# 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:

**a) *p*-cymene; 1-isopropyl-4-methylbenzene**

**and**

**b) 3-*p*-cumenyl-2-methylpropionaldehyde; 2-methyl-3-(4-isopropylphenyl)propanal [1];**

**3-(*p*-cumenyl)propionaldehyde; 3-(4-isopropylphenyl)propanal [2];**

**4-isopropylbenzaldehyde; cuminic aldehyde [3];**

**4-isopropylbenzoic acid; cuminic acid [4]**

**EC Numbers:**

a) **202-796-7**
b) **203-161-7 [1]; 231-885-3 [2]; 204-516-9 [3]; 208-642-5 [4]**

**CAS Numbers:**

a) **99-87-6**
b) **103-95-7 [1]; 7775-00-0 [2]; 122-03-2 [3]; 536-66-3 [4]**

**Index Numbers:**

a) **601-094-00-1**
b) **TBD**

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**Version number: 2** **Date: 2023-05-12**

# CONTENTS

# 1 PHYSICAL HAZARDS

Not evaluated in this dossier.

# 2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Study summaries are adapted from the publically available registration dossiers found at ECHA's dissemination site, or when scientific publications are used as references, from publically available abstracts.

## 2.1 Study 1: 3-*p*-cumenyl-2-methylpropionaldehyde

Basic toxicokinetics in vivo

Species-specificity of metabolic fate and toxicity.

Reference Type: study report

Title: Unnamed

Year: 2019

Qualifier: no guideline followed

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, sperm analysis, gross necropsy findings and histopathologic examinations (testis only), plasma concentration of Cyclamen Aldehyde; CoA-conjugate formation in testes and liver, metabolite profile in the testes, liver and plasma samples obtained on day 28 of treatment.

GLP compliance: no

Species: rat

Strain: Wistar

Remarks: WI(Han)

Sex: male

Crl: WI(Han) rats were received from Charles River Deutschland, Sulzfeld, Germany. The animals were 7 weeks old at initiation of dosing and weighed between 205 and 235 g. A health inspection was performed before the initiation of dosing.

The animals were allowed to acclimate to the Test Facility toxicology accommodation for 6 days before the commencement of dosing.

On arrival and following randomization, animals were group housed (up to 5 animals of the same sex and same dosing group together) in polycarbonate cages (Makrolon type IV, height 18 cm) containing appropriate bedding (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. Animals were separated during designated procedures/activities.

Environmental Conditions

Target temperatures of 18 to 24°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 21°C with an actual daily mean relative humidity of 39 to 54%.

Food: Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) was provided ad libitum throughout the study, except during designated procedures.

Water: Municipal tap water was freely available to each animal via water bottles.

Route of administration:oral: gavage

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.

Vehicle:corn oil

Supplier: Sigma-Aldrich

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 weekly, filled out in daily portions and stored at room temperature. Formulations were stirred for at least 30 minutes before use. If practically possible, the dosing formulations and vehicle were continuously stirred during dosing. Adjustment was made for specific gravity of the vehicle and test item. Any residual volumes were discarded.

Sample Collection and Analysis

The Sponsor provided data that demonstrated that the test article was stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data provided by the Sponsor have been retained in the study records.

Analytical verification of doses or concentrations: no

Duration of treatment / exposure: Rats dosed for 28 days.

Frequency of treatment: Once daily.

Dose / conc.: 30 mg/kg bw/day (nominal)

Dose concentration: 6 mg/mL

Dose Volume: 5 mL/kg

Dose / conc.: 100 mg/kg bw/day (nominal)

Dose concentration: 20 mg/mL

Dose Volume: 5 mL/kg

Dose / conc.: 300 mg/kg bw/day (nominal)

Dose concentration: 60 mg/mL

Dose Volume: 5 mL/kg

No. of animals per sex per dose: 5

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test item. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives. At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist. The study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD2360020172866 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

Control animals: yes, concurrent vehicle

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. The total number of animals used in this study (5 males/group) was considered to be the minimum required to properly characterize the effects of the test item. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

Male Wistar Han rats, approximately 7 weeks of age on treatment Day 1 (for exact details see main study report), were administered 3-p-cumenyl-2-methylpropionaldehyde via oral gavage daily for at least 28 consecutive days at dose levels of 30, 100 and 300 mg/kg bw/d.

Animals were assigned to groups by a stratified randomization scheme to achieve similar group mean body weights, with all animals within ± 20% of the sex mean. The dose levels were selected based on information provided by a14-day oral gavage study in rabbits.

Positive control: No

Observations and examinations performed and frequency: 5 males/group

BIOANALYSIS:

Bioanalytical Sample Collection:

Prior to necropsy, blood was collected from the jugular vein. Blood samples at a target volume of 0.5 mL were collected into tubes containing K2-EDTA as anticoagulant. 4.9.1.2. Bioanalytical Sample Processing Samples were centrifuged within 2 hours after blood sampling at approximately 2000g for 10 minutes at 4-8 °C. Immediately after centrifugation, plasma was stored in labeled polypropylene tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) at ≤ -75□C until shipped on dry ice to the Sponsor. Samples were used to determine the circulating blood concentration of metabolites of Cyclamen aldehyde in plasma sampled at the end of a 28 days range finder gavage study in rats by GC-MS. Collection on ice.

Testis, epididymis and liver were collected for metabolite analysis to determine the CoA-conjugate formation in tissue samples of both the testes and the liver, and to determine the metabolite profile in tissue samples of both the testes and the liver and in plasma samples obtained on day 28 using high-resolution LC-MS analysis.

Necropsy

At necropsy, the left testis, right epididymis and liver were collected, stored in plastic bags and snap frozen into liquid nitrogen. Samples were stored at ≤ -75 C until shipment on dry ice to the Sponsor for metabolite analysis.

Plasma was collected to determine circulating blood concentration of metabolites of Cyclamen aldehyde at day 28. Testes, liver and plasma were collected to determine the CoA-conjugate formation. Metabolite profile were evaluated for plasma, testes and liver.

Bioanalysis

Plasma:Levels of Cyclamen aldehyde and Cyclamen alcohol were below detection limit in all plasma samples including the non-diluted plasma samples. 4-iPBA plasma concentrations were below detection limit in plasma samples collected from the control group and at 30 mg/kg bw/day. 4-iPBA was detected in all plasma samples at 100 and 300 mg/kg bw/day, ranging from 13.2 to 26.6 µM (100 mg/kg bw/day) and 151.1 to 385.0 µM (300 mg/kg bw/day). Average 4-iPBA concentrations were 18.8 ± 5.1 µM and 264.6 ± 85.4 µM at 100 and 300 mg/kg bw/day, respectively, corresponding to a 14-fold difference between the animals receiving the medium and the highest dose.

