group, the testes were reduced in size.

Body weight gain and food consumption: An increase in food consumption was reported for the females of the 1000 mg/kg, but for both, the males and the females, body weight was reduced. At 300 mg/kg, body weight reduction only concerned the males.

Hematological/biochemical parameters: In the females of the 1000 mg/kg group, erythrocytes, hemoglobin, hematocrit and thromboplastin time (Hepato Quick test) were decreased. In contrast, leucocytes, platelets, eosinophilic granulocytes and neutrophilic polymorphonuclears were increased. The alkaline phosphatase and gamma-glutamyltransferase were increased, as well as calcium, total protein, globulins and cholesterol. The triglycerides were decreased. In the males of the 1000 mg/kg group, the alkaline phosphatase, gamma-glutamyltransferase and alanine aminotransferase were increased, whereas the triglycerides were decreased. At 300 mg/kg, the hemoglobin and hematocrit of the females were decreased whereas the leucocytes, eosinophilic granulocytes, neutrophilic polymorphonuclears and calcium content of the blood were increased.

Neurotoxic effects: Neither functional defects nor any other signs of neurotoxicity could be observed.

Necropsy: necropsy revealed increased absolute kidney and liver weights in the females of the 1000 mg/kg group. In males, the absolute and relative testes weights were decreased (see table below) and histopathology revealed marked diffuse atrophy of the testicular parenchyma. Furthermore, the females displayed skin lesions such as scaling. At 300 mg/kg, necropsy revealed decreased absolute and relative testes weights as well as diffuse atrophy of the testicular parenchyma (see table below). The testicular findings were considered to be substance related, although a clear dose-response relationship was not visible: as can be seen in the tables below, there is no significant difference between the absolute and relative testes weights of the 300 and 1000 mg/kg dose groups. The grading of the testicular atrophy of these dose groups is also comparable.

In the 100 mg/kg dose group, one animal exhibited moderately reduced spermiogenesis. All animals of this dose group showed a minimal to moderate vacuolar degeneration of spermatogonia in some seminiferous tubules. These lesions and the focal atrophy findings were also seen in the control group up to the same grading and are not considered to be substance related. The moderately reduced spermiogenesis of one animal in the 100 mg/kg dose group with no convincing transitional steps up to a diffuse atrophy as seen in the 300 and 1000 mg/kg dose groups is interpreted as an incidental, spontaneous occurring event and not considered to be induced by the test substance.

The NOAEL established by the study was considered to be 100 mg/kg bw/day. [4]

The following tables show the individual results of the pathology report:

Table 3: Body and testes weight development; results of testes pathology

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	--	--

Table 4: Incidence and grading of microscopic findings

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)	--	--	--	--

5.6.1.3 Confirmatory 28-day study in conjunction with a 90-day study

A 28-day and a 90-day repeated dose study was carried out with 41-43 day old male Wistar rats (3 and 10 per group for the 28-day and 90-day study, respectively). This confirmatory study was conducted to reproduce the above stated effects on the testes with a new batch of the test substance. The purity of the substance was determined to be 99.3%. Doses were 0 and 1000 mg/kg bw/day of the test substance in carboxymethyl cellulose (0.5% aqueous solution) which were applied to the animals 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 assessed for the whole body and particularly for the testes. Gross lesions as well as the testes were withdrawn, fixed in 4% formaldehyde, embedded in paraffin, sectioned and hematoxylin-eosin stained for histological examination.

Result of the 28-day test:

The terminal mean body weight for the treated group was 244.833 g versus 251.833 g for the control group. For the testes, the absolute mean weight was 3.193 g versus 3.14 g, whereas the relative mean weight was 1.307 g versus 1.249 g. This indicates no substance-related effects of the weight parameters. No gross or histopathologic lesions were observed.

