Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

EC Number: 278-355-8

CAS Number: 75980-60-8

Index Number: 015-203-00-X

Contact details for dossier submitter:

Swedish Chemicals Agency

Esplanaden 3a, P.O Box 2

SE-172 13 Sundbyberg, Sweden

kemi@kemi.se

+46 8 519 41 100

Version number: 1.0 **Date: 2020-06-30**

CONTENTS

1 PHYSICAL HAZARDS

Not relevant.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

There are no studies available.

3 HEALTH HAZARDS

3.1 Skin sensitisation

3.1.1 Animal data

3.1.1.1 Skin sensitisation. in vivo. Key study 2012

Study reference:
Study report (2012) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:
Test type
Skin sensitisation: in vivo (LLNA) according to OECD Guideline 429 (Skin Sensitisation: Local Lymph Node Assay) adopted July 22nd, 2010; EU Method B.42 (Skin Sensitisation: Local Lymph Node Assay) adopted May 30th, 2008. GLP compliant.

Test substance
- (Diphenylphosphinyl)-(2,4,6-trimethylphenyl)methanone.
- Degree of purity – 99.5% (w/w).
- Impurities – not recorded.
- Batch number – 110053.
- Physical state – yellowish solid.

Test animals
- Species – mouse; strain – CBA mouse; sex – female.
- No. of animals per sex per dose – 5 (2 in the range finding test).
- Age at study initiation – Pretest: 9-10 weeks; main experiment: 8-9 weeks.
- Weight at study initiation – 18.6 - 22.5g.

Administration/exposure
- Induction
  Concentration: 10%, 25%, 50% (w/w) (verified analytically).
  induction vehicle (acetone/olive oil (4:1 v/v)).
- Sonicating and warming to 37°C were used to formulate the test item. Preparations were freshly made before dosing.

Results and discussion

- LLNA endpoints – stimulation index (SI); EC3; disintegrations per minute.
  No significant increase in ear weights, as well as no signs of local or systemic toxicity were observed.
- The test substance was found to be a sensitizer, the EC3 value was calculated to be 27%.

Parameter	Test group	Value
SI	10%	2.22
SI	25%	2.96
SI	50%	3.45
Disintegrations per minute	control	792.1
Disintegrations per minute	10%	1757.5
Disintegrations per minute	25%	2347.1
Disintegrations per minute	50%	2742.3

For the LLNA study, provide the following additional information:

- statistical comparisons of groups mean DPMs compared to controls – not recorded.

3.1.2 Human data – no data

3.2 Reproductive toxicity

3.2.1 Animal data

3.2.1.1 Toxicity to reproduction. Key study 2019

Study reference:

Study report (2019a) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:

Test type

Reproduction / Developmental Toxicity Screening Test according to OECD Guideline 421, 2016; according to EPA OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test 2000. GLP compliant.

Test substance

- Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.
- Degree of purity - 99.32%.
- Test item storage – At room temperature.

Test animals

- Species - rat common species; Strain - Wistar rat; Sex - male/female.
- The Wistar Han rat was chosen as the animal model for this study as it is an accepted rodent species for toxicity testing by regulatory agencies. Charles River Den Bosch has general and reproduction/developmental historical data in this species from the same strain and source. This animal model has been proven to be susceptible to the effects of reproductive toxicants.

- 5 animals per sex per dose.
- Weight at study initiation: males 134 - 173 g, females: 105 - 151 g.

Administration/exposure

- Route of administration – oral, gavage.
- The test item and vehicle were administered to the appropriate animals by once daily oral gavage.
- Dosing - 7 days a week for a minimum of 12 weeks.
- Doses – 60, 200, 600 mg/kg bw/day (actual dose received).
- Control group - concurrent vehicle.
- Vehicle: water 1% Aqueous carboxymethyl cellulose .
  - Water (Elix) - Supplier: Millipore, Molsheim, France.
  - Carboxymethyl cellulose – Supplier: Fagron, Capelle aan de IJssel, The Netherlands.
- Test item dosing formulations (w/w) were homogenized to visually acceptable levels at appropriate concentrations to meet dose level requirements. The dosing formulations were prepared daily as a suspension and dosed within 5 hours after completion of the preparation of the formulation. Details of the preparation and dispensing of the test item have been retained in the Study Records. Item dosing formulations were kept at room temperature until dosing. From 16 Jul 2018 onwards, dosing formulations were protected from light. If practically possible, the dosing formulations and vehicle were continuously stirred until and during dosing. No adjustment was made for specific gravity of the vehicle and test item. No correction was made for the purity/composition of the test item. Any residual volumes were discarded.

Description of test design:

- Premating exposure period for males and females – 10 weeks.
- After 10 weeks of treatment, animals were cohabitated on a 1:1 basis within the same treatment group, avoiding sibling mating.
- Detection of mating was confirmed by evidence of sperm in the vaginal lavage or by the appearance of an intravaginal copulatory plug.
- This day was designated Day 0 post-coitum.
- Once mating had occurred, the males and females were separated.
- A maximum of 14 days was allowed for mating, after which females who have not shown evidence of mating were separated from their males. Since less than 9 females in Group 4 showed evidence of mating, each non-mated female was re-mated once with a male of proven fertility of the same group for a maximum of 7 days.
  - Males were treated for 85-92 days, up to and including the day before scheduled necropsy. This included a minimum of 10 weeks prior to mating (with the objective of covering at least one spermatogenic cycle) and during the mating period.
  - Females that delivered were treated for 113-127 days, i.e. 10 weeks prior to mating (with the objective to cover at least two complete oestrous cycles), the variable time to conception, the duration of pregnancy and at least 20 days after delivery, up to and including the day before scheduled necropsy.
  - Females which failed to deliver or had a total litter loss were treated for 99-117 days.
- To reduce variability among the litters, on PND 4 eight pups from each litter of equal sex distribution (if possible) were selected. Blood samples were collected from two of the surplus pups (if possible, from one male and one female pup). Selective

elimination of pups, e.g. based upon body weight or AGD, was not done. Whenever the number of male or female pups prevented having four of each sex per litter, partial adjustment (for example, five males and three females) was acceptable.

- Parameters assessed for F0 generation:
  - Mortality/Moribundity Checks – Throughout the study, animals were observed for general health/mortality and moribundity twice daily, in the morning and at the end of the working day. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings. Animals showing pain, distress or discomfort which was considered not transient in nature or is likely to become more severe, were sacrificed for humane reasons based on OECD guidance document on humane endpoints (ENV/JM/MONO/ 2000/7). The circumstances of any death were recorded in detail.
  - Clinical Observations – Clinical observations were performed at least once daily, beginning during the first administration of the test item and lasting throughout the dosing periods up to the day prior to necropsy. During the dosing period, these observations were performed at least after dosing. The time of onset, grade and duration of any observed sign was recorded. Signs were graded for severity and the maximum grade was predefined at 3 or 4. Grades were coded as slight (grade 1), moderate (grade 2), severe (grade 3) and very severe (grade 4). For certain signs, only its presence (grade 1) or absence (grade 0) was scored. In the data tables, the scored grades were reported, as well as the percentage of animals affected in summary tables.
  - Body Weights – Animals were weighed individually on the first day of treatment (prior to dosing), and weekly thereafter. Mated females were weighed on Days 0, 4, 7, 11, 14, 17, and 20 post-coitum and during lactation on PND 1, 4, 7, 13 and 21 (see deviation in Appendix 7). A terminal weight was recorded on the day of scheduled necropsy.
  - Food Consumption – Food consumption was quantitatively measured weekly, except for males and females which were housed together for mating and for females without evidence of mating. Food consumption of mated females was measured on Days 0, 4, 7, 11, 14, 17, and 20 post-coitum and during lactation on PND 1, 4, 7, 13 and 21.
  - Water Consumption – Water consumption was monitored on regular basis throughout the study by visual inspection of the water bottles.
  - Functional Tests – Functional tests were performed on the selected 5 males and females during the last week of the premating period (i.e. week 10 of treatment). These tests were performed after completion of clinical observations. The following tests were performed (abbreviations mentioned in the respective tables are indicated between brackets):
    - Hearing ability (HEARING) (Score 0 = normal/present, score 1 = abnormal/absent).
    - Pupillary reflex (PUPIL L/R) (Score 0 = normal/present, score 1 = abnormal/absent).
    - Static righting reflex (STATIC R) (Score 0 = normal/present, score 1 = abnormal/absent).

- Fore- and hind-limb grip strength, recorded as the mean of three measurements per animal (Series M4-10, Mark-10 Corporation, J.J. Bos, Gouda, The Netherlands).
- Locomotor activity (recording period: 1-hour under normal laboratory light conditions, using a computerized monitoring system, Kinder Scientific LLC, Poway, USA). Total movements and ambulations were reported. Ambulations represent movements characterized by a relocation of the entire body position like walking, whereas total movements represent all movements made by the animals, including ambulations but also smaller or finer movements like grooming, weaving or movements of the head.

- Cohabitation/Mating Procedure – After 10 weeks of treatment, animals were cohabitated on a 1:1 basis within the same treatment group, avoiding sibling mating. Detection of mating was confirmed by evidence of sperm in the vaginal lavage or by the appearance of an intravaginal copulatory plug. This day was designated Day 0 post-coitum. Once mating had occurred, the males and females were separated. A maximum of 14 days was allowed for mating, after which females who have not shown evidence of mating were separated from their males. Since less than 9 females in Group 4 showed evidence of mating, each non-mated female was re-mated once with a male of proven fertility of the same group for a maximum of 7 days.
- General Reproduction Data – From the mating period onwards, the following parameters were recorded for each female: male number paired with, mating date, confirmation of pregnancy and delivery day. Females were allowed to litter normally. Postnatal day (PND) 1 was defined as the day when a litter was found completed (i.e. membranes and placentas cleaned up, nest built and/or feeding of pups started). The day prior to PND 1 was considered to be the day when the female started to deliver and is defined as PND 0 and used for recording of delivery. Females that were littering were left undisturbed. Cage debris of pregnant females was examined for evidence of premature delivery and pregnant females were examined to detect signs of difficult or prolonged parturition or deficiencies in maternal care.
- Oestrous cycle determination – Oestrous cycles were evaluated by examining the vaginal cytology of samples obtained by vaginal lavage. Daily vaginal lavage was performed for all females beginning 14 days prior to the mating period, and during mating until evidence of copulation was observed. Vaginal lavage was continued for those females with no evidence of copulation until termination of the mating period. On the day of necropsy, a vaginal lavage was also taken to determine the stage of oestrus. This was done for all females, except for females that had to be euthanized in extremis or died spontaneously.

- Parameters assessed for F1 generation
  - Mortality/Moribundity Checks – Pups were observed daily for general health/mortality. The number of live and dead pups was determined on PND 1 and daily thereafter. Pups were not removed from the cage during observation, unless necessary for identification or confirmation of possible findings.
  - Clinical Observations – Clinical observations were performed at least once daily for all pups. Only days on which clinical signs were present between the first and last litter check were given in the respective report tables.

- Body Weights – Live pups were weighed individually on PND 1, 4, 7, 13 and 21.
- Sex – Sex was externally determined for all pups on PND 1 and 4.
- Anogenital Distance – Anogenital distance (AGD) was measured for all live pups on PND 1. The AGD was normalized to the cube root of body weight.
- Areola/Nipple Retention – All male pups in each litter were examined for the number of areola/nipples on PND 13.
- Culling – To reduce variability among the litters, on PND 4 eight pups from each litter of equal sex distribution (if possible) were selected. Blood samples were collected from two of the surplus pups (if possible, from one male and one female pup). Selective elimination of pups, e.g. based upon body weight or AGD, was not done. Whenever the number of male or female pups prevented having four of each sex per litter, partial adjustment (for example, five males and three females) was acceptable.

- Clinical Pathology
  - Blood of F0-animals (except for animals which were sacrificed in extremis or found dead and females with total litter loss) was collected on the day of scheduled necropsy. Samples were collected, between 7.00 and 10.30 a.m., from the retro-orbital sinus under anaesthesia using isoflurane in the animal facility. Both F0-males and F0-females were not fasted overnight before scheduled necropsy.
  - Blood of F1-animals was collected on PND 4 and PND 21-23, if possible. This was performed in the necropsy room. On PND 4 at culling, blood was collected from two surplus pups per litter (if possible) by decapitation, between 7.00 and 10.30 a.m., and samples were pooled to one sample per litter. If available, blood was collected from one male and one female pup. If only one surplus pup per litter was available at culling, as much blood as possible was collected from this single pup. On PND 21-23, separate blood samples were collected from two pups per litter (from one male and one female). Blood was drawn, between 7.00 and 10.30 a.m., by aorta puncture under anaesthesia using isoflurane as part of the necropsy procedure.

Group No.	**No. Animals**		**Scheduled Euthanasia**	**Necropsy procedures**			**Histology and histopathology**
	M	**F**		**Necropsy**	**Tissue collection**	**Organ weights**	
1	≤ 10	≤ 10	Males: after a minimum of 12 weeks of administration Females: PND 21-23	X	X[1]	X[a]	Full list (+ remaining reproductive tissues[c])
2	≤ 10	≤ 10					Gross lesions, Target tissues[2], Reproductive tissues[c]
3	≤ 10	≤ 10					Gross lesions, Target tissues[b],

[1] See Tissue Collection and Preservation table for listing of tissues.