Cyclamen acid concentrations were below detection limit in plasma samples collected from the control group. Cyclamen acid was detected in all plasma samples of all test item groups and ranged from 0.1 to 0.3 µM (30 mg/kg bw/day), 0.3 to 1.2 µM (100 mg/kg bw/day) and 1.4 to 6.4 µM (300 mg/kg bw/day). Average Cyclamen acid concentrations were 0.2 ± 0.1 µM, 0.7 ± 0.4 µM and 3.2 ± 2.1 µM at 30, 100 and 300 mg/kg bw/day, respectively. Plasma concentrations of Cyclamen acid were 3.5-fold higher at 100 mg/kg bw/day compared to 30 mg/kg bw/day, whereas concentrations were 4.6-fold higher at 300 mg/kg bw/day compared to 100 mg/kg bw/day. However, plasma levels of Cyclamen acid were 27-fold lower (100 mg/kg bw/day) and 83-fold lower (300 mg/kg bw/day), respectively, compared to 4-iPBA.

Testes and liver:

In animals dosed with 30 mg/kg bw/day, trace amounts 4-iPBA-CoA were detected in the testes of only one individual. At 100 mg/kg bw/day, the conjugate was detectable at low levels in testes samples from all animals. At 300 mg/kg bw/day, 5-6 times higher levels (0.724 ± 0.222 nmol/gtissue) than at 100 mg/kg

bw/day were observed, indicating that at the toxic dose this metabolite is significantly formed in the reproductive tissue. The concentration in the liver is clearly higher (> 500 fold), and strong accumulation in the liver of this metabolite as previously shown in the in vitro studies.

Metabolites:

The major metabolite based on peak area observed by LC-MS analysis in the tissue samples and in the blood plasma is the acyl-glucuronide conjugate of iPBA (M25). The second most abundant peak is U1, an unknown metabolite with a mass of 208.1099 which was detected as the most abundant peak in plasma at 30 and 100 mg/kg bw/day and the second most abundant metabolite at 300 mg/kg bw/day. Further abundant metabolites were 4-iPBA (M3), hydroxylated 4-iPBA (M8), hydroxylated 4-iPBA-acylglucuronide (M37) and the glycine conjugate of 4-iPBA (M36).

Cyclamen aldehyde is easily oxidized to the corresponding acid (M2), but this is only a minor intermediate as shown before by GC-MS analysis of plasma samples and it was not detectable by LC-MS, neither in tissue nor in plasma samples. The acid is either directly degraded to iPBA or it is hydroxylated, putatively at the isopropyl-side chain. Hence the hydroxylated Cyclamen acid (M5) is a further important metabolite in this analysis especially in the plasma samples, where also the product of a further oxidation step is observed (di-acid, M6, detected in plasma mainly). M5 can be degraded to the hydroxylated iPBA (M8), which is found in tissue and plasma samples. However, M5 could be also formed from Cyclamen aldehyde by side chain degradation to 4-iPBA followed by hydroxylation. In both cases, the hydroxylated metabolites (M5 and M8) are then again conjugated, especially to glucuronic acid and esp. the glucuronide of M8 (M37) is quite abundant.

Next to the glucuronide, different iPBA conjugates are detected in the plasma and in testes and liver (glycine-, taurine-, carnitine- and glutamic acid-conjugates; M36, M28, M30 and M34). Thus in summary, the key metabolic pathways observed are formation of iPBA and subsequent conjugation mainly with glucuronic acid and glycine and/or hydroxylation and further oxidation.

Conclusions:

The key metabolic pathways for Cyclamen Aldehyde observed are formation of iPBA and subsequent conjugation mainly with glucuronic acid and glycine and/or hydroxylation and further oxidation. 4-iPBA is efficiently conjugated to coenzyme A in the liver leading to high levels of this CoA conjugate. In the testes, iPBA-CoA was also found, but at substantially lower concentrations. Different iPBA conjugates are detected in the plasma and in testes and liver (glucuronide-, glycine-, taurine- carnitine- and glutamic acid-conjugates) with glucuronides as major Phase II metabolites.

## 2.2 Study 2: 3-*p*-cumenyl-2-methylpropionaldehyde and 3-(p-cumenyl)propionaldehyde

Basic toxicokinetics in vitro / ex vivo

Reference Type: publication

Title: Unnamed

Year: 2017

Publication: Laue et al. 2017. p-Alkyl-Benzoyl-CoA conjugates as relevant metabolites of aromatic aldehydes with rat testicular toxicity- studies leading to the design of a safe new fragrance chemical. Toxicological Sciences 160(2), 2017, 244-255.

Conflict of interest: This work was entirely funded by Givaudan. Givaudan is a company a producing and using 3-*p*-cumenyl-2-methylpropionaldehyde.

**Materials and methods**

Objective of study: metabolism,toxicokinetics

Metabolism studies in rat hepatocytes in suspension:

Cryopreserved primary hepatocytes from male rats (pool, Sprague-Dawley) for use in suspension, CHRM (Cyropreserved Hepatocytes Recovery Medium), Williams E Medium (WEM; without Phenol Red, L-

glutamine and HEPES) and supplements (Cell Maintenance Supplement Pack) were purchased from Gibco (Life Technologies Europe B.V., Zug, Switzerland). Rat hepatocytes were thawed in CHRM, washed and resuspended in supplemented WEM medium (cocktail solution of penicillinstreptomycin, insulin, transferrin, selenium complex, BSA and linoleic acid; GlutaMAX, HEPES and Dexamethasone). Viable cells were counted using trypan blue (Sigma-Aldrich, Buchs, Switzerland) staining. Viability was between 82% and 93%. Closed 10 ml headspace glass vials (BGB Analytik, Böckten, Switzerland) were used for the incubations due to evaporation losses observed in initial experiments with BMHCA. Hepatocytes (400 µl; 1x10E6 viable cells/ml) were pre-incubated on a shaker for 5 min at 37°C and 5% CO2 prior to closing the vials. Test chemicals (final concentration: 100 µM) were added to the vials containing the cell suspensions, the vials were tightly closed and incubated at 37°C on a shaker. For each replicate and time point one vial was used. Controls with the test chemicals in hepatocyte medium without cells were carried out to assess chemical stability. Samples (200 µl) were removed from the vials after 0, 1, and 4h and stopped with ice cold 1 M HCl (200 µl) containing ibuprofen as internal standard (50 µM). Samples were extracted with 200 µl MTBE, centrifuged (15 000 x g, 5 min, room temperature (RT), and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The remaining sample volume (100 µl from each vial was pooled) was used for liquid chromatography coupled to mass spectrometry (LC-MS) analysis of phase II metabolites after purification using solid phase extraction (OASIS HLB µElution plate 30 µm, Waters, Baden-Dättwil, Switzerland).