Result for the 90-day test:

The terminal mean body weight for the treated group was 298.1 g versus 332.21 g for the control group. For the testes, the absolute mean weight was 2.1 g versus 3.286 g, whereas the relative mean weight was 0.718 g versus 0.996 g, which is indicative of a substance-related effect on these weight parameters. Moreover, the testes of 8/10 rats were reduced in size compared to control; they also showed a loss of turgor. The reduction in size also affected the epididymes. In one case, the thoracic region of the rat showed sparse hair. From a histopathological point of view, a slight to severe diffuse atrophy (mostly bilateral) of the seminiferous tubules of the testes was seen in all animals. In four cases, edemas as well as a minimal to slight hyperplasia of the Leydig cells were also seen. In the epididymes with reduced size, histopathology revealed oligo- to azoospermia (i.e. reduction in or absence of mature sperms). The following tables show the individual results of the ten animals that were subjected to a macroscopic and histopathological examination. Lesions were bilateral unless indicated otherwise [5]:

Table 5: Macroscopic findings

Reduction in organ size	**slight**	**moderate**
Testes	1/10	7/10
Epididymes	8/10	--

Loss of turgor	8/10

Table 6: Histopathological findings

Grading/finding	1 (minimal	2 (slight)	3 (moderate)	4 (marked)	5 (severe)
Epididymes: reduction/absence of mature sperm	--	--	1/10	6/10	1/10
Testes: atrophy of seminiferous tubules	--	1/10	3/10	6/10	--
Testes: Leydig cell hyperplasia	3/10*	1/10	--	--	--
Intestinal edema	2/10*	2/10	--	--	--

* one unilateral case

5.6.2 Repeated dose toxicity: inhalation

No data available.

5.6.3 Repeated dose toxicity: dermal

No data available.

5.6.4 Other relevant information

No data available.

5.6.5 Summary and discussion of repeated dose toxicity

The first 28-day subacute study revealed a number of adverse effects on body weight development, food and water consumption, blood, liver and kidney. A number of clinical signs of toxicity, macroscopic abnormalities and histopathological treatment-related changes in the liver, kidney and testes were also reported. Not only did some of these changes occur exclusively in the highest dose group (750 mg/kg bw/day), most changes were also reversible as demonstrated by a satellite group that was observed for 14 days after the cessation of treatment. A 90-day study that was intended to look for neuropathological effects of the test substance also showed that treatment with Lucirin TPO leads to atrophy of the testes whereas neither neurotoxic signs nor functional defects of the animals were reported. The NOAEL determined by this study was 100 mg/kg bw/day Lucirin TPO whereas the LOAEL was 300 mg/kg bw/day. The temporary unavailability of the report of the initial 28-day study prompted an investigation of these effects in a second 28-day study with a new batch of the test substance. In contrast to the first test, no adverse effects were observed on the body and testis weight of rats treated with 1000 mg/kg bw/day of Lucirin TPO. Histopathology, too, did not reveal any lesions. The 90-day study that was conducted with the same dose and batch of test substance,

however, did reveal a reduction in size of the testes and epididymes, which was substantiated by the histopathologic lesions found.

Based on the results of one 28-day and the 90-day studies there is clear evidence for lesions of the testes as the only indication that Lucirin TPO may lead to reduced fertility of the animals. The conclusion therefore is included in section 5.9.5 Summary and discussion of reproductive toxicity.

5.7 Mutagenicity

5.7.1 In vitro data

Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and Escherichia coli strain WP2UvrA$^-$ were treated with Lucirin LR 8728 by the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolizing system. The dose range was determined in a preliminary toxicity assay and was 8 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day using different cultures of the bacterial strains and fresh chemical solutions. In this case the dose range of Lucirin LR 8728 was 312.5 to 5000 µg/plate. The solvent (ethanol) control plates gave counts of revertant colonies within the normal range. Positive control chemicals were N-methyl-N´-nitro-N-nitrosoguanidine (TA 1535, TA 100), 9-aminoacridine (TA 1537), 4-nitro-o-phenylendiamine (TA 98) and 4-nitroquinoline N-oxide (E. coli WP2 uvrA), and in case of metabolic activation, 2-aminoanthracene (all strains). All positive control chemicals gave increases in revertants both with and without a metabolizing system within expected ranges. Lucirin LR 8728 caused no reduction in the growth of the bacterial lawn at any dose level either with or without metabolic activation. Lucirin LR 8728 was therefore tested up to the maximum dose level of 5000 µg/plate. No significant increase in the numbers of revertant colonies was recorded for any of the bacterial strains at any dose level either with or without the addition of a metabolizing system. Therefore, the test substance was considered to be non-mutagenic under the test conditions chosen. [6]