[2] The testes and epididymides of all males and thyroid gland of all males and females of Groups 2 and 3, based on possible treatment-related changes in these tissues.

							Reproductive tissues[3]
4	≤ 10	≤ 10					Full list (+ remaining reproductive tissues[c])
Unscheduled deaths (sacrificed in extremis or found dead)				X	X	X	Full list

X = Conducted procedure; - = Not applicable.

- Thyroid hormone - Blood samples at a target volume of 0.9 mL (F0-males), 1.0 mL (F0-females), 0.5 mL (pooled PND 4 pups) and 1.0 mL (PND 21-23 pups) were collected into tubes without anticoagulant. Blood samples were processed for serum, and serum was analysed for total Thyroxine (T4). Measurement of total T4 was conducted for F0-males and PND 21-23 pups. For the F0-generation, assessment of T4 (females) and Thyroid Stimulating Hormone (TSH; both sexes) was considered not relevant because no adverse effects on thyroid histopathology and no treatment-related changes in thyroid weight were recorded. Assessment of T4 for PND 4 pups and TSH for PND 21-23 pups was considered not relevant because no treatment-related changes in T4 were noted in pups at PND 21-23. Serum samples retained for possible future analysis were maintained by the Test Facility in the freezer (≤-75°C). Under these storage conditions, samples are stable for 6 months. Any remaining samples will be discarded after 6 months.

- Terminal procedures for F0 generation
  - Unscheduled Deaths – necropsy was conducted for animals that died on study, and specified tissues were saved. If necessary, for humane reasons, animals were euthanized as per Test Facility SOPs. These animals were deeply anaesthetized using isoflurane and subsequently exsanguinated. They underwent necropsy, and specified tissues were retained but not weighed.
  - Scheduled Euthanasia – Animals surviving until scheduled euthanasia were weighed, and deeply anaesthetized using isoflurane and subsequently exsanguinated and subjected to a full post-mortem examination. Scheduled necropsies were conducted on the following days:
    - Males (which sired and failed to sire) – Following completion of the mating period (a minimum of 12 weeks of administration).
    - Females which delivered – PND 21-23.
    - Females which failed to deliver.
    - With evidence of mating: Post-coitum Day 25-27 (nos. 56, 68, 71, 73, 76, 77, 78, 80).
    - Without evidence of mating: 26 days after the last day of the mating period (nos. 72, 74, 79).
- Female with total litter loss – The Dam (no. 67) with no surviving pups was euthanized within 24 hours after the last pup was found dead or missing.

[3] Reproductive tissues were applicable for males that failed to sire and females that failed to deliver pups (i.e. non-mated females, non-pregnant females, females with implantation sites only or no offspring) and females with total litter loss. Based on possible treatment-related changes in these tissues, males of Groups 2 and 3 were also included. See Tissue Collection and Preservation table for listing of tissues.

- Necropsy – All animals were subjected to a full post-mortem examination, with special attention being paid to the reproductive organs. Both F0-males and F0-females were not fasted overnight before scheduled necropsy. Necropsy procedures were performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, was available. The numbers of former implantation sites were recorded for all paired females. In case no macroscopically visible implantation sites were present, non-gravid uteri were stained using the Salewski technique in order to detect any former implantation sites and the number of corpora lutea was recorded in addition.
- Organ Weights – The organs identified in the table below were weighed at necropsy for all scheduled euthanasia animals. Organ weights were not recorded for animals found dead or euthanized in poor condition or in extremis. Paired organs were weighed together. Organ to body weight ratios (using the terminal body weight) were calculated. Organs Weighed at Necropsy:
  - Epididymisa
  - Gland, coagulation[a, b]
  - Gland, parathyroid[c]
  - Gland, prostate
  - Gland, seminal vesicle[a]
  - Gland, thyroid
  - Testes[a]

[a] Paired organ weight. [b] Weighed together with the seminal vesicles. [c] Weighed together with the thyroid.

- Tissue Collection and Preservation – Representative samples of the tissues identified in the table below were collected from all animals and preserved in 10% neutral buffered formalin (neutral phosphate buffered 4% formaldehyde solution, Klinipath, Duiven, The Netherlands), unless otherwise indicated. Tissue Collection and Preservation:
  - Animal identification
  - Cervix
  - Epididymis[a]
  - Gland, coagulation
  - Gland, mammary
  - Gland, parathyroid[b]
  - Gland, pituitary
  - Gland, prostate
  - Gland, seminal vesicle
  - Gland, thyroid
  - Gross lesions/masses
  - Ovaries
  - Testes[a]
  - Uterus
  - Vagina

[a] Preserved in modified Davidson's fixative and transferred to formalin after fixation for at least 24 hours. [b] Only collected if present in the routine section of the thyroid.

- Histology – The following tissues were embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin:

- All animals: Gross lesions/masses.
- All animals of Groups 1-4: Epididymis, thyroid gland, ovaries and testes.
- Unscheduled deaths (sacrificed in extremis or found dead): Tissues identified above (except animal identification, mammary gland, parathyroid gland and pituitary gland).
- Males that failed to sire and females that failed to deliver pups: Tissues identified above (except animal identification, mammary gland, parathyroid gland and pituitary gland).
- Female with total litter loss (no. 67): Mammary gland.

- Histopathology – All tissues as defined under Histology F0-Generation were examined by a board-certified toxicological pathologist with training and experience in laboratory animal pathology. Target tissues identified by the study pathologist during microscopic evaluation were communicated to the Study Director; tissues were evaluated and reported. For the testes of all males of Groups 1 and 4, and all males that failed to sire, a detailed qualitative examination was made, taking into account the tubular stages of the spermatogenic cycle. Testes and epididymides of males and the thyroid glands from both sexes from all 60 and 200 mg/kg treated rats were also analysed, based on possible treatment-related changes in these tissues. A peer review on the histopathology data was performed by a second pathologist.

- Terminal Procedures for F1 generation
  - Method of Euthanasia – Pups, younger than 7 days were euthanized by decapitation. All remaining pups (PND 7-23), except for the two pups per litter selected for blood collection were euthanized by an intraperitoneal injection of sodium pentobarbital (Euthasol® 20%). The pups selected for blood collection on PND 21-23 were anesthetized using isoflurane followed by exsanguination.
  - Unscheduled Deaths - Pups that died before scheduled termination were examined externally and sexed (both externally and internally). The stomach of pups not surviving to the scheduled necropsy date was examined for the presence of milk, if possible. If possible, defects or cause of death were evaluated.
  - Scheduled Euthanasia – On PND 4, the surplus pups (> 8 pups per litter) were euthanized by decapitation. From two surplus pups per litter, blood was collected, if possible. For details see also section 4.11.1. All remaining pups were euthanized on PND 21-23. Sex was determined both externally and internally. Descriptions of all external abnormalities were recorded. Particular attention was paid to the external reproductive genitals to examine signs of altered development. In addition, blood was collected from two pups per litter, and the thyroid from two pups per litter (if possible one male and one female pup) was preserved in 10% buffered formalin. If possible, the pups selected for blood sampling were the same pups as selected for thyroid preservation.

- Statistical analysis - all statistical tests were conducted at the 5% significance level. All pairwise comparisons were conducted using two sided tests and were reported at the 1% or 5% levels. Numerical data collected on scheduled occasions for the listed variables were analysed as indicated according to sex and occasion. Descriptive statistics number, mean and standard deviation (or %CV or SE when deemed appropriate) were reported whenever possible. Inferential statistics were performed according to the matrix below when possible, but excluded semi-quantitative data,

and any group with less than 3 observations. The following pairwise comparisons were made:

- Group 2 vs. Group 1
- Group 3 vs. Group 1
- Group 4 vs. Group 1

- Parametric – Datasets with at least 3 groups (the designated control group and 2 other groups) were compared using Dunnett-test (many-to-one-t-test).
- Non-Parametric – Datasets with at least 3 groups was compared using a Steel-test (many-to-one rank test). The motor activity data set was compared using an overall Kruskal-Wallis.
- Incidence – An overall Fisher's exact test was used to compare all groups at the 5% significance level. The above pairwise comparisons were conducted using Fisher's exact test whenever the overall test is significant.

- Computerized systems - critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

System name	Version no.	Description of data collected and/or analysed
Tox data[a]	8.0	In-life phase (Mortality; Clinical signs; Body weights; Food consumption; Functional tests, Organ weights; Reproduction parameters; Observations pups[b]) data collection
REES centron	SQL 2.0	Temperature and humidity (animal and laboratory facilities) data collection
Motor Monitor II	15251-16GLP	Motor activity measurement data collection
Pathdata	6.2e2	Histopathology data collection
IMMULITE 1000	5.22	Thyroid hormone data collection
Deviation Information Library	2.1.61	Deviations

[a] For logistic reasons, data was captured under separate Study numbers, see Appendix 6.
[b] Only at first and last litter check, and in case of clinical pup findings also on the respective days in between.

Results and discussion

- Accuracy - The concentrations analysed in the formulations of Groups 2, 3 and 4 (samples prepared for use on 04 June 2018; Week 3) and Group 4 (samples prepared for use on 07 September 2018; Week 17) were in agreement with the target concentrations (i.e. mean accuracies between 85% and 115%). The analytical run performed by Ardena Bioanalytical Laboratory on formulation samples prepared in Week 1 (16 May 2018) was not accepted since procedural recovery samples were outside the acceptance criteria. Therefore, a second occasion for formulation sample collection and analysis of all groups was included in Week 3 (04 June 2018). No test item was detected in the Group 1 formulation.

- Homogeneity - The formulations of Group 2 and Group 4 (samples prepared for use on 04 June 2018; Week 3) were homogeneous (i.e. coefficient of variation ≤10%).
- Stability - Formulations of Group 45 (samples prepared for use on 07 September 2018; Week 17) were stable when stored either at room temperature under normal laboratory light conditions or protected from light for at least 5 hours (i.e. relative difference ≤10%).

Results: P0 (first parental animals)

- Clinical signs - effects observed, non-treatment-related
  - Treatment-related clinical signs were noted during daily detailed clinical observations. At 600 mg/kg, transient signs of abnormal behaviour and/or posture were noted during the premating period in all males and one female. Towards the end of Week 8 of treatment (premating period), all males at 600 mg/kg were noted less reactive (slightly calm/lethargic). This finding lasted for 1 week and was observed when opening the cage (before dosing) and persisted for a few minutes. One female at 600 mg/kg (no. 79) was observed on three different occasions during Week 10-11 of treatment (end of the premating period-beginning of the mating period) with transient muscle twitching in combination with hunched posture and/or piloerection on 1 or 2 occasions (directly after dosing, after handling for performing the vaginal smear or in the cage just before handling). These clinical signs were short in duration (lasting for only a few minutes), and afterwards the female completely recovered (6). Piloerection was also noted in this female no. 79 and in two other females treated at 600 mg/kg (nos. 73 and 74) for 2 to 3 consecutive days at the end of Week 13 or 16 of treatment, respectively.
  - After the second week of the mating period, female no. 79 at 600 mg/kg was additionally noted with a transient reddish liquid secretion from and around the vagina(b). At the incidence observed, this clinical sign was considered a chance finding. Salivation was observed in one control female on a single day, in several animals at 200 mg/kg and among all animals at 600 mg/kg for multiple days during the premating period (with a higher incidence and/or persistence in females) and mating period (only noted incidentally in a few males). This finding was considered to be a physiological response related to the taste of the test item rather than a sign of systemic toxicity considering the nature and minor severity of the effect and its time of occurrence (i.e. after dosing).
  - Several animals among the groups were noted with alopecia, scabs and/or wounds and other incidental findings noted included rales and reddening of the ear. These findings occurred within the range of background findings to be expected for rats of this age and strain which are housed and treated under the conditions in this study and did not show any apparent dose-related trend. At the incidence observed, these were considered signs of no toxicological relevance.
- Mortality – mortality observed, non-treatment-related
  - There were 2 preterm decedents over the study period: one control female and one female at 600 mg/kg (both during the premating period). One female of the 600 mg/kg group (no. 75) was sacrificed in extremis for animal welfare reasons, on Day 61 (Week 9 of the premating period). This female was noted with moderate lethargy, flat/hunched posture, muscle twitching, piloerection, slight

chromodacryorrhoea, slight ptosis, and red snout. She presented with normal body weight gain from start of treatment onwards, with a slight body weight loss (2%) over Weeks 7-8 of the premating period, followed by recovery in Week 9. During the macroscopic examination at necropsy, accentuated lobular pattern of liver and reduced spleen were noted. Although no definite cause of moribundity could be established from the microscopic examination of the selected tissues, a relationship to treatment could not be excluded as comparable clinical signs were noted for a surviving high dose female as well (no. 79).

- The other decedent was regarded to be unrelated to treatment with the test item: one control female (no. 45) was found dead before dosing on Day 43 (beginning Week 7 of the premating period). No relevant clinical signs were noted for this female from the start of the study period. During the macroscopic examination at necropsy, watery-clear content was observed in the thoracic cavity. Although no definite cause of death could be established during microscopic examination of the selected tissues, the macroscopic observation at necropsy of watery-clear content in the thoracic cavity could be indicative for a technical error during the oral gavage procedure.
- One female of the 200 mg/kg group (no. 67) was euthanized on lactation Day 4, as she had a total litter loss.