Formation of acyl-CoA conjugates in plated rat hepatocytes:

Plateable primary male rat hepatocytes (Sprague-Dawley, Grade P pooled cryopreserved hepatocytes, for plated assays), WEM (with Phenol Red, with GlutaMAX, without HEPES) was purchased from Gibco (Life Technologies Europe B.V.) and supplements from Biopredic International (Saint Gre´goire, France). Cells were thawed following the supplier's protocol and seeded at a density of 450 000 cells/ml in 0.25 ml seeding medium (WEM supplemented with fetal bovine serum, Dexamethasone, penicillin-streptomycin, insulin, GlutaMAX and HEPES) using 48-well plates which were coated with collagen (Collagen I Rat Protein, Tail; Gibco, Life Technologies Europe B.V.) and incubated for 4–5h to facilitate attachment. Then, the medium was changed to culturing medium (WEM supplemented with penicillin, streptomycin, insulin, hydrocortisone without serum) to prevent detachment of cells. Test chemicals were dissolved in methanol (80%–90% methanol) and directly added with the culture medium (0.8-0.9% final methanol concentration). For structure-activity studies, final concentration of test chemicals was 50 µM or 5 µM. Cells were incubated for 0.5–22 h in the presence of test chemicals. To stop the reactions, the culture medium was removed and 0.5 M citric acid (pH 2.0; 60 µl) and cold acetonitrile (60 µl) containing 2 mM decanoyl CoA as internal standard were added to each well. The attached cells were removed and disrupted by scraping the well with a pipette tip and by pipetting up and down several times, transferred in 1.5 ml tubes and frozen at -80°C. The cell lysates were defrozen, centrifuged (21 000 x g, 5 min, RT), and the supernatant was diluted with 1.36 ml Tris-buffer (4mM) containing KCl (6 mM), MgCl2 (0.3 mM), and n-heptadecanoyl CoA as internal standard (0.2 µM). Samples were neutralized by addition of NaOH (42 µl of 1 M NaOH) and loaded onto solid phase extraction cartridges (OASIS HLB mElution plate). Columns were washed with 200 µl of ammonium solution (1 M), and samples eluted sequentiallywith 50 µl acetonitrile and 50 µl water.

Formation of acyl-CoA conjugates in rat liver S9 fractions:

Incubations were performed in 50mM Tris-HCl buffer at pH 8.5 containing 72mM KCl, 3.6mM MgCl2, 1 mg/ml rat liver S9 fractions (Gibco, Life Technologies Europe B.V.), 40 µM CoA and 5mM ATP. Different carboxylic acids were dissolved in acetonitrile at 0.8mM, and 2.5 µl were added to a final volume of 200 µl incubation mixture (final concentration: 10 µM) to start the reaction. After 30 min the reaction was stopped by adding 40 µl citric acid (0.5 M, pH 2.0) and 60 µl acetonitrile. The samples were purified with SPE as described earlier.

Determination of benzoic acid metabolites by GC-MS:

A GC-MS method was used to determine the decrease of the parent chemicals and the formation of phase I metabolites. Derivatisation with trimethylsilyldiazomethane in methanol was applied to detect methyl esters of the acid metabolites. Aldehydes react also with diazomethane yielding a methyl ketone which was used for quantification of the parent chemicals. Ibuprofen (50 µM) derivatized to its corresponding methyl ester was used as internal standard. Samples were analyzed by GCMS on an HP 6890 coupled to an MSD 5973

(Agilent Technologies, Basel, Schweiz). The GC column used was a BPX5 (SGE Analytical Science), with an inner diameter of 0.22mm, a length of 12m and a film thickness of 0.25 µm. The injector temperature was set to 230°C. Samples (1 µl) were injected splitless at constant flow (1 ml/min, carrier gas: helium). The temperature of the column oven was ramped from 35°C (hold for 2 min) with 20°C/min to 240°C, then with 35°C/min to 270°C (hold for 3 min). The MS analysis was performed with electron ionization at 70 eV and a mass range of m/z 25-460. Single ion monitoring was applied for the quantification of the analytes. To quantify the decrease of test chemicals and formation of metabolites, calibration standards with parent chemicals and putative metabolites (carboxylic acids and benzoic acid metabolites) were prepared in incubation medium.

Detection of CoA conjugates by LC-HRMS:

CoA conjugates were analyzed with LC-HRMS on a Dionex UltiMate 3000 RS HPLC system coupled to a Q-Exactive orbitrap mass spectrometer (Thermo Scientific, Reinach, Switzerland) with electrospray ionization in both positive and negative ionization mode. For liquid chromatography separation an Agilent Zorbax 300Extend-C18 column with dimensions 2.1 x 50mm and particle size of 3.5 µm with a 2.1 x 10mm precolumn of the same material was used. The flow rate was 0.4 ml/min. Eluent A consists of water containing 0.0025% ammonia (pH around 9.8) and eluent B consists of acetonitrile containing 0.0025% ammonia. A linear gradient was run from 95% eluent A (hold for 1 min) to 100% eluent B within 6 min (hold for 1 min), back to 95% eluent A within 0.5 min followed by 1.5 min equilibration time. The injection volume of the sample was 10 µl. The mass resolution of the HRMS spectra was set to 70 000. The mass accuracy was <5 ppm. Data-dependent high-resolution product ion spectra (HR-MS/MS) were recorded at a resolution of 17 500. Ion source parameters adjusted were as follows: sheath gas flow (30 arbitrary unit), auxiliary gas flow (10 arbitrary unit), capillary temperature (270°C), and source voltage (4 kV in positive mode, -3 kV in negative mode). Fragmentation was obtained from dissociation in an octopole collision cell using higher energy collision dissociation settings at 35 and 45 (arbitrary unit). The mass scan range was set from 120 to 1800 m/z. Calibration standards were prepared with CoA, acetyl-CoA, benzoyl-CoA, tBBA-CoA, and octanoyl-CoA to determine the concentrations of CoA conjugates. Decanoyl-CoA and heptadecanoyl-CoA were used as internal standards. The limit of detection with this method is 0.005 µM for CoA conjugates.

GLP compliance: no

**Test material**

Key Chemicals of the study:

-BMHCA (CAS 80-54-6) - 2-(4-*tert*-butylbenzyl)propionaldehyde

-m-BMHCA (CAS 62518-65-4)

-BMHCA-acid (CAS 66735-04-4)

-BHCA (CAS 18127-01-0)

**-PMHCA (CAS 103-95-7) -3-*p*-cumenyl-2-methylpropionaldehyde**

**-PHCA (CAS 7775-00-0) - 3-(p-cumenyl)propionaldehyde**

-m-iP2MHCA (CAS 125109-85-5)

-iBMHCA (CAS 6658-48-6)

-p-tert-butyltoluene (CAS 98-51-1)

-tBBA (CAS 98-73-7) - tert-butylbenzoic acid

**-p-isopropylbenzoic acid (CAS 536-66-3) - 4-iPBA**

-ibufenac (CAS 1553-60-2)

-ibuprofen (CAS 15687-27-1)

Radiolabelling:no

**Test animals**

Species:rat

Strain:Sprague-Dawley

Sprague-Dawley strain for metabolism in vitro studies and Wistar strain for OECD 407 study

Sex:male

**Results and discussion**

Main ADME results

Type: metabolism

Results: Chemicals causing reprotoxicity in male rats formed p-alkyl-benzoic acids (PABA) in rat hepatocytes in suspension. Compounds metabolized to PABA led to accumulation of p-alkyl-benzoyl-CoA conjugates at high and steady levels in plated rat hepatocytes.