In a standard plate Ames test with Salmonella typhimurium strains TA1535, TA100, TA1537, TA1538, and TA98, Initiator 554 was used in the dose range from 4 – 2500 µg/plate. Two experiments were done with 4 test plates per dose or per control. 0.1 ml of the bacterial suspension was added to 0.1 ml of test substance solution and, in case of metabolic activation, 0.5 ml of S-9 mix. The mixture was then added to 2ml of soft agar in test tubes kept in a water bath at 45 °C. The whole mixture was then poured onto minimal agar plates. After incubation in the dark at 37°C for 48 hours, the his+ revertant bacterial colonies were counted. Methanol was used as the solvent in which the test substance was completely soluble. A bacteriotoxic effect (reduced his$^-$ background growth) was observed with S9-mix (all tester strains) and without S-9 mix (TA1535 only) at 2500 µg/plate. An increase in the number of his$^+$ revertants was not observed either without S-9 mix or after the addition of a metabolizing system. According to the results of this study, the test substance is therefore considered non-mutagenic in the Ames test under the conditions chosen. [7]

In a chromosomal aberration test according to Japanese MOL/MHW/MITI, Chinese hamster lung (CHL) cells were treated with Lucirin LR 8728 at four dose levels, in duplicate. The dose range was selected based on the results of three preliminary toxicity tests and was 15 to 25 µg/ml for the 6-hour treatment without S-9, 20 to 30 µg/ml for the 6-hour treatment with S-9, 5 to 20 µg/ml for the 24-hour treatment and 2 .5 to 20 µg/ml for the 48-hour treatment.
Lucirin LR 8728 was accurately weighed and dissolved in dimethyl sulfoxide and appropriate dilutions were made. Negative and positive controls were used in parallel with the test material. Solvent treatment groups were used as the negative controls and the positive control materials were as follows: Mitomycin C 0.075 µg/ml for cultures treated for 24 or 48 hours In the absence of metabolizing enzymes. Cyclophosphamide (CP) 10 µg/ml for cultures treated for 6 hours both with and without S-9 mix.
Cultures were established approximately 24 hours prior to treatment, 0.5×10^6 cells and 0.25×10^6 cells were seeded per flask for 24 and 48-hour cultures, respectively. The cells were exposed to four doses of the test material, negative and positive controls, both with and without metabolic activation.

The treatment regimens were as follows:

A. Without Metabolic Activation:

1) 6 hours exposure to the test material, a phosphate buffered saline wash and then a further 18 hours culture in treatment-free media prior to cell harvest.
2) 24 hours continuous exposure to the test material prior to cell harvest.
3) 48 hours continuous exposure to the test material prior to cell harvest.

B. With Metabolic Activation:

1) 6 hours exposure to the test material and S-9 mix (0.5 ml per 4.5 ml culture medium, of 10% S-9 in standard cofactors). A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest.

After cell harvest, the cells were fixed, left to air-dry on slides, and stained in 2% Gurrs Giemsa R66. Where possible the first 100 consecutive well-spread metaphases from each culture were counted, and if the cell had 23 to 27 chromosomes, any gaps, breaks or rearrangements were noted according to the simplified system of Savage (1976) recommended in the UKEMS guidelines for mutagenicity testing.
The negative (solvent) controls gave frequencies of aberrations within the range expected for the CHL cell line. All the positive control treatments, except cyclophosphamide without S-9, gave highly significant increases in the frequency of aberrations indicating the satisfactory performance of the test and of the activity of the metabolizing system.
Lucirin LR 8728 demonstrated no significant, dose-related increases in the frequency of aberrations in any of the treatment cases. Lucirin LR 8728 was shown to be highly toxic to CHL cells in vitro in all four treatment cases, with a very steep dose-response curve. Lucirin LR 8728 was shown to be non-clastogenic to CHL cells in vitro. [8]

5.7.2 In vivo data

No data available.