- Body weight and weight changes - effects observed, non-treatment-related
  - A dose-dependent test item-related effect on body weight and body weight gain was observed in males at 200 and 600 mg/kg during both the premating and mating period. Males treated at 600 mg/kg had a slightly reduced mean body weight gain from start of treatment onwards (reaching statistical significance on Day 8 and from Day 57 onwards), resulting in a 13% lower mean body weight when compared with control values at the end of treatment.
  - A slight reduction (non-significant) in mean body weight and body weight gain was also observed in males at 200 mg/kg from Day 29 (Week 5 of the premating period) onwards, that was mainly attributed to 2 animals in this group (nos. 22 and 29) that presented with lower body weight gain. At necropsy, a statistically significant decrease in mean body weight (9%) was observed at 200 mg/kg vs mean control.
  - No toxicologically relevant changes in body weight or body weight gain were observed in males treated at 60 mg/kg and females treated up to 600 mg/kg over the entire treatment period.
  - The lower body weight gain noted in females at 600 mg/kg during the gestation period was considered to be related to the non-pregnancy status of all the females at this high dose level, and as such not to reflect a systemic toxic effect of the test item (see Individual Data Tables).
  - The slightly reduced mean body weight and body weight gain observed at 200 mg/kg during the last phase of the gestation period was mainly attributed to one animal in this group (no. 68) that presented with only resorptions.
- Food consumption and compound intake - effects observed, non-treatment-related
  - No toxicologically relevant changes in food consumption (before or after correction for body weight) were recorded over the treatment period in all treated animals of both sexes.

- The lower mean value for absolute food consumption recorded in females at 600 mg/kg from Day 14 post-coitum onwards, was considered to be related to their non-pregnancy status. After allowance for body weight, food consumption remained within normal range.

- Clinical biochemistry findings – no effects observed
  - Thyroid hormone analyses - serum levels of T4 in F0-males were considered not to be affected by treatment up to 600 mg/kg.
- Behaviour – effects observed, non-treatment-related
  - Functional tests were performed at the end of the 10-week premating period for all groups (5 animals/sex/group) to further investigate the abnormal behaviour and muscle twitching observed during clinical observations in Group 4 animals towards the end of the premating period. Hearing ability, pupillary reflex, static righting reflex, and grip strengths of fore and hind limb were considered to be unaffected by treatment up to 600 mg/kg.
  - For treated males, a decrease in fore and hind limb grip strength was observed, reaching statistical significance for fore limb grip strength in all treated groups. However, as all values remained well within the available historical control range for male rats of this strain and age(a), and no dose-related trend could be established, this finding was considered unrelated to treatment. It should be noted that mean control value for fore limb grip strength was at the upper limit of the historical control data. This could explain the statistically significant changes seen in treated males. Also, in females, fore and hind limb grip strength was considered to be unaffected by treatment as all mean values remained within the normal range for female rats of this strain and age(a).
  - A dose-dependent decrease of total movements and ambulations was noted in females, but changes did not reach statistical significance and all mean values remained within the available range of historical control range(b). Therefore, no toxicological relevance was attached to this finding. Also in males, a slight decrease in motor activity (non-significant) was observed at 600 mg/kg but mean values remained within the normal range(b). Although, this decrease was considered not toxicologically relevant, a relationship with treatment could not be discarded based on the clinical signs observed at this dose level.

  (a) Grip Strength Historical Control Data in Wistar Han rats (2013-2018):

  - Fore leg (male): mean = 1073, P5-P95 = 623-1676 (N = 199).
  - Hind leg (male): mean = 690, P5-P95 = 368-999 (N = 199).
  - Fore leg (female): mean = 893, P5-P95 = 525-1324 (N = 198).
  - Hind leg (female): mean = 529, P5-P95 = 310-758 (N = 208).

  (b) Motor Activity Historical Control Data in Wistar Han rats (2012-2018):

  - Total movements (male): mean = 3648, P5-P95 = 2123-5617 (N = 369).
  - Ambulations (male): mean = 733, P5-P95 = 305-1198 (N = 369).
  - Total movements (female): mean = 4858, P5-P95 = 2561-8187 (N = 379).
  - Ambulations (female): mean = 1328, P5-P95 = 629-2281 (N = 369).

- Organ weight findings including organ / body weight ratios – effects observed, treatment-related

- Test item-related lower testes and epididymides weights (absolute and relative to body weights) were noted in the 600 mg/kg treated males.

  Mean Percent Organ Weight Differences from Control Groups

Males		Dose level (mg/kg bw/day)		
		60	200	600
Testes	Absolute	0	4	- 52 **
	Relative to body weight	4	15*	-45**
Epididymides	Absolute	-2	-11*	-43**
	Relative to body weight	1	-1	-34**

  *: P<0.05, **: P<0.01

- There was a decrease in mean epididymis weight (absolute) at 200 mg/kg which was normalized after correction for terminal body weight.
- Mean organ:body weight ratios of thyroid and seminal vesicles weights were statistically significantly higher in males at 600 mg/kg. These organ weight differences were statistically significant when compared with the control group but were considered to be the result of a test item-related effect on final body weight.

- Gross pathological findings – effects observed, treatment-related
  - Macroscopic observations at necropsy revealed test item-related alterations in the reproductive organs of males at 600 mg/kg: macroscopic findings were present in the testes as flaccid (8/10 animals) and reduced in size (10/10 animals) and in the epididymides as reduced in size (9/10 animals).
  - The remainder of the recorded macroscopic findings were within the range of background gross observations encountered in rats of this age and strain. Watery fluid in the uterus, found in one female in each group including controls, is related to a stage in the oestrous cycle and is a normal finding.
- Histopathological findings: non-neoplastic – effects observed, treatment-related
  - Test item-related microscopic findings after treatment with Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide were noted in the thyroid gland, testes and epididymides and are summarized below.

Summary Test Item-Related Microscopic Findings – Males and Females

	Males				Females			
	Dose level (mg/kg bw/day)							
	0	60	200	600	0	60	200	600
Thyroid glands[a]	10	10	10	10	9	10	10	10
Hypertrophy follicular cell								
Minimal	3	5	2	5	5	3	3	4
Slight	-	-	4	3	-	-	-	2
Colloid alteration								
Minimal	-	-	3	2	-	-	-	2
Slight	-	-	-	4	-	-	-	1

a = Number of tissues examined from each group.

- Thyroid gland – Hypertrophy follicular cell was present at increased incidence and severity in males treated at 200 and 600 mg/kg and in females treated at 600 mg/kg up to slight degree. Colloid alteration was present in males treated at 200 and 600 mg/kg and in females treated at 600 mg/kg up to slight degree.

Summary Test Item-Related Microscopic Findings – Males

	Dose level (mg/kg bw/day)			
	0	**60**	**200**	**600**
Testes[a]	10	10	10	10
Atrophy tubular				
Massive	-	-	-	10
Atypical residual bodies				
Slight	-	-	9	-
Moderate	-	-	1	-
Multinucleated giant cells				
Moderate	-	-	1	-
Degeneration germ cells				
Moderate	-	-	1	-
Depletion germ cells				
Moderate	-	-	1	-
Epididymides[a]	10	10	10	10
Cell debris				
Minimal	-	-	-	2
Slight	-	-	-	5
Moderate	-	-	1	1
Reduced sperm				
Slight	-	-	1	-
Massive	-	-	-	10

a = Number of tissues examined from each group.

- Testes – Tubular atrophy was present in all males treated at 600 mg/kg at massive degree. This correlated with the decreased testes weight and with the macroscopic finding flaccid and/or reduced in size. Atypical residual bodies were present in all males treated at 200 mg/kg at slight to moderate degree. Multinucleated giant cells were present in a single male treated at 200 mg/kg and in a single male treated at 600 mg/kg at moderate degree. Degeneration and depletion germ cell were present in a single male treated at 200 mg/kg at moderate degree.
- Epididymides – Cell debris was present in a single male treated at 200 mg/kg at moderate degree and in most males treated at 600 mg/kg up to moderate degree. Reduced sperm was present in a single male treated at 200 mg/kg at slight degree and in all males treated at 600 mg/kg at massive degree. This correlated with the decreased epididymides weight and with the macroscopic finding of reduced in size.
- The remainder of the recorded microscopic findings were within the range of background pathology encountered in rats of this age and strain. There was no test item related alteration in the prevalence, severity, or histologic character of those incidental tissue alterations.

Reproductive performance/function

Group	Dose level (mg/kg bw/day)	Female/Male nos.	In-life reason	Histopathology
1	0	-	-	-
2	60	56/16	Not pregnant	-
3	200	67/27	Total litter loss	-
		68/28	Implantation sites only	Testes: moderate degeneration and depletion germ cells
4	600	72/32 and 36	No evidence of mating	Testes: massive tubular atrophy Epididymides: massive reduced sperm
		74/34 and 38	No evidence of mating	
		79/39 and 40	No evidence of mating	
		71/31	Not pregnant	
		73/33	Not pregnant	
		76/36	Not pregnant	
		77/37	Not pregnant	
		78/38	Not pregnant	
		80/40	Not pregnant	

- There were 9/9 couples treated at 600 mg/kg, compared to 2/10 at 200 mg/kg and 1/10 at 60 mg/kg, that failed to deliver healthy pups. The males treated at 600 mg/kg all showed massive tubular atrophy in the testes and reduced luminal sperm with luminal cell debris in the epididymides which accounted for the lack of offspring. The lack of offspring for one couple treated at 200 mg/kg could be explained by the moderate depletion and degeneration of sperm cells with multinucleated giant cells in the testes and moderate cell debris and slight reduced sperm in the epididymides. The rest of the males treated at 200 mg/kg all showed atypical residual bodies, which apparently did not affect their fertility.
- There were no morphological findings in the reproductive organs of the females which could be attributed to the test item and stage aware evaluation of the testes of males treated at 60 mg/kg did not show any indication for abnormal spermatogenesis.

- Reproductive function: oestrous cycle – effects observed, treatment-related
  - Length and regularity of the oestrous cycle were not considered to be affected by treatment up to 200 mg/kg.
  - All females had regular cycles of 4 days. Extended di-oestrus during the mating period occurred in one female at 60 mg/kg (no. 53) and 3 females at 600 mg/kg (nos. 72, 74 and 79). An extended di-oestrus occasionally occurs at low incidence in untreated controls. Therefore, and due to the absence of a dose-related incidence at 200 mg/kg and based on this low dose female had a normal litter, the single occurrence at 60 mg/kg was considered a chance finding. However, for the

three high dose females with irregular cycle during the mating period a relation to treatment with the test item could not be excluded. For all three females mating could not be confirmed, even though they had been cohoused for another 7 days, after 14 days of unsuccessful mating, with a male of the same group for which mating was already confirmed.

- Reproductive performance - effects observed, treatment-related
  - Mating index was considered to be affected by treatment at 600 mg/kg: 3 out of 9 high dose female (nos. 72, 74 and 79) showed no evidence of mating after a prolonged cohabitation period of a total of 21 days with two different males.
  - For all females at 60 and 200 mg/kg mating could be confirmed.
  - The mating indices were 67% at 600 mg/kg and 100% for all the other groups. Mating index at 600 mg/kg was below the 5th percentile of the historical control range (mean=99%, P5-P95=90-100%, N=98).
- Precoital Time – Precoital time was considered not to be affected by treatment up to 600 mg/kg for all mated females. Most of these females showed evidence of mating within 4 days. One control female (no. 41) and one low dose female (no. 60) had a precoital time of 5-6 days. Another low dose female (no. 53, presenting with extended di-oestrus) had a precoital time of 14 days. In the absence of a dose-related response, this increase in the precoital time was considered unrelated to treatment.
- Number of Implantation Sites – At 600 mg/kg, all 6 mated females presented with 0 implantation sites (and 0 corpora lutea). At 60 and 200 mg/kg, mean number of implantation sites remained in the same range of controls. All mean values were within the historical control range(10). However, at the individual level, there was one female at 200 mg/kg (no. 68) with only 5 implantation sites. Such low numbers are occasionally seen in females of this strain and age, but as the lack of offspring for this animal could be explained by the effects observed on the male sperm (moderate depletion and degeneration of sperm cells with multinucleated giant cells in the testes and moderate cell debris and slight reduced sperm in the epididymides), it was considered to be related to treatment.
- Fertility index was considered to be affected by treatment at 600 mg/kg: all 6 mated females in the high dose group were not pregnant (0 implantation sites).
  - All mated females at 60 and 200 mg/kg were pregnant, except for one low dose female (no. 56). As this was an isolated finding in the low dose group only, it was considered to be unrelated to treatment.
  - The fertility indices were 100%, 90%, 100% and 0% for the control, 60, 200 and 600 mg/kg groups, respectively.
- Historical Control Data in Wistar Han rats (2015-2018) – Number of implantation sites: mean = 12.3, P5-P95 = 6-16 (N = 920).
- Gestation Index and Duration – Gestation index and duration of gestation were considered not to be affected by treatment up to 200 mg/kg. Except for one female at 200 mg/kg (no. 68) with implantation sites only, all pregnant females had live offspring. The gestation indices were 100%, 100% and 90% for the control, 60 and 200 mg/kg groups, respectively.
- Parturition/Maternal Care – No signs of difficult or prolonged parturition were noted among the pregnant females by treatment up to 200 mg/kg. Examination of cage

debris of pregnant females revealed no signs of abortion or premature birth. No deficiencies in maternal care were observed.