Metabolite characterisation studies

Metabolites identified: yes

The data from the suspension hepatocyte experiments indicate that the chemicals cited above with male reprotoxic effects in the rat are metabolized to p-alkyl benzoic acids, supporting the hypothesis that these are the primary toxic metabolites.

Enzymatic activity

It is not fully clear which enzymes are involved in benzoic acid formation, but one possibility is that the aldehydes are oxidized to the corresponding acids, conjugated to CoA and then undergo b-oxidation to form the corresponding alkyl benzoic acid metabolite.

Conclusions:

All of the test chemicals causing reproductive adverse effects in male rats formed p-alkyl-benzoic acids in rat hepatocytes in suspension. Compounds metabolized to p-alkyl-benzoic acids led to accumulation of p-alkyl-benzoyl-CoA conjugates at high and steady levels in plated rat hepatocytes, whereas CoA conjugates of most other xenobiotic acids were only transiently detected in this in vitro system.

## 2.3 Study 3: 3-*p*-cumenyl-2-methylpropionaldehyde

Title: Unnamed

Year: 2020

Publication: Laue et al. 2020. Benzoyl-CoA conjugate accumulation as an initiating event for male reprotoxic effects in the rat? Structure-activity analysis, species specificity, and in vivo relevance. Archives of Toxicology 94:4115–4129.

Funding: This work was entirely funded by Givaudan, no external funding was received.

Conflict of interest: All authors are employees of Givaudan, a company that is producing and using 3-p-cumenyl-2-methylproprionate.

**Materials and methods**

Objective of study: metabolism, tissue distribution, toxicokinetics

Plateable primary male rat hepatocytes (Sprague–Dawley, Grade P pooled cryopreserved hepatocytes, for plated assays, lots HEP134033 and HEP134040) were purchased from Biopredic International. Cell culture media and supplements were purchased from Gibco™ (Thermo Fisher Scientific). Cells were thawed in Leibovitz L-15 medium supplemented with GlutaMAX (Thermo Fisher Scientific), centrifuged at 115 × g for 1 min at room temperature, and suspended in Williams E Medium with GlutaMAX (WEM; Thermo Fisher Scientific) supplemented with penicillin, streptomycin, bovine insulin and FCS (Additives for

hepatocytes seeding medium, ADD221, Biopredic International). Rat hepatocytes were seeded at a density of 450,000 cells/ mL in 250 µL medium in collagen coated 48-well plates (Laue et al. 2017) and incubated for 4–5 h at 37 °C to facilitate attachment. Then, the medium was changed to culturing medium (WEM with GlutaMAX supplemented with penicillin, streptomycin, bovine insulin and hydrocortison; ADD222, Biopredic International) to prevent detachment of cells.

Plateable primary cryopreserved human hepatocytes from five donors of mixed gender, (HMCPP5 pooled hepatocytes, for plated assays; lot HPP1870744) were purchased from Gibco™ (Thermo Fisher Scientific). Cells were thawed in Cryopreserved Hepatocyte Recovery Medium (CHRM; Gibco™, Thermo Fisher Scientific), centrifuged at 100 × g for 10 min at room temperature and seeded at a density of 700,000 cells/mL in 0.2 mL seeding medium (WEM with GlutaMAX™, supplemented with 5% fetal bovine serum, dexamethasone, and thawing/plating cocktail A (CM3000, Thermo Fisher Scientific) in collagen coated 48-well plates. After 5–6 h incubation to facilitate attachment of cells, medium was changed to culturing medium (WEM with GlutaMAX™, supplemented with dexamethasone and cell maintenance cocktail B (CM4000, Thermo Fisher Scientific).

Primary cryopreserved hepatocytes from male New Zealand White Rabbits (three donors; M00405, lot TDV) were purchased from BioIVT. The same media were used as described for the human hepatocytes except that the rabbit hepatocytes were directly suspended in the seeding medium without centrifugation step to a cell density of 700,000 cells/mL. Test chemicals were dissolved in methanol (80–90% methanol) and directly added to the attached rat, human or rabbit hepatocytes with the corresponding culture medium (0.8–0.9% final methanol concentration). For structure–activity studies, final concentration of test chemicals was 50 µM or 5 µM and at least two independent experiments were done in plated rat, human hepatocytes, and rabbit hepatocytes. Cells were incubated for 0.5–22 h in the presence of test chemicals. To stop the reactions, the culture medium was removed and 0.5 M citric acid (pH 2.0; 60 µL) and cold acetonitrile (60 µL) containing 2 µM decanoyl-CoA as internal standard were added to each well. The attached cells were removed and disrupted by scraping the well with a pipette tip and by pipetting up and down several times, transferred in 1.5 mL tubes and frozen at − 80 °C. The cell lysates were defrozen, centrifuged (21,000 × g, 5 min, room temperature), and the supernatant was diluted with 1.36 mL Trisbuffer (4 mM) containing KCl (6 mM), MgCl2 (0.3 mM) and n-heptadecanoyl-CoA as internal standard (0.2 µM). Samples were neutralized by addition of NaOH (42 µL of 1 M NaOH) and loaded onto solid phase extraction (SPE) cartridges (OASIS HLB µElution plate, Waters). Columns were washed with 200 µL of ammonium solution (1 M), and samples eluted sequentially with 50 µL acetonitrile and 50 µL water and analysed by LC-HRMS. As no synthetic standards of all the different formed benzoyl-CoA conjugates are available for quantification and to account for possible variability between different experiments, BMHCA was tested as a control in each experiment, and the signal of the CoA conjugates formed from test chemicals was normalized to the amount of p-tBBA-CoA formed from BMHCA at a given time point and test concentration(i.e. p-tBBA-CoA formed from BMHCA was set to 100%) for the detailed SAR analysis. The amount of p-tBBA-CoA formed from BMHCA in the different experiments using different batches of rat hepatocytes ranged from 1 to 2 µM.