5.7.3 Human data

No data available.

5.7.4 Other relevant information

No data available.

5.7.5 Summary and discussion of mutagenicity

The available in vitro data show that Lucirin TPO is non-mutagenic in the Ames test as well as non-clastogenic in a chromosomal aberration test in CHL cells.

5.8 Carcinogenicity

Carcinogenicity has not been considered here due to lacking data.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

See section 5.6 Repeated dose toxicity.

5.9.2 Developmental toxicity

See section 5.6 Repeated dose toxicity.

5.9.3 Human data

No data are available.

5.9.4 Other relevant information

No data available.

5.9.5 Summary and discussion of toxicity for reproduction

Based on the results of one 28-day and the 90-day studies there is clear evidence for lesions of the testes as the only indication that Lucirin TPO may lead to reduced fertility of the animals. Due to an administration of the test substance by oral gavage, it cannot be excluded that this may have been a bolus effect. To investigate the toxicokinetics of the substance by a more continuous exposure of the animals, the design of the study was

changed to administration of the test substance via the feed. However, the rats avoided the Lucirin TPO-treated feed for palatability reasons, which led to excessive weight loss of the animals such that they had to be sacrificed on humane grounds. An additional encapsulation to improve acceptance of the test substance did not solve the palatability problem.

Classification:

According to the classification criteria of 67/548/EEC as well as the GHS, classification with Cat. 2 or Cat. 1B, respectively, requires demonstration of the impairment of fertility in *in vivo* studies. As clear evidence for testes lesions is a valid but nevertheless only indirect indicator that Lucirin TPO may lead to reduced fertility, classification with either Repro. Cat. 3, R62 (EU criteria) and Repro Cat. 2 (GHS criteria) seems appropriate.

Moreover, the testes lesions were only found after gavage but not diet application because of reasons of test substance palatability.

Endpoint	**Quantitative dose descriptor (appropriate unit) or qualitative assessment**		**Associated relevant effect**	**Remarks on study**
	Local effect	Systemic effect		
Repeated dose toxicity sub-chronic oral	--	NOAEL 100 mg/kg bw/day LOAEL 300 mg/kg bw/day	-- Atrophy of the testes	[9]

5.10 Other effects

Not relevant for this dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES

Not relevant for this dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier.

8 PBT, vPvB AND EQUIVALENT CONCERN ASSESSMENT

Not relevant for this type of dossier.

9 JUSTIFICATION FOR ACTION AT COMMUNITY LEVEL

It is proposed that the substance is classified as Repro. Repro. Cat. 3, R62. Harmonised classification and labelling for reprotoxicants is considered a Community-wide action under Article 114 and it is recommended that the classification proposal is considered for inclusion on Annex I of Directive 67/548/EEC.

OTHER INFORMATION

This substance will be registered under REACH. The producer company has been contacted during the production of this dossier and they provided the available industry reports for the substance.

References

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[5] BASF AG (2001). Studie zur Hodentoxizität in Wistar-Ratten. Verabreichung per Schlundsonde bis zu 3 Monaten. Department of Toxicology, unpublished data, report No. 51C0293/99096, 19 Jan 2001.

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[7] BASF AG (1979). Report on the study of Initiator 554 in the Ames test. Department of Toxicology, unpublished data, report No. 79/85, 12 June 1979.

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[9] BASF AG (2001). Studie zur Hodentoxizität in Wistar-Ratten. Verabreichung per Schlundsonde bis zu 3 Monaten. Department of Toxicology, unpublished data, report No. 51C0293/99096, 19 Jan 2001.