- Litter Size – Litter size was considered not to be affected by treatment up to 200 mg/kg. Live litter sizes were 10.6, 10.8 and 10.4 living foetuses/litter for the control, 60 and 200 mg/kg groups, respectively. One female (no. 67) at 200 mg/kg had only 1 living pup.

Effect levels P0

Dose descriptor		Effect level	Based on	Sex	Basis for effect level
Parental toxicity	NOAEL	200 mg/kg bw/day	actual dose received	male	clinical signs
					body weight and weight gain
Reproduction	NOAEL	60 mg/kg bw/day	actual dose received	male	reproductive performance

Target system/organ toxicity

Critical effects observed	Lowest effective dose	System	Organ	Treatment related
Yes	60 mg/kg bw/day	male reproductive system	testes	yes

Results: Developmental Data

- Clinical signs – effects observed, non-treatment-related
  - No clinical signs occurred among pups that were considered to be related to treatment. For the 2 pups of control litter no. 42 which were found dead at first litter check on PND 1, and for the pup of litter no. 53 (at 60 mg/kg) which was found dead on PND 2, absence of milk in the stomach was noted at first litter check.
  - One pup from litter no. 62 (at 200 mg/kg) was noted with a (little) swollen circle at the tail apex over lactation Days 13-22. The nature and incidence of this and other clinical signs remained within the range considered normal for pups of this age and were therefore considered to be of no toxicological relevance.
- Mortality / viability – mortality observed, non-treatment-related
  - Live Birth Index – The number of live offspring on Day 1 after littering compared with the total number of offspring born was considered not to be affected by treatment up to 200 mg/kg. Live birth indices (number of live offspring on PND 1 as percentage of total number of offspring born) were 97% for the control and 99% for the 60 and 200 mg/kg groups. Two pups of the control group (no. 42) were found dead at first litter check. As the control group was treated with the vehicle alone, a relation to treatment with the test item could be excluded.
  - Viability Index – The number of live offspring on Day 4 before culling compared with the number of offspring on Day 1 was considered unaffected by treatment up to 200 mg/kg. Viability indices (number of live offspring on PND 4 before culling as percentage of number of live offspring on PND 1) were 99% for all the groups. One control pup (litter no. 43) and one pup at 60 mg/kg (litter no. 53) were missing or found dead at PND 2. In addition, one female (no. 67) at 200 mg/kg

had lost her single pup on PND 4. The pups missing were most likely cannibalised. Lobuloalveolar development was observed in the mammary gland of animal no. 67 which could not explain the total litter loss in this female. No toxicological relevance was attributed to these dead/missing pups since the mortality incidence did not show a dose-related trend and remained within the range considered normal for pups of this age.

- Body weight and weight changes - no effects observed
  - Body weights of pups were considered not to be affected by treatment up to 200 mg/kg.
- Food consumption and compound intake – effects observed, non-treatment-related
  - The number of live offspring on Day 20 after littering compared to the number of live offspring on Day 4 (after culling) was considered not affected by treatment. No pups were found dead/missing between lactation Days 5 and 20, resulting in a lactation index of 100% for all groups.
- Clinical biochemistry findings – no effects observed
  - Serum T4 levels in male and female PND 21-23 pups were considered not to be affected by treatment up to 200 mg/kg.
- Gross pathological findings – effects observed, non-treatment-related
  - No macroscopic findings were noted among pups that were considered to be related to treatment. The nature and incidence of macroscopic findings remained within the range considered normal for pups of this age and were therefore considered to be unrelated to treatment. No milk in the stomach was noted for the 2 pups of control litter no. 42 which were found dead at first litter check on PND 1. As the control group was treated with the vehicle alone, a relation to treatment with the test item could be excluded.
  - Note: As female no. 56 at 60 mg/kg was not pregnant, developmental data are available of only 9 females in the low dose group. As all females at 600 mg/kg were not mated or non-pregnant, no developmental data were available for evaluation in the high dose group.
- Post-Implantation Survival Index – The total number of offspring born compared to the total number of uterine implantations was considered unaffected by treatment up to 200 mg/kg.
  - Post-implantation survival index (total number of offspring born as percentage of total number of uterine implantation sites) was 94%, 90% and 85% for the control, 60 and 200 mg/kg groups, respectively. The slightly lower post-implantation survival index observed at 200 mg/kg was mainly attributed to a single female in this group (no. 67) that had only 1 living pup out of 6 implantation sites. At the isolated incidence, and as all mean values were within the historical control range (mean=92%, P5-P95=83-99%, N=98), no toxicological relevance was attached to this finding. For control female no. 44, the number of pups (11) was slightly higher than the number of implantations (10). This phenomenon is observed from time to time and is caused by normal resorption of these areas during lactation. No toxicological relevance was attached to this finding in the current study.

- Sex Ratio – At 60 and 200 mg/kg, sex ratio (% of males/females) was reduced (43/57 and 41/59, respectively) when compared with concurrent controls (57/43), reaching statistical significance at 200 mg/kg. This decrease in the % of living males was mainly attributed to single females at 60 mg/kg (no. 52) and 200 mg/kg (no. 67) that presented with a low % of males/females. Therefore, this finding was considered unrelated to treatment.
- Anogenital Distance – Anogenital distance (absolute and normalized for body weight) in male and female pups was considered to be unaffected by treatment up to 200 mg/kg.
- Areola/Nipple Retention – Treatment up to 200 mg/kg had no effect on areola/nipple retention. For none of the examined male pups nipples were observed at PND 13.

Dose descriptor		Effect level	Based on	Sex	Basis for effect level
Developmental	NOAEL	200 mg/kg bw/day	actual dose received	male/female	No developmental toxicity was observed by treatment up to 200 mg/kg. At 600 mg/kg, no litters were available for evaluation due to the non-pregnancy status of females.

Overall reproductive toxicity

Reproductive effects observed	Lowest effective dose	Treatment related	Relation to other toxic effects	Dose response relationship
Yes	60 mg/kg bw/day	yes	reproductive effects in the absence of other toxic effects	yes

3.2.1.2 Developmental toxicity/teratogenicity. Key study 2016

Study reference:

Study report (2016) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:

Test type

Developmental toxicity according to OECD Guideline 414 (Prenatal Developmental Toxicity Study) (Jan. 2001); according to EU Method B.31 (Prenatal Developmental Toxicity Study) (May 2008); according to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study) (August 1998). GLP compliant.

Test substance

- Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.
- Degree of purity – 99.5 %.
- Impurities – not reported.

- Batch number - not reported.

Test animals

- Rat, Wistar Rat, female.
- 22 females per dose.
- Age and weight at the study initiation - approximately 10 to 14 weeks; no information about weight.

Administration/exposure

- Route of administration – oral: gavage
- Duration and frequency of test/exposure period - Once daily for 7 days per week, approximately the same time each day with a maximum of 6 hours difference between the earliest and latest dose from days 6 to 20 post-coitum, inclusive.
- 50, 150 and 500 mg/kg bw/day.
- Dose selection rationale: Dose levels were selected by the Sponsor and based on data from a maternal toxicity study, (BASF Project 10R0490/03X053, WIL Project 510610). In this study, the pregnant female rats treated at 500 mg/kg bw/day showed reduced body weight gain arising from reduced food consumption. 5/6 animals showed hunched posture, piloerection and/or salivation and increased liver weights were apparent at termination. No clear signs of toxicity were observed in pregnant female rats treated at 150 mg/kg bw/day.
- Control group and treatment – concurrent vehicle.
- Historical control data if available – not reported.
- Vehicle: CMC (carboxymethyl cellulose) 1% in water, 5ml/kg.
- Details on analytical verification of doses or concentrations – Samples of dose preparations were taken at the test facility on a single occasion during the treatment period (formulations were prepared and sampled on 12 October 2015). The samples were dispatched on dry ice to ABL where they were analysed to assess accuracy of preparation (all groups), homogeneity (lowest and highest concentration) and stability in vehicle over 5 hours at room temperature (lowest and highest concentration).
    - No test item was detected in the Group 1 formulations.
    - The concentrations analysed in the formulations of Group 2, 3 and 4 were in
    - agreement with the target concentrations (i.e. mean accuracies between 85% and 115%).
    - The formulations of Group 2 and Group 4 were homogeneous (i.e. coefficient of variation ≤ 10%).
    - Formulations at the entire range were stable when stored at room temperature under normal laboratory light conditions for at least 5 hours (i.e. relative difference ≤ 10%).

Description of test design:

- Impregnation procedure: purchased timed pregnant. Proof of pregnancy: vaginal plug referred to as day 0 of pregnancy premating exposure period for males and females (P and F1) dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate.
- Standardization of litters – no.

Maternal examinations

- Cage side observations time schedule: twice daily.
- Detailed clinical observations time schedule: once daily.
- Body weight – time schedule for examinations: Days 2, 6, 9, 12, 15, 18 and 21 post-coitum.

- Food consumption – Food consumption for each animal determined and mean daily diet consumption calculated. Time schedule for examinations: Days 2-6, 6-9, 9-12, 12-15, 15-18 and 18-21 post-coitum.
- Water consumption – Subjective appraisal was maintained during the study, but no quantitative assessment was introduced.
- Post-mortem examinations – Yes. Sacrifice on gestation day # 21 post-coitum (or within 24h of abortion or early delivery). Organs examined: All macroscopic abnormalities (external, thoracic, abdominal) were recorded, collected and fixed in 10% buffered formalin.
- Ovaries and uterine content – the ovaries and uterine content was examined after termination. Examinations included:
    - Gravid uterus weight.
    - Number of corpora lutea.
    - Number and distribution of implantations (In case implantations were not macroscopically visible, the uterus was stained using the Salewski technique in order to determine any former implantation sites).
    - Number of early resorptions.
    - Number of late resorptions.
- Other: sex and weight of each foetus, weight of each placenta (of live foetuses only).

Foetal examinations

- External examinations [all per litter]
- Soft tissue examinations [half per litter]
- Skeletal examinations [half per litter]
- Head examinations [half per litter, i.e., the foetuses also used for visceral examination]

Statistics - The following statistical methods were used to analyse the data:

- If the variables could be assumed to follow a normal distribution, the Dunnett-test (Ref. 6) (many-to-one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex.
- The Steel-test (Ref. 7(many-to-one rank test)) was applied if the data could not be assumed to follow a normal distribution.
- The Fisher Exact-test (Ref. 8) was applied to frequency data.
- The Mann Whitney test (Ref. 9) was used to compare mean litter proportions (percent of litter) of the number of viable and dead foetuses, early and late resorptions, total resorptions, pre- and post-implantation loss, and sex distribution.
- Mean litter proportions (percent per litter) of total foetal malformations and developmental variations (external, visceral and skeletal), and each particular external, visceral and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test (Ref. 10) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test (Ref. 11) was used to compare the compound-treated groups to the control group. All tests were two-sided and in all cases $p < 0.05$ was accepted as the lowest level of significance.

- Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables. Test statistics were calculated on the basis of exact values for means and pooled variances. Individual values, means and standard deviations might be rounded off before printing. Therefore, two groups might display the same printed means for a given parameter, yet display different test statistics values.
- No statistics were applied for data on maternal survival, pregnancy status, group mean numbers of dead foetuses, early and late resorptions, and pre- and post-implantation loss.
- Indices
    - Pre-implantation loss = (number of corpora lutea - number of implantation sites) / number of corpora lutea.
    - Post-implantation loss = (number of implantation sites - number of live foetuses) / number of implantation sites.
    - Viable foetuses affected/litter = number of viable foetuses affected/litter / number of viable foetuses/litter.

Results and discussion

- General toxicity (maternal animals)
    - Clinical signs – effects observed, treatment-related. Five females delivered early on Day 21 post-coitum (one vehicle control, one 50 mg/kg bw/day treated female, two 150 mg/kg bw/day treated females and one 500 mg/kg bw/day treated female). Additionally, one female in the 500 mg/kg bw/day group had an early delivery on Day 20 post-coitum. The incidence and persistence of salivation showed a dose related increase. In 14/22 females treated with 150 mg/kg bw/day salivation was observed for one to a few days at the end of treatment. For one to several days, salivation was noted in 19/22 females in the highest dose group (500 mg/kg bw/day). Since no correlated findings were noted, this was attributed to the taste of the test item and of no toxicological relevance. Furthermore, piloerection in 7/22 females and hunched posture in 4/22 females was observed for one to several days in females treated at 500 mg/kg bw/day.
    - Mortality – no mortality observed.
    - Body weight and weight changes - effects observed, treatment-related. Mean body weight gain was significantly reduced in the highest dose group compared to vehicle controls from Day 9 post-coitum onwards. Consequently, on Day 21 post-coitum mean body weights and weight gain corrected for uterus weight were significantly lower in 500 mg/kg bw/day treated females compared to the controls (mean body weight on day 21 post coitum was 285 g compared to 305 g in controls). Body weights and body weight gain were unaffected at 50 and 150 mg/kg bw/day.
    - Food consumption and compound intake – effects observed, treatment-related. At the beginning of treatment (Day 6 to Day 12 post-coitum), food consumption was significantly reduced in Group 4 compared the control Group (maximum approximately 20%). This fully recovered to similar levels as controls from Day 12 post-coitum onwards. Food consumption was unaffected at 50 and 150 mg/kg bw/day.