Cell viability assays:

To verify that the observed decrease of the CoA conjugates over time was not caused by cell lysis or reduced capacity for CoA conjugation, cell viability was assessed by the Presto Blue® assay which is a resazurine based assay measuring metabolic activity. The culture medium was removed from the plated rat, human or rabbit hepatocytes incubated with the solvent control (0.8% methanol in culture medium) and with 50 µM BMHCA (lysmeral) or 50 µM PMHCA (3-p-cumenyl-2-methylpropionaldehyde) (only with rat hepatocytes) for 0.5, 4, 8 (only with rabbit hepatocytes) and 22 h. The Presto Blue® Cell Viability Reagent (Thermo Fisher Scientific) was diluted 1:10 in PBS and 250 µL added to the cells. The plates were incubated for 30 min at 37 °C and analysed in a plate reader by measuring the fluorescence at 560 nm/590 nm. The attachment of cells was verified by microscopic observation prior to the sampling for CoA analysis. Additionally, formation of endogenous CoA conjugates in the solvent controls was evaluated in parallel by LC-HRMS. The most prominent endogenous CoA conjugates detected were two C8-CoAs based on the exact mass of 893.2197 and 891.2040, respectively, and were quantified using commercial octanoyl-CoA or p-tBBA-CoA as references as described below.

Detection of CoA conjugates by LC-HRMS:

CoA conjugates were analysed with high-resolution LC–MS (LC-HRMS) on a Dionex UltiMate 3000 RS HPLC system coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) with electrospray ionization (ESI) in positive ionization mode. For liquid chromatography separation, an Agilent Zorbax 300Extend-C18 column with pore size of 300 Å, dimensions 2.1 mm × 150 mm and particle size of 3.5 µm with a 2.1 mm × 12.5 mm pre-column with particle size of 5 µm of the same material was used. The flow rate was 0.4 mL/min. Eluent A consisted of water containing 0.0025% ammonia (pH around 9.8) and eluent B consisted of acetonitrile containing 0.0025% ammonia. A linear gradient was run from 95% eluent A (hold for 1 min) to 100% eluent B within 6 min (hold for 1 min), back to 95% eluent A within 0.5 min followed by 1.5 min equilibration time. The injection volume of the sample was 10 µL. The mass resolution of the HR-MS spectra was set to 70,000. The mass accuracy was < 5 ppm. Data-dependent high-resolution product ion spectra (HR–MS/MS) were recorded at a resolution of 17,500. Ion source parameters adjusted were as follows: sheath gas flow (30 arbitrary unit), auxiliary gas flow (ten arbitrary unit), sweep gas flow (five arbitrary unit), capillary temperature (270 °C), and source voltage (4 kV in positive mode). Fragmentation was obtained from dissociation in an octopole collision cell using higher energy collision dissociation settings at NCE = 35 (arbitrary unit). The mass scan range was set from 120 to 1800 m/z. Calibration standards were prepared with CoA, acetyl-CoA, benzoyl-CoA, p-tBBA-CoA, and octanoyl-CoA to determine the concentrations of CoA conjugates. Decanoyl-

CoA and heptadecanoyl-CoA were used as internal standards. The limit of detection with this method is 0.005 µM for CoA conjugates.

Determination of parent and phase I metabolites of BMHCA (lysmeral) and PMHCA (3-p-cumenyl-2-methylpropionaldehyde) in plated rat and human hepatocytes:

In addition to the quantification of the CoA conjugates, the decrease of the parent and formation of selected phase I metabolites was evaluated in plated rat and human hepatocytes exposed to BMHCA and PMHCA using GC–MS analysis. Primary cryopreserved rat and human hepatocytes were grown as monolayers and incubated in presence of 50 µM BMHCA and PMHCA as described above. The culture medium was sampled (200 µL) at 0.5, 4 and 22 h, mixed with an equal volume of 5 µM ibufenac as internal standard in 1 M HCl and extracted with MTBE (200 µL). Derivatisation with (trimethylsilyl)diazomethane in methanol was applied to detect methyl esters of the acid metabolites and ibufenac as internal standard. The fragrance aldehydes were detected as methyl ketones and acids as methyl ester. Calibration standards with BMHCA, PMHCA and putative metabolites (alcohols, carboxylic acids, and benzoic acids) were prepared in incubation medium (Laue et al. 2017). Decrease of the parent aldehydes and formation of acid metabolites was quantified by GC–MS on a Trace 1310 gas chromatograph coupled to a TSQ8000 triple quadrupole MS (Thermo Scientific). The GC column used was a HP-5MS UI (Agilent Technologies), with an inner diameter of 0.25 mm, a length of 15 m and a film thickness of 0.25 µm. The injector temperature was set to 230 °C. Samples (1 µL) were injected splitless at constant flow (2 mL/min, carrier gas: helium). The temperature of the column oven was ramped from 35 °C (hold for 2 min) with 10 °C/min or 20 °C/min to 240 °C, then with 35 °C/min to 270 °C (hold for 3 min). The MS analysis was performed with electron ionization at 70 eV and a mass range of m/z 25–460. Single ion monitoring (SIM) was applied for the quantification of the analytes.

Determination of additional phase I and phase II metabolites of BMHCA and PMHCA in plated rat and human hepatocytes:

Additional phase I and phase II metabolites in plated rat and human hepatocytes were determined by LC-HRMS. Cells were disrupted by a repeated freeze–thaw cycle (three times), removed by scraping the well with a pipette (200 µL), transferred to 1.5 mL tubes and mixed with ibufenac (rat hepatocytes; 200 µL 5 µM) or diclofenac (human hepatocytes; 200 µL 1 µM) as internal standard. The cell lysates were centrifuged (21,000 × g, 5 min, RT) and the supernatantloaded onto solid phase extraction cartridges. Columns were washed with 5% methanol and samples eluted sequentiallynwith 50 µL methanol and 50 µL water. Samples were analysed with LC-HRMS and phase I and phase II metabolites were tentatively identified based on their exact mass.

A range finder 28 days study (Study report 2019) was performed on 3-(4-isopropylphenyl)-2-methylpropanal (PMHCA) prior to a 90 days subchronic toxicity study, and animals were used after necropsy for analytical investigations. Male animals (n = 5) were treated for 28 days by oral gavage with 0, 30, 100, and 300 mg/kg bw/day with PMHCA. One day after last dosage and before sacrifice, blood samples were taken for

metabolite analysis. Immediately after sacrifice, one testis and the liver were excised and snap-frozen in liquid nitrogen. The frozen organs were stored and shipped on dry ice.

Serum sample analysis for benzoic acid formation:

Plasma samples (100 µL, shipped on dry ice and kept at -80 °C) were incubated for 2 h at 37 °C in presence of β-glucuronidase from Helix pomatia (final 5000 units/mL plasma) in 500 mM acetate buffer (pH 5.0) containing Ibufenac (800 µM) as internal standard. 200 µL of saturated NaCl solution and 10 µL of 1 M HCl were added, samples were mixed shortly and 500 µL of MTBE was added for extraction of the analytes. A sample of the organic phase (150 µL) was separated and incubated with 30 µL (trimethylsilyl)diazomethane (792 mM) in methanol at 40 °C for 1 h. Samples were diluted 1:10 in MTBE and carboxylic acids (p-iPBA, PMHCA acid and ibufenac) were detected as methyl esters; PMHCA was detected as methyl ketone by GC–MS as described above. Selected samples were additionally analysed with and without β-glucuronidase treatment.