- Gross pathological findings – no effects observed. No macroscopic findings were noted that were considered to be treatment related.

- Maternal developmental toxicity
  - Number of abortions – no effects observed. There were no effects on the number of pregnant females, corpora lutea, implantation site and pre- or post-implantation loss noted with treatment up to 500 mg/kg bw/day.
  - Pre- and post-implantation loss – no effects observed. There were no effects on the number of pregnant females, corpora lutea, implantation site and pre- or post-implantation loss noted with treatment up to 500 mg/kg bw/day.
  - Early or late resorptions - no effects observed. There were no effects on the number of pregnant females, corpora lutea, implantation site and pre- or post-implantation loss noted with treatment up to 500 mg/kg bw/day.
  - Changes in number of pregnant – effects observed, non-treatment-related. Four animals were found not pregnant, (two 50 mg/kg bw/day treated females, one 150 mg/kg bw/day treated female and one 500 mg/kg bw/day treated female). All other females were pregnant and had litters with viable foetuses.

Dose descriptor	Effect level	Based on	Basis for effect level
NOAEL	150 mg/kg bw/day	actual dose received	maternal toxicity
			reduced body weight

- Maternal abnormalities – no abnormalities observed.
- Foetuses
  - Foetal body weight changes - effects observed, treatment-related. At 500 mg/kg bw/day, female foetal weights were slightly, but significantly lower compared to the control group (4.8 gram versus 5.1 gram in controls). A similar, but not significant, reduction was observed in male foetal weights (5.0 gram versus 5.3 gram in controls) and consequently combined foetal weights. The placenta weights were unaffected by treatment up to 500 mg/kg bw/day.
  - Changes in sex ratio – no effects observed. The male:female ratio was unaffected by treatment up to 500 mg/kg bw/day.
  - Changes in litter size and weights – no effects observed. Litter size was unaffected by treatment up to 500 mg/kg bw/day. The numbers of foetuses (litters) available for morphological examination on Day 21 post-coitum were 233 (22), 200 (20), 202 (21) and 202 (20) in Groups 1, 2, 3, and 4, respectively.
  - External malformations - effects observed, non-treatment-related. There were no treatment related effects on external morphology following treatment up to 500 mg/kg bw/day. The only external malformations observed, were noted in two foetuses from a litter at 500 mg/kg bw/day. Foetus A086-05 had no tail and foetus A086-06 had a tail that was filamentous, which was confirmed at skeletal examination. Both these tail malformations were not seen

previously among historical controls, however, because they pertain to the same group of tail abnormalities and occurred in one litter, they can be considered genetic in origin and are not related to treatment.

- Skeletal malformations – effects observed, treatment-related.
  - There was an increase in the number of foetuses with bent limb bones in the highest dose group. Ten foetuses from 5 litters were affected with this skeletal malformation, compared to one control foetus (bent limb bones were not observed at 50 and 150 mg/kg bw/day). In all cases one or both scapulae were bent and in three foetuses also humeri were involved. The incidence of bent limb bones at 500 mg/kg bw/day was far above the upper limit in historical control foetuses (10.6% versus 0.7% per litter, respectively) and therefore the bent limb bones were considered to be treatment related. The higher incidence of bent limb bones at 500 mg/kg bw/day coincided with an increased litter incidence for bent ribs in this group. Mean litter proportions for this skeletal variation were 13.5%, 23.5%, 22.1% and 69.9% per litter in Group 1, 2, 3 and 4, respectively. The incidence in Group 4 was statistically significantly increased and all foetuses with bent limb bones also had bent ribs.
  - At 500 mg/kg bw/day, the incidence of the skeletal variations reduced ossification of the skull and unossified metatarsals and metacarpals were significantly increased compared to the control group. Mean litter incidences for reduced ossification of skull bones were 12.4%, 12.5%, 21.1%, 45.9% and for unossified metatarsals and metacarpals 5.4%, 6.6%, 3.2% and 21.0% per litter in the control, 50, 150 and 500 mg/kg bw/day group, respectively. Both these parameters are indicative of retarded skeletal ossification which is in line with the lower foetal body weights in this dose group. Both findings are considered to be related to the decreased maternal body weights (-7%).
- Visceral malformations - no effects observed. There were no treatment related effects on visceral morphology following treatment up to 500 mg/kg bw/day.

Dose descriptor	Effect level	Based on	Sex	Basis for effect level
NOAEL	150 mg/kg bw/day	actual dose received	male/female	skeletal malformations
				bent limbs

- Foetal abnormalities – skeletal, forelimb and hindlimb
- In the absence of gross limb malformations, and in the presence of retardation as a consequence of maternal toxicity, bent limb bones could be considered temporary variations rather than malformations. It is hypothesized, that during development the increase in muscle mass puts stress on the bones. If ossification is delayed, the bones might not be able to counteract this pressure and appear bowed until ossification is finalized. Bone development in rats continues long after birth, extending into young adulthood. In a few studies, pups were followed sequentially after birth, and bent long

bones and scapulae were transient in nature and appeared normal by the time of weaning. References:

- De Schaepdrijver L., Delille P., Geys H., Boehringer-Shahidi C., Vanhove C. In vivo longitudinal micro-CT study of bent long limb bones in rat offspring. Reprod Toxicol 46, 91–97 (2014).
- Mitchard T.L., French J. Apparent postnatal recovery of chondrodystrophy in the Harlan Han Wistar rat. Reprod Toxicol 32, 169–70 (2011).
- Kimmel C.A., Garry M.R., DeSesso J.M. Review Articel: Relationship Between Bent Long Bones, Bent Scapulae, and Wavy Ribs: Malformations or Variations? Birth Defects Research (Part B) 101:379–392 (2014).

3.2.1.3 Developmental toxicity/teratogenicity. Key study 2018

Study reference:
Study report (2018) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:
Test type
Developmental toxicity, according to OECD Guideline 414 (Prenatal Developmental Toxicity Study) 2001; according to EU Method B.31 (Prenatal Developmental Toxicity Study) 2008; according to EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study) 1998. GLP compliant.

Test substance
- Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.
- Degree of purity - 99.32%.
- Appearance – Pale yellow crystalline powder.
- Test item storage – At room temperature.
- Impurities – not reported.
- Batch number – not reported.

Test animals
- Rabbit; New Zealand White rabbit; female
- 22 females per dose
- Age and weight at the study initiation – 17-19 weeks old and weighed between 2900 and 4060 g at the initiation of dosing

Administration/exposure
- Route of administration – oral, gavage.
- Duration and frequency of test/exposure period - 7 days a week from Day 6 to Day 28 post-coitum, inclusive.
- Doses/concentration levels – 10, 30 and 100 mg/kg bw/day (actual dose received).
- Rationale for dose level selection - The oral route of exposure was selected because this is a possible route of human exposure during manufacture, handling or use of the test item. The dose levels were selected based on the results of the dose range finder

and in an attempt to produce graded responses to the test item control group and treatment.

- Historical control data for New Zealand White (Crl:KBL (NZW)) rabbits (period 2012-2017; n=495):
  Corrected body weight gain (gram): Mean: -245; p5 - p95: -557.3 – 45.4
  Corrected body weight gain (%): Mean: -6; p5 - p95: -13.8 - 1.3
  Weight of the uterus (gram): Mean: 524; p5 - p95: 360.3 - 699.8
- vehicle - water 1% aqueous carboxymethyl cellulose
  Water, Supplier: Elix, Millipore S.A.S., Molsheim, France.
  Carboxymethyl cellulose, Supplier: Fagron, Capelle aan de IJssel, The Netherlands.
- Preparation of Test Item – Test item dosing formulations (w/w) were homogenized to visually acceptable levels at appropriate concentrations to meet dose level requirements. The dosing formulations were prepared daily as a suspension and dosed within 5 hours after adding the vehicle to the test item.
    - Dose Formulation Sample Collection Schedule:
        - Occasion: Week 1 of treatment: 18 Dec 2017; Concentration: All groups; Homogeneity: Groups 2 and 4(a); Stability: Groups 2 and 4
        - Occasion: Week 4 of treatment: 10 Jan 2018; Concentration: Group 2; Homogeneity:- ; Stability:-
        - (a)The homogeneity results obtained from the top, middle and bottom for the Group 2 and 4 preparations were averaged and utilized as the concentration results. Stability samples were kept at room temperature under normal laboratory light conditions for 5 hours, and then placed on dry ice. All other samples were stored on dry ice immediately after sampling. All samples to be analysed were shipped on dry ice to ABL BV on the following dates: 19 Dec 2017 and 10 Jan 2018. The analytical laboratory was notified before shipment of the samples. Upon receipt at the analytical laboratory, the samples were stored in the freezer ≤ -70 °C until analysis. Based on the results of the Week 1, additional Group 2 samples collected in Week 4 of treatment. However, in consultation with the Sponsor and after re-evaluation of the results samples were not analysed. Samples were stored at -70°C for a maximum of 37 days (available long-term stability information) and will be discarded after the expiry date. Residual samples were discarded after completion of the sample analysis.
    - Analytical Method – Analyses were performed by using a validated analytical procedure (ABL No. 15222; Test Facility Study No. 510160).
    - Concentration Analysis – Duplicate sets of samples (approximately 500 mg) for each sampling time point were sent to the analytical laboratory. Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% for suspensions of target concentration.
    - Homogeneity Analysis – Duplicate sets of samples (approximately 500 mg) for each sampling time point were sent to the analytical laboratory. Homogeneity results were considered acceptable if the coefficient of variation (CV) of concentrations was ≤ 10%.

- Stability Analysis – During the course of this study at one occasion during the treatment phase, stability of the prepared formulation was determined for 5 hours at room temperature. Duplicate sets of each sample (approximately 500 mg) were sent to the analytical laboratory. Stability results were considered acceptable if the sample analysis results were within or equal to ±10% of the concentration determined by the initial analysis of each formulation. actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test.

Description of test design:

- details on mating procedure - time-mated female New Zealand White rabbits were received from Charles River (Chatillon sur Chalaronne, France). The females arrived on Days 1, 2, 3 or 4 post-coitum (Day 0 post-coitum is defined as the day of successful mating).
- premating exposure period for males and females (P and F1) – no premating exposure.
- standardization of litters – no data.

Maternal examinations - In-life Procedures, Observations, and Measurements – The in-life procedures, observations, and measurements listed below were performed for parental animals.

- Mortality/Moribundity Checks – Throughout the study, animals were observed for general health/mortality and moribundity twice daily, in the morning and at the end of the working day. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings. Animals showing pain, distress or discomfort which was considered not transient in nature or was likely to become more severe, were sacrificed for humane reasons based on OECD guidance document on humane endpoints (ENV/JM/MONO/ 2000/7). The circumstances of any death were recorded in detail.
- Clinical Observations – Clinical observations were performed at least once daily, beginning on Day 6 post-coitum and lasting up to the day prior to necropsy. The time of onset, grade and duration of any observed sign was recorded. Signs were graded for severity and the maximum grade was predefined at 3 or 4. Grades were coded as slight (grade 1), moderate (grade 2), severe (grade 3) and very severe (grade 4). For certain signs, only its presence (grade 1) or absence (grade 0) was scored. In the data tables, the scored grades were reported, as well as the percentage of animals affected in summary tables. Cage debris was examined to detect abortion or premature birth.
- Body Weights – Animals were individually weighed on Days 6, 9, 12, 15, 18, 21, 24, 27 and 29 post-coitum.
- Food Consumption – Food consumption was quantitatively measured for Days 6-9, 9-12, 12-15, 15-18, 18-21, 21-24, 24-27 and 27-29 post-coitum.
- Water Consumption – Subjective appraisal was maintained during the study, but no quantitative investigation introduced as no effect was suspected.
- Terminal Procedures – Terminal procedures are summarized in the following table.

Group No.	No. of Females	Scheduled Euthanasia Day	Necropsy Procedures			Histology and Histopathology
			Necropsy	Collection of Gross Lesions	Organ Weights	
1	22	29 post-coitum	X	X	-	-
2	22					-
3	22					-
4	22					-
Unscheduled deaths			X	X	-	-

X = Procedure to be conducted; - = Not applicable.

- Unscheduled Deaths – If necessary, for humane reasons, animals were euthanized as per Test Facility SOPs. These animals were euthanized by intravenous injection of pentobarbital (approx. 1 mL/kg Euthasol® 20%), underwent necropsy, and specified tissues were retained.
- Scheduled Euthanasia – Animals surviving until scheduled euthanasia were euthanized by intravenous injection of pentobarbital (approx. 1 mL/kg Euthasol® 20%). No body weight was recorded at necropsy. Scheduled necropsy was conducted on the following days:
    - Females surviving to planned necropsy: Day 29 post-coitum.
    - Females with early delivery: (no. 66, 85, 87, 88): Within 24 hours of early delivery.
- Necropsy – All animals (including animals sacrificed before planned necropsy and females with early delivery) were subjected to an external, thoracic and abdominal examination, with special attention being paid to the reproductive organs. All macroscopic abnormalities were recorded, collected and fixed in 10% buffered formalin (neutral phosphate buffered 4% formaldehyde solution). No organs (except for the uterus) were weighed. Each ovary and uterine horn of all animals was dissected and examined as quickly as possible to determine:
    - The number of corpora lutea.
    - The weight of the (gravid) uterus (not for animals sacrificed before planned necropsy, except for female no. 49).
    - The number and distribution of live and dead foetuses.
    - The number and distribution of embryo-foetal deaths.
        - For animals sacrificed before planned necropsy, these findings were reported in the individual data tables only. Necropsy procedures were performed by qualified personnel with appropriate training and experience in animal anatomy and gross pathology. A veterinary pathologist, or other suitably qualified person, was available.

Foetal examinations

- Method of Euthanasia – Live foetuses were euthanized by administration of sodium pentobarbital (Euthasol® 20%) into the oral cavity using a small metal feeding tube. Foetuses of animals sacrificed before planned necropsy were externally examined in detail and euthanized by sodium pentobarbital.
- Foetal Examinations – External, visceral, and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have

no significant biological effect on animal health or body conformity and/or represent slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

- External Examinations – Each viable foetus was examined in detail to detect macroscopic visible abnormalities and their weight was determined. Nonviable foetuses (the degree of autolysis was minimal or absent) were examined and weighed. Weights were not determined for foetuses of animals sacrificed before planned necropsy or females with early delivery, except for foetuses of female no. 49 (sacrificed on Day 28 post-coitum). For late resorptions and recognizable foetuses of females euthanized in extremis, a gross external examination was performed.
- Visceral Examinations– All foetuses were internally sexed and examined for visceral anomalies by dissection in the fresh (non-fixed) state. The thoracic and abdominal cavities were opened and dissected using a technique described by Stuckhardt and Poppe1. This examination included the heart and major vessels. Foetal kidneys were examined and graded for renal papillae development as described by Woo and Hoar. The heads were removed from approximately one-half of the foetuses in each litter and placed in Bouin's solution for soft-tissue examination of all groups using the Wilson sectioning technique3. After examination, the tissues without variation or malformations were discarded. Selected tissues with variations or malformations were stored in 10% formalin. The heads from the remaining one-half of the foetuses in each litter of all groups were examined by a mid-coronal slice. All carcasses, including the carcasses without heads, were eviscerated, skinned, labelled and fixed in 96% aqueous ethanol for subsequent examination of skeletons.
- Skeletal Examinations – All eviscerated foetuses, following fixation in 96% aqueous ethanol, were macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson. Subsequently, the skeletal examination was done on all foetuses from Groups 1 and 4. Since no treatment related effects in the high dose group were seen, skeletal examination was not extended to the foetuses from the low and mid dose group. All specimens were archived in glycerin with bronopol as preservative. A few bones were not available for skeletal examination because they were accidentally damaged or lost during processing. The missing bones were listed in the raw data; evaluation by the foetal pathologist and Study Director determined there was no influence on the outcome of the individual or overall skeletal examinations, or on the integrity of the study as a whole.
- Statistical analysis
    - All statistical tests were conducted at the 5% significance level. All pairwise comparisons were conducted using two sided tests and were reported at the 1% and 5% levels. Numerical data collected on scheduled occasions for the listed variables were analysed as indicated according to sex and occasion. Descriptive statistics number, mean and standard deviation (or %CV or SE when deemed appropriate) were reported whenever possible. Inferential statistics were performed according to the matrix below when possible, but excluded semi-quantitative data, and any group with less than 2 observations.
    - The following pairwise comparisons were made:
        - Group 2 vs. Group 1
        - Group 3 vs. Group 1

- Group 4 vs. Group 1

- Parametric – Datasets with at least 3 groups (the designated control group and 2 other groups) were compared using Dunnett-test (many-to-one-t-test).
- Non-Parametric – Datasets with at least 3 groups were compared using a Steel-test (many-to-one rank test). Mean litter proportions (percent of litter) of the number of viable and dead foetuses, early and late resorptions, total resorptions, pre- and post-implantation loss, and sex distribution were compared using the Mann Whitney test. Mean litter proportions (percent per litter) of total foetal malformations and developmental variations (external, visceral and skeletal), and each particular external, visceral and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the compound-treated groups to the control group.
- Incidence – An overall Fisher's exact test was used to compare all groups at the 5% significance level. The above pairwise comparisons were conducted using a two-sided Fisher's exact test at the 5% significance level if the overall test was significant. No statistics were applied for data on maternal survival, pregnancy status, group mean numbers of dead foetuses, early and late resorptions, and pre- and post-implantation loss.

- Computerized systems – Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

System name	Version No.	Description of Data Collected and/or Analysed
ToxData	8.0	In-life phase (Mortality; Clinical signs; Body weights; Food consumption; Reproduction parameters) Data Collection
REES Centron	SQL 2.0	Temperature and Humidity (animal and laboratory facilities) Data Collection
WTDMS	Details are included in the Test Facility SOP	Reproduction and Foetal Pathology Data Collection

Results and discussion
Maternal animals

- Clinical signs - no effects observed
  - No treatment-related clinical signs were noted up to 100 mg/kg. Any clinical signs noted during the treatment period occurred within the range of background findings to be expected for rabbits of this age and strain which are housed and treated under the conditions in this study and did not show any apparent dose-related trend. At the incidence observed, these were considered to be unrelated to treatment.

- Mortality - no mortality observed
    - No mortality occurred during the study period that was considered to be related to treatment with the test item. Two females from the mid dose group (30 mg/kg) were euthanized prematurely. Female no. 64 was euthanized on Day 26 post-coitum, as she was found lethargic with hunched posture, piloerection and ptosis. Additionally, reduced faeces production, faeces containing mucus and red fluid were observed on the manure tray. From post-coitum Day 21 onwards, this female had consumed no food and showed slight body weight loss. At necropsy, intussusception of the caecum with dark red discolouration was noted, which correlated with the poor clinical status of this female. Based on the single occurrence of this finding, it was considered a chance finding and unrelated to treatment.
    - Female no. 49 was euthanized on Day 28 post-coitum, as she experienced laboured respiration on two subsequent days and gasping on one day. At necropsy, the left caudal lobe of the lungs was found perforated. Additionally, a reddish contents of the trachea, many dark red foci on the lungs and a reddish, watery-clear fluid in the thoracic cavity were noted. Taken together, these findings were indicative of a dosing related incident and the preterm sacrifice of this female was considered unrelated to the test item.
    - Four females (one at 30 mg/kg and three at 100 mg/kg) were sacrificed prematurely after an early delivery, for details see maternal pregnancy data).
- Body weight and weight changes - no effects observed
    - Body weights and body weight gain of treated animals remained in the same range as controls over the study period. Body weight gain corrected for weight of the gravid uterus was slightly lower in 100 mg/kg treated females compared to the concurrent controls. However, as the effect was minimal, not statistically significant, and remained within the historical control range it was considered unrelated to treatment.
- Food consumption and compound intake - no effects observed
    - No toxicologically relevant changes in food consumption before or after correction for body weight were recorded up to 100 mg/kg. Any statistically significant changes in food consumption were considered to be unrelated to treatment since no trend was apparent regarding dose.
- Gross pathological findings - effects observed, non-treatment-related
    - Macroscopic observations at necropsy did not reveal any alterations that were considered to have arisen as a result of treatment. Incidental findings among control and treated animals included emaciated appearance, cysts of the ovaries or oviducts, alopecia, scabs or scars. These findings are occasionally seen among rabbits used in these types of study and in the absence of a dose relationship were considered unrelated to treatment.
- Number of abortions - effects observed, non-treatment-related
    - Note: In order to enter female numbers into WTDMSTM an adjustment in the numbering was made, for example: female 1 was reassigned as female A001, female 2 as A002 etc. Also numbering of foetuses was changed; foetus 1 of female 1 was reassigned as A001-01 etc. Overall, the number of pregnant

females, corpora lutea and implantation sites, and pre-and post-implantation loss in the control and treatment groups were similar and in the range of normal biological variation.

- One female (no. 66) treated at 30 mg/kg and three females (nos. 85, 87 and 88) treated at 100 mg/kg delivered early on Day 27 or 28 post-coitum. These females had relatively low to no food consumption in the six days prior to the early delivery. Except for cannibalism of two foetuses (one at 30 mg/kg and one at 100 mg/kg), no external abnormalities were observed for the premature litters.

Dose descriptor	Effect level	Based on	Basis for effect level
NOAEL	> 100 mg/kg bw/day	actual dose received	clinical signs
			mortality
			body weight and weight gain
			food consumption and compound intake
			gross pathology
			necropsy findings

Foetuses

- Foetal body weight changes - effects observed, non-treatment-related
  - There were no toxicologically relevant effects on foetal body weights (both sexes) noted by treatment up to 100 mg/kg. The combined foetal weights in the 100 mg/kg dose group was slightly decreased compared to the concurrent controls. However, as the decrease was only slight (5% lower than concurrent controls), in the absence of corroborative findings of growth retardation at skeletal examination (see 9.4.3) and without statistical significance, this finding was considered not toxicologically relevant.
- Changes in sex ratio - no effects observed
  - The male:female ratio was unaffected by treatment up to 100 mg/kg. Mean sex ratios (males:females) were 48:52, 48:52, 49:51 and 46:54 for the control, 10, 30 and 100 mg/kg groups, respectively.
- Changes in litter size and weights - no effects observed
  - There were no treatment-related effects on litter size of any group. Mean litter sizes were 9.1, 9.1, 9.1 and 9.5 foetuses/litter for the control, 10, 30 and 100 mg/kg groups, respectively.
- External malformations - effects observed, non-treatment-related
  - There were no treatment related effects on external morphology following treatment up to 100 mg/kg. External malformations were observed in two, three and one foetus(es) of respectively the control, 10 and 30 mg/kg groups, but not at 100 mg/kg. Carpal and/or tarsal flexures (all without apparent skeletal origin) occurred in the 30 mg/kg foetus (A048-09) and two control foetuses (A007-01 and A008-04) of which foetus A008-04 also had an omphalocele and multiple malformations viscerally. An omphalocele was

also observed in 10 mg/kg foetus A038-11 and the other externally affected foetuses at 10 mg/kg either had cyclopia (foetus A029-03) or meningocele (foetus A030-02). As the above malformations occurred singly in the 10 and 30 mg/kg groups, not at 100 mg/kg and/or were seen in concurrent and historical control foetuses (except for cyclopia), they were considered chance findings and were not related to treatment. External variations were not observed in this study.

- Skeletal malformations - effects observed, non-treatment-related
    - A statistically significant increase in the incidence of malaligned sternebra(e) was observed at 100 mg/kg; 9.2% per litter compared to 3.8% for the concurrent controls. The value at 100 mg/kg remained within the maximum value of the available historical control data (10.2% per litter). All remaining variations noted, were not considered treatment related as they occurred infrequently and/or at frequencies that were within the range of available historical control data. Only one skeletal malformation was observed in this study, a vertebral anomaly with associated rib anomaly, and as it occurred in two control foetuses only (A004-10 and A013-04), it was as such considered to be spontaneous in origin.
- Visceral malformations - effects observed, non-treatment-related
    - There were no treatment related effects on visceral morphology following treatment up to 100 mg/kg. Visceral malformations occurred in 2 (2), 3 (3), 2 (2) and 1 (1) foetuses (litters) in the control, 10, 30 and 100 mg/kg groups, respectively.
    - At 100 mg/kg, foetus A078-07 had abnormal lobation of the liver, while at 30 mg/kg, foetus A058-11 had both kidneys and testes malpositioned. Malpositioning of kidneys and/or testes were also observed in 30 mg/kg foetus A057-04 (one kidney), 10 mg/kg foetus A036-10 (both kidneys and testes) and control foetus A008-04 (both testes). In foetus A036-10 this was found together with an intestine diverticulum and the affected control foetus had multiple other malformations, namely interrupted aortic arch, liver anomaly, anomalous content of kidneys and ureters and haemorrhagic eyes.
    - The two remaining viscerally malformed foetuses were 10 mg/kg foetus A029-03, which had transposition of the great vessels and control foetus A013-05 with a narrow aorta and ventricular septum defect. The very low incidence and group distribution of these malformations does not indicate a treatment relationship. Moreover, all but two of the malformations observed in test item treated groups (transposition of the great vessels and intestine diverticulum) were noted previously in historical controls.
    - All the variations noted, were considered unrelated to treatment as they occurred infrequently, in the absence of a dose-related incidence trend and/or occurred at frequencies that were within the range of available historical control data.

Dose descriptor	Effect level	Based on	Sex	Basis for effect level
NOAEL	> 100 mg/kg bw/day	actual dose received	male/female	changes in sex ratio
				foetal/pup body weight changes
				changes in litter size and weights
				skeletal malformations
				visceral malformations

In conclusion, based on the results in this prenatal developmental toxicity study the maternal and developmental No Observed Adverse Effect Level (NOAEL) for Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide was established as being at least 100 mg/kg. Higher doses were not tolerated as 3/6 females treated at 200 mg/kg in the Dose Range Finder were sacrificed in extremis.

3.2.2 Human data – no data

3.3 Specific target organ toxicity – repeated exposure

3.3.1 Animal data

3.3.1.1 Repeated dose toxicity. oral. Key study 1989 (Short-term)

Study reference:
Study report (1989d) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:
Test type
Short-term repeated dose toxicity: oral according to Japanese Ministry of Health and Welfare (M.H.W.) guidelines 1986 for a twenty-eight day repeat dose oral toxicity study. GLP compliant.