CoA conjugate analysis of liver and testes tissue samples:

CoA conjugates were extracted with a method modified from Mangino et al. (1992) and Kasuga et al. (2004) Tissue samples were pulverized with a mortar and pestle on liquid nitrogen. Aliquots (10 mg for liver and 50 mg for testes) were then homogenized on ice and suspended in 460 µL ammonium acetate buffer (2 M, pH 5.3) and amended with 20 µL of H2O containing 6.25 µM of decanoyl-CoA as internal standard. Additional samples from control animals were prepared, containing as additional internal standard p-tBBA-CoA (0.165–50 µM) to determine extraction efficacy. Samples were extracted with 3 mL of chloroform: methanol (1:2) and centrifuged (15 min, 3000 × g, 4 °C). The supernatant was transferred to a new tube and the pellet resuspended in 400 µL methanol and 100 µL ammonium acetate buffer. Then, 900 µL ammonium acetate buffer and 1 mL chloroform were added. The mixture was further extracted by adding the supernatant from the first extraction step. After centrifugation (15 min, 3000 × g, 4 °C), the upper aqueous layer containing putative CoA conjugates was separated and concentrated under a stream of nitrogen to a final volume of 1.5 mL. The samples were then diluted with 2 mL water containing 0.5 µM heptadecanoyl-CoA and 0.0625 µM benzoyl-CoA as internal standards and loaded onto SPE columns (OASIS HLB µElution plate), washed, eluted and analysed by LC-HRMS as described for the hepatocyte samples. Quantification of the p-iPBA-CoA conjugate was based on the synthetic, structurally very closely related p-tBBA-CoA as reference standard.

Analysis liver, testes, and plasma samples for additional metabolites:

Pooled extracted tissue samples and plasma samples were analysed for additional phase I and phase II metabolites by LC-HRMS.

GLP compliance: no

**Test material**

Chemicals tested in the study were:

-BMHCA, 3-(4-tert-Butylphenyl)-2-methylpropanal, CAS 80-54-6

-m-BMHCA, 3-(3-tert-Butylphenyl)-2-methylpropanal, CAS 62518-65-4

-BMHCA acid, 3-(4-tert-Butylphenyl)-2-methylpropanoic acid, CAS 66735-04-4

-BMHCA alcohol, 3-(4-tert-Butylphenyl)-2-methylpropanol, CAS 56107-04-1

-BHCA, 3-(4-tert-Butylphenyl)-propanal, CAS 18127-01-0

-**PMHCA**, 3-(4-Isopropylphenyl)-2-methylpropanal, CAS 103-95-7

-**PHCA**, 3-(4-Isopropylphenyl)-propanal, CAS 7775-00-0

-m-iP2MHCA, 3-(3-Isopropylphenyl)-3-methylpropanal, CAS 125109-85-5

-4-tert-Butyltoluene, CAS 98-51-1

-p-tBBA, 4-tert-Butylbenzoic acid, CAS 98-73-7

**-p-iPBA, 4-Isopropylbenzoic acid, CAS 536-66-3**

-Floralozone®, 4-Ethyl-alpha,alphadimethyl-benzenepropanal, CAS 67634-15-5

-Tropional®, 3-(Benzo[d][1,3]dioxol-5-yl)-2-methylpropanal, CAS 1205-17-0

-Fennaldehyde, 3-(4-Methoxyphenyl)-2-methyl-propanal, CAS 5462-06-6

-Jasmorange®, 3-(4-Methylphenyl)-2-methylpropanal, CAS 41496-43-9

-Nympheal®, 2-Methyl-4-(2-methylpropyl)-benzenepropanal, CAS 1637294-12-2

-Benzoic acid, CAS 65-85-0

-4-Hydroxy-benzoic acid, CAS 99-96-7

-Ethyl paraben, Ethyl 4-hydroxybenzoate, CAS 120-47-8

Radiolabelling:no

**Test animals**

Species: other: rat, rabbit and human hepatocytes

Strain: other: Sprague–Dawley (rat) and New Zealand (rabbit)

Sex: male/female

**Results and discussion**

Main ADME results

Type: metabolism

Results: p-benzoyl-CoA accumulation was found to be specific to the rat hepatocytes, not occurring in human hepatocytes. There was also very limited accumulation in hepatocytes from rabbits.

Toxicokinetic / pharmacokinetic studies

Details on distribution in tissues:

Tissues of rats treated with 3-(4-isopropylphenyl)-2-methylpropanal (lysmeral) were analysed and p-isopropyl-benzoyl-CoA conjugates were detected in the liver and in the testes in animals at toxic doses indicating that the metabolism observed in vitro is relevant to the in vivo situation and the critical metabolite does also occur in the reproductive tissue.

Metabolite characterisation studies

Metabolites identified: yes

Details on metabolites:

In addition to the study performed in 2017 (Laue et al. 2017), additional chemicals for which no reprotoxic effects were observed in male rats were tested. For these ten benzoic acids and their precursors, a clear lower initial p-benzoyl-CoA conjugate formation was observed compared to BMHCA, and, more importantly, the levels of the CoA conjugates decreased substantially over time. After 22 h incubation, there is a very strong difference in p-benzoyl-CoA conjugate accumulation between the reprotoxic derivatives and the non-reprotoxic derivatives lacking the substituent in para-position for eight of the chemicals. Although there was an accumulation of the corresponding benzoyl-CoA conjugates detected for m-BMHCA and Fennaldehyde, much lower levels (1–24%) compared to BMHCA and the other reprotoxic chemicals were detected and only at the high 50 µM dose. This strong correlative SAR between reprotoxic outcome and p-benzoyl-CoA accumulation is an indication, that the observed metabolic fate in rat hepatocytes may be linked to the observed reproductive toxicity and that a threshold of CoA conjugate formation needs to be exceeded to cause adverse effects in vivo.

While, qualitatively, the p-benzoyl-CoA conjugates were also formed in human and rabbit hepatocytes, in general (especially in human hepatocytes) lower levels were initially formed for BMHCA, and a strong clearance over time was noted for both BMHCA and PMHCA in cells of both rabbit and human origin. Thus

lower levels of the p-benzoyl-CoA conjugates were detected after 22h in both rabbit and human hepatocytes, while sustained accumulation at higher levels was found in rat hepatocytes.

## 2.4 Study 4: 3-*p*-cumenyl-2-methylpropionaldehyde

Basic toxicokinetics in vitro / ex vivo

Reference type: publication

Title: Unnamed

Year: 2021

Publication: Natsch, A., Nordone, A., , Adamson, GM., Laue, H. 2021. A species specific metabolism leading to male rat reprotoxicity of Cyclamen aldehyde: in vivo and in vitro evaluation. Food Chem Toxicol. 2021 Jul;153:112243. doi: 10.1016/j.fct.2021.112243.