Test substance
- (Diphenylphosphinyl)-(2,4,6-trimethylphenyl)methanone.
- Degree of purity – 99%.
- Impurities – not reported.
- Batch number – 010213.
- Physical state – pale yellow powder.

Test animals
- Rat; Sprague Dawley Rat; male/female.
- 5 animals per sex per dose.
- Age and weight at the study initiation - 152 - 192 g for males, 155 - 189 g for females.

Administration/exposure

- Route of administration – oral, gavage.
- Duration and frequency of test/exposure period – daily for 28 days.
- Doses/concentration levels – 50, 250 and 750 mg/kg bw/day (actual dose received).
- Rationale for dose level selection - In a pretest study the test substance was administered to groups of 3 male and 3 female rats at a dose level of 20, 250, 500, 750 and 1000 mg/kg bw/day for 14 days. Body weight, clinical signs and macroscopic findings were recorded. Animals of the highest dose group were killed in extremis. Whereas at 750 and 500mg/kg bw, adverse clinical signs and reduction in body weight gain was detected, this was reduced to minor adverse clinical signs at 250 mg/kg bw. No abnormalities were observed in the control group and at 50 mg/kg bw.
- post exposure observation period - satellite groups: 5 male and 5 female animals of the control and the high dose group. Rationale for selecting satellite groups: recovery examination. Post-exposure recovery period in satellite groups: 14 days.
- Vehicle – arachis oil.
- Concentration and volume used - 4 ml/kg bw.
- Justification of choice of vehicle - The test substance is hardly soluble in water. Administration via diet is impossible due to palatability reasons.
- Control group and treatment – yes, concurrent vehicle.
- The stability of the test material formulation for at least nine days was determined by Safepharm Analytical Laboratory prior to the start of the study. Since no differences were detected in concentration from samples taken from the top, the middle, or the bottom, the formulation was homogenous. Concentrations were checked at weekly intervals, i.e. for every new formulation. All results were between 91% and 109% of the target concentration.
- Statistics - Data were processed to give group mean values and standard deviations where appropriate. Relative organ weights, haematological and blood chemical data were analysed by one-way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogenous variances were analysed using Kruskal Wallis non-parametric analysis of variance and Mann Whitney U-Test. Probability values were calculated as follows: $p < 0.001$, ***; $p < 0.01$, **; $p < 0.05$, * and $p > 0.05$, not significant.

Examinations

- Clinical signs - Time schedule: immediately before dosing and one and five hours on weekdays, before dosing and one hour after dosing on weekends and public holidays. During the treatment-free period satellite group animals were observed twice daily, morning and afternoon (once daily on weekends). Parameters: overt signs of toxicity, ill-health, behavioural changes
- Body weight – Time schedule for examinations: Individual body weights were recorded on the day before the start of treatment (day 0) and on days 7, 14, 21 and 28 and in the case of satellite group animals on days 35 and 44. Body weights were also recorded at necropsy.
- Food consumption – weekly
- Water consumption – Time schedule for examinations: water intake was observed daily by visual inspection of the water bottles per group.

- Haematology – Time schedule for collection of blood: at the end of the treatment period and in satellite group animals at the end of the treatment-free period (blood samples were obtained by orbital sinus puncture). Anaesthetic used for blood collection - (diethyl ether). Animals not fasted. How many animals: all animals. Parameters examined: haematology (haematocrit (Hct), haemoglobin (HB), erythrocyte count (RBC), total leucocyte count (WBC), erythrocyte indices [mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC)]); blood chemistry (blood urea, total protein, albumin, albumin/globulin ratio [by calculation], sodium, potassium, chloride, calcium, inorganic phosphorus, creatinine, alkaline phosphatase [AP], alanine aminotransferase [ALAT], aspartate aminotransferase [ASAT], glucose, gamma glutamyl transpeptidase (γGT), triglycerides, total cholesterol and bilirubin). In satellite group animals only those parameters were examined, that showed possible treatment-related changes after 28 days.
- Urinalysis – urinalysis was performed on all animals within the week prior to necropsy (week 4 or week 6 (satellite group)). Metabolism cages used for collection of urine: Yes, urine was collected over a period of 16 hours. The animals were maintained under conditions of normal hydration during collection, but without access to food. Parameters examined: volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, reducing substances, blood.
- Gross pathology – the following organs, dissected free from fat, were weighed before fixation: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen
- Histopathology – Samples were removed from all animals and preserved in 10% buffered formalin. All animals were assessed by gross pathology, followed by histopathological examinations: adrenals, aorta (thoracic), bone and bone marrow (femur), brain, caecum, colon, duodenum, eyes, gross lesions, heart, muscle (skeletal), pancreas, pituitary, rectum, sciatic nerve, skin (hind limb), spleen, stomach, testes, thymus, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical and mesenteric), thyroid/parathyroid, trachea, urinary bladder, oesophagus and ovaries. The following preserved tissues from all high dose and control group animals were prepared as paraffin blocks, sectioned at nominal thickness of 5 µm and stained with haematoxylin and eosin: adrenals, heart, kidneys, liver, spleen and testes. Macroscopically observed lesions were also processed in addition to the above. Since there were indications of treatment-related changes in the liver, kidneys and testes, examination of these tissues was subsequently extended to the remaining groups, body weight and body weight changes.

Results and discussion

- Clinical signs – effects observed, treatment-related
    - 750mg/kg: The following clinical signs were observed from day three onward: increased salivation, red/brown staining around the snout and mouth, wet fur, red/brown staining of the fur, hair loss, piloerection, hunched posture, lethargy, ptosis, diuresis, diarrhoea and abdominal distension, single incidence of vocalisation. Satellite high dose animals recovered immediately following cessation of dasing and appeared normal throughout the treatment-free period.
    - 250mg/kg: Animals from this group showed the same clinical signs, though with less severity, from day four onward, which were observed in the high

dose group, with the exception of diarrhoea, abdominal distension, and vocalisation.

- 50mg/kg: no clinical signs were observed.

- Mortality - mortality observed, non-treatment-related
  - 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.
- Body weight and weight changes - effects observed, treatment-related
  - 750mg/kg: Bodyweight and bodyweight gains were significantly reduced in week four in males and females. Four females lost bodyweight within this week. Females of the satellite group were not affected. Weight gains quickly recovered in satellite high dose males during the treatment-free period. Reduced weight gain was also observed in high dose males throughout weeks 1 and 2, while an increase in bodyweight gain was observed in females in week 3.
  - 250mg/kg: Bodyweight gains were reduced in males and females throughout week 4. No adverse effect on bodyweight was noted in animals treated with 50 mg/kg/day.
- Food consumption and compound intake - no effects observed
  - No differences in food consumption were observed.
- Haematological findings - effects observed, treatment-related
  - High dose animals of both sexes and intermediate dose females showed reduced haemoglobin levels. Calculation of erythrocyte indices showed reductions in mean corpuscular volume and mean corpuscular haemoglobin. Platelet counts were also slightly reduced in high dose females but clotting potential (by 'Hepato-Quick') was unaffected. Most values for the above parameters were within the normal range, but occasionally individual or mean values were slightly outside the normal range. These minor changes were probably associated with the general condition of the animals rather than being direct haematopathological effects.
  - Males treated with 750 mg/kg/day showed a statistically significant increase in leucocyte counts compared with controls (p =<0 .001) and a slight increase was also noted in intermediate dose males. The increase was predominantly in the lymphocyte fraction and a slight increase in lymphocyte counts was also apparent for high and intermediate dose females. Lymphocyte and total leucocyte count for individual animals were all entirely within the normal range for rats of this strain and age. However, a slight increase in neutrophil counts involved one value outside the normal range. Neither lymphocytosis nor granulocytosis have been associated with specific chemical insults and, in the absence of any other evidence of infection, the intergroup differences were considered to be entirely fortuitous.
- Clinical biochemistry findings - effects observed, treatment-related
  - Toxicologically significant blood chemical changes were observed, indicative of cholestatic hepatic injury and renal obstruction.

- Animals of both sexes treated with 750 mg/kg/day showed marked elevations in cholesterol and gamma glutamyl transpeptidase with males also showing increased alkaline phosphatase. Bilirubin was increased in high dose animals of both sexes and in mid-dose females.
- Creatinine levels were substantially elevated in males and females treated with 750 mg/kg/day with some individual animals showing extremely high values. This change was accompanied in males by increased blood urea. Males from the intermediate dose group also showed a slight, but statistically significant increase in this parameter. Calcium levels were elevated in mid and high dose males, and high dose animals of both sexes showed reduced levels of aspartate aminotransferase, but the biological significance of this change is unclear.
- Effects in satellite groups were confined to a slight increase in cholesterol (females) and calcium (males), thus changes in blood chemistry were reversible in this study. No treatment related changes were observed in low dose animals.

- Urinalysis findings – effects observed, treatment-related
  - An increased incidence of ketones in the urine was noted for high dose animals of both sexes and intermediate dose males. High dose animals also showed increased urine volume and reduced specific gravity. These changes were not apparent in satellite group animals after the treatment-free period.
- Organ weight findings including organ / body weight ratios effects observed, treatment-related
  - Relative liver weights were markedly increased in the high and mid dose in both sexes. Relative kidney weights were also elevated in high dose animals and mid dose males. in the satellite group, an increase in liver weight was still observed, but kidney weights had returned to normal.
- Gross pathological findings – effects observed, treatment-related
  - Animals of the high dose group had enlarged livers, which were occasionally dark or showed accentuated lobular pattern, pallor of the adrenals, and abnormally small testes.
  - Satellite group animals showed patchy pallor of the liver and compacted contents in the caecum and distal ileum. The stomach and small intestine were haemorrhagic with ulceration of the glandular gastric mucosa and white thickening in the nonglandular region of the stomach. Small testes were noted in one animal.
- Histopathological findings: non-neoplastic – effects observed, treatment-related
  - Treatment-related changes were observed in the liver, kidneys and testes.
  - Liver: Periportal hepatocyte vacuolation was observed in two female rats dosed at 750 mg/kg/day. Similar changes were not observed in control animals, nor amongst those from remaining treatment groups, including satellite group animals.
  - Kidneys: Basophilia and, in some instances, associated dilatation of distal tubules was observed for male and female rats dosed at 750 mg/kg/day.

Similar changes were not observed in control animals, nor amongst those from other treatment groups, including satellite group animals.

- Testes: Testicular atrophy, frequently bilateral, was seen in all male rats dosed at 750 mg/kg/day. Control animals did not demonstrate similar changes. Although one animal from each of the remaining treatment groups also exhibited a minimal degree of testicular atrophy, this was considered to be spontaneous in origin and unrelated to treatment at these dose levels. Testicular atrophy was also present amongst satellite 750 mg/kg/day animals, although the incidence was reduced (3/5).

Dose descriptor	**Effect level**	**Based on**	**Sex**	**Basis for effect level**
NOAEL	50 mg/kg bw/day	actual dose received	male/female	clinical signs
				body weight and weight gain
LOAEL	250 mg/kg bw/day	actual dose received		based on marked reduction in bodyweight gain, increase in liver (app. +25%) and kidney (app. +13%) weight

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
	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

Incidence 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

Overall remarks

- Oral administration of test substance under the conditions of this study, resulted in treatment-related changes at dose levels of 250 and 750 mg/kg/day. During the dosing period animals treated with these dose levels showed changes in behaviour and physical condition together with a marked reduction in bodyweight gain. These effects were distinctly dose-related in severity. However, there was no concomitant effect on food consumption, implying that he bodyweight changes resulted from a direct toxicological effect of the test material and were not simply related to lethargy and consequent failure to eat normally. Possibly associated with the general loss of condition in these animals were several minor haematological changes which, in themselves, are unlikely to be of any substantial toxicological significance.
- Blood chemical determinations demonstrated a number of abnormalities which were strongly suggestive of renal and hepatic changes. In particular, elevations in bilirubin, cholesterol, gamma glutamyl transpeptidase and alkaline phosphatase were indicative of some form of cholestatic injury although this was not entirely substantiated by the histopathological changes seen in the liver. In addition to this, increased levels of creatinine and urea in the plasma suggested renal obstruction which may be supported by elevated kidney weights and by the histopathological evidence of basophilia and tubular dilatation. Adverse liver changes are further indicated by the presence of ketones in the urine which, in association with reduced glucose and increased triglyceride levels in the blood, may suggest some defect in carbohydrate metabolism. A marked hepatomegaly was also apparent in animals treated with 250 and 750 mg/kg/day.
- Males from the 750 mg/kg/day dose group showed reduced testicular size which was identified microscopically as testicular atrophy.
- Treatment-related histopathological changes were confined to animals treated with 750 mg/kg/day but organ weight and blood chemical data strongly suggest that renal and hepatic changes were also present in the 250 mg/kg/day dose group, albeit at a lower severity level. To a large extent the effects seen in the high dose group appeared to regress in satellite group animals over the fourteen-day treatment-free period. However, testicular atrophy and hepatomegaly were persistent.

3.3.1.2 Repeated dose toxicity: oral. Key study 1991 (Sub-chronic)

Study reference:
Study report (1991) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:
Test type
Sub-chronic toxicity: oral equivalent or similar to ECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) based on EPA-TSCA Guideline "Functional Observational Battery" & EPA-TSCA Guideline " Neuropathology" (Federal Register Vol. 50, No. 188). GLP compliant.