Conflict of interest: The authors are all employees of Givaudan SA, a company producing and using Cyclamen aldehyde (3-*p*-cumenyl-2-methylpropionaldehyde).

**Materials and methods**

**Test material**

EC Number: 203-161-7

EC Name: 3-p-cumenyl-2-methylpropionaldehyde

Cas Number: 103-95-7

Molecular formula: C13H18O

Test material form: liquid

Radiolabelling: no

**Test animals**

Species:other: rats, rabbits and human hepatocytes

**Results and discussion**

Main ADME results 1

Type: metabolism

Results: The effect on spermatogenesis appears to be linked to the metabolite p-isopropyl-benzoic acid (p-iPBA).

Main ADME results 2

Type: metabolism

Results: Studies in rat, rabbit and human suspended hepatocytes indicated species differences with p-iPBA detected in rat hepatocytes only.

Main ADME results 3

Type: metabolism

Results: In plated rat hepatocytes, p-iPBA is conjugated to Coenzyme A (CoA) and p-iPBA-CoA accumulates to stable levels over 22 h. In plated rabbit and human hepatocytes p-iPBA-CoA doesn’t accumulate.

Main ADME results 4

Type: metabolism

Results: p-iPBA-CoA is formed in vivo in liver and testes of rats dosed with Cyclamen Aldehyde

Metabolite characterisation studies

Metabolites identified: yes

Details on metabolites: The effect on spermatogenesis associated to Cyclamen Aldehyde (CA) appears to be linked to the metabolite p-isopropyl-benzoic acid (p-iPBA):

-studies in rat, rabbit and human suspended hepatocytes indicated species differences with p-iPBA detected in rat hepatocytes only.

- conjugation of p-iPBA to CoA (p-iPBA-CoA) was investigated in plated rat, human and rabbit hepatocytes by LC-HRMS, using a structurally related CoA conjugate as synthetic reference (ptBBA-CoA) for quantification as described previously (Laue et al., 2020). In rat hepatocytes, p-iPBA-CoA is rapidly formed and remains at constant levels for the entire duration of the experiment (22 h). Dose-dependent formation of p-iPBA-CoA was observed in rat hepatocytes exposed with different concentrations of CA (0–50 µM). Similar concentrations of the CoA conjugate (0.95–1.42 µM) were detected with 5, 10 and 50 µM CA and the levels at 22 h were very similar to the levels observed at 0.5 h.

In rabbit and human cells, an initial formation of p-iPBA-CoA is also detected. However, this CoA conjugate is cleared over time, and only low levels are found at 22 h. This CoA conjugate appears to be cleared by rabbit and human cells, while it is persistent in the rat cells.

These in vitro results correlate with the in vivo findings:

-p-iPBA-CoA is formed in vivo in liver and testes of rats dosed with CA.

## 2.5 Study 5: 3-(*p*-cumenyl)propionaldehyde

Basic toxicokinetics in vitro / ex vivo

Title: Unnamed

Year: 2012

Objective of study: metabolism

Qualifier: no guideline followed

GLP compliance: yes

**Test material**

EC Number: 231-885-3

EC Name: 3-(p-cumenyl)propionaldehyde

Cas Number: 7775-00-0

Molecular formula: C12H16O

Test material form: liquid

Radiolabelling: no

**Test animals**

Source: All cryopreserved hepatocytes were obtained from Celsis In Vitro Technologies.

Strains: Mouse CD-1, Rat Sprague-Dawley, Rabbit New Zealand White, Human N/A.

**Administration / exposure**

Route of administration: in vitro application in liquid medium

Vehicle: ethanol

Details on exposure: For each species, components were mixed together in the following order, to give a total incubation volume of 1.0 mL:

- Foetal calf serum (100 µL)

- Test substance (10 µL of solution in ethanol),

- Supplemented Williams' Medium E

- Hepatocyte suspension (mouse 0.3 × 10^6 viable cells, other species 1 × 10^6 viable cells).

Duration and frequency of treatment / exposure:0, 1 and 4 h

Dose / conc.:1 other: µM

Dose / conc.:10 other: µM

Dose / conc.:100 other: µM

INCUBATION CONDITIONS

The hepatocytes were incubated for 0, 1 and 4 h in polyethylene vials in an orbital shaking water bath (set at 60 rpm and 37°C), under an atmosphere of humidified 95% oxygen : 5% carbon dioxide. Reactions were terminated after the appropriate incubation time by placing the incubation vessels on ice followed immediately by addition of chilled acetonitrile (1 mL) and vortex mixing. Samples were then homogenised by ultrasonic disruption (approximately 20 s) using an ultrasonic probe. The resulting samples were stored briefly at approximately -70°C (to ensure rapid freezing) followed by storage at approximately -20°C pending processing for analysis.

CONTROL INCUBATIONS

On each occasion, control incubations in the absence of hepatocytes were conducted in parallel at each substrate concentration for 4 h only. Additionally, control incubates were conducted with hepatocytes in the absence of test substance for 4 h only. Positive control incubations for metabolic activity were conducted with 7-ethoxy[14C]coumarin (7-EC) as substrate.

NUMBER OF REPLICATES:

Incubations were conducted in triplicate, with the exception of the control hepatocytes in the absence of test substance which were conducted singly.

MEASUREMENT OF LDH LEAKAGE

Incubations with test substance were conducted in parallel to those not containing any test substance, so as to assess whether the inclusion of test substance in the incubation medium had any effect on the leakage of LDH from the hepatocytes. The incubations without test substance comprised of duplicate samples containing 0.3 ×10^6 viable cells/mL (mouse) or 1 x 10^6 viable cells/mL (rat, rabbit, human) in supplemented Williams' Medium E in the presence of 1% (v/v) ethanol. These were directly compared to samples containing the same number of viable hepatocytes, supplemented Williams' Medium E and test substance (at final concentration of 100 µM) and 1% (v/v) ethanol. Aliquots (0.5 mL) of the incubation volume were removed at 0, 1 and 4 h and centrifuged at 3,500 rpm (1,068 x g) for 1 min. The supernatant was carefully separated from the cell pellet. The cell pellets from all samples were lysed in 5 mL final volume of 1% (v/v) Triton X-100 in PBS. All pellets and supernatants were stored at approximately 4°C until assay.

The rate of change of absorbance at 340 nm was determined over 1 min in a Shimadzu UV-1700 series spectrophotometer with temperature controlled cell chamber. The assay was performed in a 1 mL, 1 cm light path cuvette at 37°C and consisted of the following components (all pre-warmed to 37-38 °C prior to use): 100 µL cell medium or cell lysate diluted appropriately (1:4, v/v) with PBS, 800 µL NADH in PBS (200 µg/mL) and 100 µL pyruvate in PBS (150 µg/mL). The activity of LDH was measured in LDH units. The magnitude of enzyme leakage into the supernatant was expressed as a percentage of the total cellular LDH activity.