Test substance

- (Diphenylphosphinyl)-(2,4,6-trimethylphenyl)methanone.
- Degree of purity – 94.8%.
- Impurities – not reported.
- Batch number – 49/0193.

Test animals

- Rat; Wistar rat; male/female.
- 10 animals per sex per dose.
- Age and weight at the study initiation – 5 weeks approximately, 191 (177 - 210) g for males, 156 (143 - 217) g for females.

Administration/exposure

- route of administration – oral, gavage.
- Duration and frequency of test/exposure period – 3 months (total of 64 applications); once daily on workdays (5days/week, excepted for 3 working days over the whole experimental period).
- Doses/concentration levels – 100, 300, 1000 mg/kg bw/day (actual dose received).
- Rationale for dose level selection – the doses were selected based on the results of a test study where a dose of 1000 mg/kg bw/day was applied 10 times in 14 days and did not lead to marked toxicity besides a minimal reduction in body weight (6 %) in male but not in female animals if compared to controls.
- Vehicle – CMC (carboxymethyl cellulose) 0.5% in water.
- Control group and treatment – yes, concurrent vehicle.
- Test substance formulation preparation – Stability of the test substance in the vehicle was verified for a period of 24h. The concentrations from the beginning and the end of the study were verified via UV spectroscopy at 379 nm and found to be within 10% of the target concentration.
- Statistical methods - the statistical assessment of the findings was based on the analysis of variance (ANOVA) followed by a Dunnett´s test (Dunnett CW, J. Amer. Statist. Assoc., 50, 1096-1121, 1955,´Dunnett CW, Biometrics, 20, 482-491, 1964.

Examinations

- Detailed clinical observations – twice daily for any evident signs of toxicity / mortality; once weekly for exact clinical signs.
- Body weight– once a week.
- Food consumption for each animal – once a week.
- Haematology - collection of blood: in the morning on days 33 and 87 after the beginning of administration. Animals not fasted. All animals examined. Parameters examined: leukocytes, erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets and thromboplastin time.
- Clinical chemistry – in the morning on days 33 and 87 after the beginning of administration. Animals not fasted. All animals examined. Parameters examined: 1) enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-γ-glutamyltransferase: 2) blood chemistry (sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol).

- Neurobehavioral examination – once prior to the start of the study (Oct 16 1987), at 1, 6, and 24 hours after the first administration and before the daily exposure on days 7 (Oct 26 1987) and 14 (Nov 2 1987) and then monthly to day 91 (Nov 30 1987; Dec 21 1987 and Jan 18 1988). Dose groups that were examined: all animals.
    - Battery of functions tested (observation of present/absent): tremors, convulsions, lacrimation/secretion of pigmented tears, salivation, piloerection, pupil size (constriction/dilatation), diarrhoea, vocalization, paresis, paralysis, and ataxia.
    - Appearance of impairments referring to: general appearance, posture, skin colour, locomotor activity, respiration, urination (volume, odour, colour), pupillary reflex, winking reflex, righting reflex, behaviour, grip strength, body tone, vision, olfaction, audition, sensitivity of the body surface, pain perception (hot plate), tail Pinch, toe Pinch, visual placing response.
- Sacrifice and pathology
    - Perfusion fixation – Organs of the first three animals per group and sex were fixed by perfusion using 2.5% glutaraldehyde and 4% formaldehyde.
        - Gross pathology – brain, gasserian ganglia of both sides, dorsal root ganglia (C3-C6 and L1-L4), proximal sciatic nerve (mid-thigh and sciatic notch), gastrocnemius muscle, kidneys, adrenal glands, skin, medulla oblongata, spinal cord at cervical (C3-C6) and lumbar swelling (L1-L4), dorsal and ventral root fibres (C3-C6 and L1-L4), sural and tibial nerve (knee region), liver, spleen, testes, all gross lesions.
        - Histopathology – all groups: gastrocnemius muscle, skin (auricle), skin (paw region), liver, kidneys, testes, spleen, all gross lesions (if observed), gasserian ganglia, dorsal root ganglia, dorsal and ventral root fibres, proximal sciatic nerve, sural and tibial nerve. high dose and control: brain (forebrain, cerebrum, midbrain, cerebellum, pons), medulla oblongata, spinal cord at C3-C6 and at L1-L4.
    - Immersion fixation – Organs of the remaining 7 animals per group and sex were necropsied and assessed by gross pathology prior to fixation in 4% formaldehyde solution.
        - Gross pathology – yes.
        - Histopathology – all groups: liver, kidney, spleen, adrenals, testes, left hind extremity with all skeletal muscles and sciatic nerve roots, all gross lesions high dose and control: gasserian ganglia of both sides, spinal cord at cervical and lumbar swelling (C3-C6 and L1-L4), dorsal root ganglia (C3-C6 and L1-L4), dorsal and ventral root fibres (C3-C6 and L1-L4), proximal sciatic nerve (mid-thigh and sciatic notch), sural and tibial nerve (knee region), brain, medulla oblongata.

Results and discussion

- Clinical signs – effects observed, treatment-related
    - 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 males and females of the 1000 mg/kg

group. In males of both, the 300 and the 1000 mg/kg group, the testes were reduced in size, which was palpable from week 6 onwards.

- Mortality – mortality observed, non-treatment-related
  - Two females of the 1000 mg/kg group died during the experimental period.
- Body weight and weight changes – effects observed, treatment-related
  - For males and females of the 1000 mg/kg group body weight was reduced (23% in males, 8% in females). At 300 mg/kg, body weight reduction only concerned the males (10%). Body weight gain was also reduced by 38% (high dose males), 16% (mid dose males), and 16% (high dose females).
- Food consumption and compound intake – effects observed, treatment-related
  - An increase in food consumption was reported for the females of the 1000 mg/kg.
- Haematological findings – effects observed, treatment-related
  - In the females of the 1000 mg/kg group, erythrocytes, haemoglobin, haematocrit and thromboplastin time (Hepato Quick test) were decreased. Haemoglobin and haematocrit values were also found to be reduced in females exposed to 300 mg/kg. In contrast, leucocytes, platelets, eosinophilic granulocytes, neutrophilic polymorphonuclears, and calcium levels were increased in mid and high dose females.
- Clinical biochemistry findings – effects observed, treatment-related
  - Serum alkaline phosphatase and gamma-glutamyltransferase activity were significantly increased in both sexes of the high dose group (1000mg/kg). In addition, alanine aminotransferase activity was significantly increased in high dose males. Triglyceride levels were decreased in both sexes of the high dose group, while cholesterol levels were increased, and clotting time was decreased in females only. These findings are attributed to liver dysfunction.
- Organ weight findings including organ / body weight ratios – effects observed, treatment-related
  - Necropsy revealed increased absolute and relative kidney and liver weights in the females and increased relative kidney and liver weight in males of the 1000 mg/kg group and females of the 300mg/kg group. In all males in the mid and high dose group, the absolute and relative testes weights were decreased, on average by about 50%.
- Neuropathological findings – no effects observed
  - Neither functional defects nor any other signs of neurotoxicity were observed.
- Histopathological findings: non-neoplastic – effects observed, treatment-related
  - Despite altered blood and urine parameters and increased liver and kidney weight, histopathology revealed no damage on these organs. In the testes of mid and high dose males marked diffuse atrophy of the testicular parenchyma and a slight to moderate interstitial oedema was detected. No further substance related effects were found. Especially, no damage was detected in any samples taken from nervous tissues.

Dose descriptor	Effect level	Based on	Sex	Basis for effect level
NOAEL	100 mg/kg bw/day	nominal	male/female	clinical signs
				body weight and weight gain
LOAEL	300 mg/kg bw/day	actual dose received	male/female	haematology
				clinical biochemistry
				organ weights and organ / body weight ratios

Body and testes weight development; results of testes pathology:

	Dose group/ parameter	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
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	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
	Oedema	-	-	10 / 10	10 / 10
	Focal atrophy	2/10	1/10	-	-
	Vacuolar degeneration	7/10	10/10	-	-
	Reduced spermiogenesis	-	1/10	-	-

Incidence and grading of microscopic findings:

Dose group	Grading	0 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
Diffuse atrophy	1 (minimal)	-	-	-	-
	2 (slight)	-	-	-	-
	3 (moderate)	-	-	1	-
	4 (marked)	-	-	9	10
	5 (severe)	-	-	-	-
oedema	1 (minimal)	-	-	-	-
	2 (slight)	-	-	3	7
	3 (moderate)	-	-	7	3
	4 (marked)	-	-	-	-
	5 (severe)	-	-	-	-
Focal atrophy	1 (minimal)	-	-	-	-
	2 (slight)	-	-	-	-

	3 (moderate)	1	-	-	-
	4 (marked)	1	1	-	-
	5 (severe)	-	-	-	-
Vacuolar degeneration	1 (minimal)	3	3	-	-
	2 (slight)	1	6	-	-
	3 (moderate)	3	1	-	-
	4 (marked)	-	-	-	-
	5 (severe)	-	-	-	-
Reduced spermiogenesis	1 (minimal)	-	-	-	-
	2 (slight)	-	-	-	-
	3 (moderate)	-	1	-	-
	4 (marked)	-	-	-	-
	5 (severe)	-	-	-	-

3.3.1.3 Repeated dose toxicity: oral. Supporting study 2001 (Sub-chronic)

Study reference:
Study report (2001) as summarised in the publicly disseminated REACH Registration for Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide https://echa.europa.eu/registration-dossier/-/registered-dossier/13110 accessed 27 May 2020.

Detailed study summary and results:
Test type
Sub-chronic toxicity: oral equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) – deviation: only one dose group, only male animals, evaluation of testis. Not GLP compliant.

Test substance
- (Diphenylphosphinyl)-(2,4,6-trimethylphenyl)methanone.
- Degree of purity – 99.3%.
- Impurities – not reported.
- Batch number – 99-0029.

Test animals
- Rat; Wistar rat; male.
- Single dose – 3 animals (4 weeks exposure), 10 animals (3 months exposure).
- Age at the study initiation - 41-43 days in the 4-week exposure group, 34 days in the 3-months exposure group.
- Weight at the study initiation – not reported.

Administration/exposure
- Route of administration – oral, gavage.
- Duration and frequency of test/exposure period – once daily for 4 weeks/ 3 months.
- Doses/concentration levels – 1000 mg/kg bw/day.
- Rationale for dose level selection – based on formerly performed 28-day and 90-day study.

- Vehicle – CMC (carboxymethyl cellulose) 0.5% in water; Concentration in vehicle: 10g/100mL; Amount of vehicle (if gavage): 10mL/kg/bw.
- control group and treatment – concurrent vehicle control for the 4-weeks exposure, concurrent without treatment for the 3 months exposure.
- Test substance formulation/diet preparation achieved concentration by sex and dose level, stability and homogeneity of the preparation – not specified.
- Statistical methods - The weight parameters were assessed statistically by means of the non-parametric one-way analysis using KRUSKAL-WALLIS test; the WILCOXON test was added in case of a p-value equal or below 0.05.

Examinations

- Cage side observations – 2 times daily on working days, once daily on weekends and holidays.
- Detailed clinical observations – daily.
- Body weight – at the end of the study.
- Haematology – no.
- Clinical chemistry – no.
- Urinalysis – no.
- Gross pathology – Yes.
- Histopathology – Yes (testes, gross lesions), animals were fasted for 16-20h prior to necropsy.

Results and discussion

- Clinical signs and mortality – No adverse effects reported.
- Body weight and weight gain – Body weight reduction (10%) after exposure for 3 months.
- Organ weights – The mean absolute and relative testes weights showed a statistically significant decrease after 3 months of treatment. No effect was observed after 4 weeks of treatment.
- Gross pathology
    - Testes – reduced organ size and loss of turgor was noted for 8 of 10 animals after 3 months of treatment, but not after 4 weeks of treatment.
    - Epididymides – a reduced organ size was noted for 8 of 10 animals after 3 months, but not after 4 weeks, of treatment.
    - Skin – one high dose test animal showed sparse hair at the thoracic region after 3 months of treatment only.
- Histopathology: non-neoplastic
    - 3-months exposure – All testes showed a diffuse atrophy of seminiferous tubules with gradings of 2 (slight) up to 4 (severe). In 4 test animals, an oedema and Leydig cell hyperplasia of minimal to slight degree were recorded in addition. All 8 epididymides with a reduced organ size were examined histopathologically and correlated with an oligozoospermia up to grade 5(azoospermia).
    - 4-weeks exposure – No histomorphological changes were detected.

Effect level	Based on	Sex	Basis for effect level
1000 mg/kg bw/day	nominal	male	test substance induced testicular atrophy after 90 days (only concentration tested)

The absolute mean weights are summarized below:

Treatment period	4 weeks		3 months	
Dose (mg/kg bw)	0	1000	0	1000
Body weight	251.8±19.6	244.8±7.18	332.2±28.9	298.1±29.6
Testes weight	3.14±0.14	3.19±0.27	3.29±0.28	2.1±0.70

The incidence of microscopic findings is resumed below:

Treatment period	4 weeks		3 months	
Dose (mg/kg bw)	0	1000	0	1000
Testes	3	10	3	10
Diffuse atrophy	-	-	-	10
Leydig cell hyperplasia	-	-	-	4
Oedema	-	-	-	4
Epididymides	-	-	-	8
Oligozoospermia	-	-	-	8
Skin	-	-	-	1

3.3.2 Human data – no data