METABOLITE PROFILING

HPLC system: Waters Acquity UPLC (including binary pump, autosampler and UV detector)

Mass spectrometer: Waters Q-Tof micro

HPLC conditions:

- Column: Phenomenex Luna C8(2), 3 μm, 150 × 4.6 mm

- Guard column: Phenomenex Luna C8, 4 × 2 mm

- Column oven temperature: 40°C

- Mobile phase A: 0.005% (v/v) formic acid in purified water

- Mobile phase B: Acetonitrile

- Flow rate: 0.7 mL/min

- UV detection: 220 nm

- Gradient (time : % eluent B): 0 min: 50%, 10 min: 90%, 12 min: 100%, 12.1 min: 50%

- MS settings: split ration 1:9, ionisation: negative, desolvation gas: nitrogen 450 L/h, cone gas: argon 50 L/h, scan rate: 1/s

**Results and discussion**

Metabolite characterisation studies

Metabolites identified:yes

METABOLITE IDENTIFICATION

A total of eight separate metabolites of the substance were observed and identified, labelled C1 to C8. Interspecies differences in the metabolite profiles were typically small at each incubation time.

There are 8 metabolites detected.

In summary, Cyclemax is mostly conjugated. There are two non-conjugated metabolites. The first is Cyclemax propyl acid, where the aldehyde is oxidised into the acid mainly present at the start of the incubationand present in all species (C8). The second acid is the Cyclemax methyl acid (iso-propyl benzoic acid), present at high dosing >=10 umol in low concentrations in rat, mouse and rabbit but not in humans, potentially formed when the conjugation system becomes saturated.

METABOLITE IDENTIFICATION

A total of eight separate metabolites of the substance were observed and identified, labelled C1 to C8. Interspecies differences in the metabolite profiles were typically small at each incubation time.

A total of eight components (labelled C1 to C8) were detected following hepatocyte incubations with the test substance, with similar results obtained for each species.

- Metabolite C6 (glucuronide conjugate of the alcohol) was the largest metabolite in most of the 1 h and 4 h hepatocyte incubations, although the intermediate (alcohol) was not observed.

- Metabolite C8 (the aldehyde turned into acid) was observed widely and was the second largest component in most mouse, rabbit and human hepatocyte incubations. It was also the only component detected in the 0 h (control) incubations, although levels were low.

- Metabolite C5 (glucuronide conjugate) was also detected in most incubations and tended to be the second largest component in rat hepatocyte incubations.

- Most of the remaining metabolites were present at low levels and/or in a limited number of incubations

- Although metabolite C4 (hydroxylated substance) was detected in most incubations.

- Metabolite C1 eluted with a typical retention time of 3.14 min. It was detected in hepatocyte incubations from the mouse (plus one human profile, 100μM, 4 h incubation) and levels were variable (not detected to <20% of total peak area). The molecular ion [M-H]- was 369 and the molecular weight was 370. The metabolite was identified as the glucuronide conjugate of the hydroxylated alcohol, although the position of the hydroxylation could not be confirmed.

- Metabolite C2 was detected in some profiles from all species, but typically only at low levels. The typical retention time was 3.10 min (similar to C1) and the molecular ion [M-H]- was 207, giving a molecular weight of 208. This component was identified as a hydroxylated acid, although it was not possible to determine the position of the hydroxylation.

- Metabolite C3 eluted with a typical retention time of 3.64 min and was observed at variable levels in some mouse profiles (plus one rabbit profile, 10µM, 1 h incubation). The molecular ion [M+HCl+H]- was 375, which indicated a molecular weight of 340, and C3 was identified as a hexose conjugate of conjugate of the alcohol.

- Metabolite C4 was detected at variable levels in some hepatocyte incubations at 1 and 4 h from all species, typically at higher substance concentrations. The typical retention time was 4.10 min, the molecular ion [M-H]- was 207 and the molecular weight was 208. This component was identified as a hydroxylated acid (similar to C2), although it was not possible to determine the position of the hydroxylation.

- Metabolite C5 eluted with a typical retention time of 4.29 min and was observed in some hepatocyte incubations at 1 and 4 h from all species, generally at higher substance concentrations. Variable levels of metabolite C5 were observed in the profiles. The molecular ion [M-H]- was 351, which indicated a molecular weight of 352, and C5 was identified as the glucuronide conjugate of the substance.

- Metabolite C6 had a typical retention time of 4.76 min and was observed in the majority of 1 h and 4 h hepatocyte incubations from all species. It was the largest metabolite in most 1 h and 4 h incubations. The molecular ion [M-H]- was 353, giving a molecular weight of 354, and the metabolite was identified as the glucuronide conjugate of the alcohol.

- Metabolite C7 eluted with a typical retention time of 5.18 min. It was typically observed as a minor metabolite (<5% of total peak area) in 4 h mouse, rat and rabbit hepatocyte incubations at a substance concentration of 100µM. It was not detected in any human hepatocyte incubations. The molecular ion [M-H]- was 163, which indicated a molecular weight of 164. The identity of C7 was determined as 4-isopropylbenzoic acid, a finding that was supported by analysis of the reference substance.

- Metabolite C8 was detected in several hepatocyte incubations from all species. It was the only component in half of the 0 h incubations and was present at variable levels in a number of 1 h and 4 h incubations. The molecular ion [M-H]- was 191, the molecular weight was 192 and C8 was identified as an acid.

MOUSE HEPATOCYTES

A total of eight metabolites were observed and identified by LC-MS following incubations of the substance with mouse cryopreserved hepatocytes. These were C1 (glucuronide conjugate of the hydroxylated alcohol), C2 and C4 (hydroxylated acid), C3 (hexose conjugate of an alcohol), C5 (glucuronide conjugate), C6 (glucuronide conjugate of the alcohol), C7 (4-isopropylbenzoic acid) and C8 (acid). No metabolites were observed in 0 h (control), 1 h and 4 h hepatocyte incubations of the substance at a concentration of 1µM. Similarly, no metabolites were detected in the control 0 h incubations at 10µM and only C8 was present in the control 100µM incubations.

At a concentration of 10µM, the largest component following the 1 h incubations was metabolite C6 (49.9% of total peak area), followed by C8 (18.0%) and C5 (10.5%). Metabolites C1, C3 and C4 were also observed (each between approximately 6% and 8%). For the 100µM incubations, metabolite C6 (63.6%) was also the largest component, followed by C8 (17.0%), C3 and C5 (each between 6% and 7%). Other metabolites detected were C1, C4 and C7 (each <5%).

The profiles and levels of metabolites (in terms of total peak area) remained similar following the 4 h hepatocyte incubations. For the 10µM concentration, C6 (47.1%) was the largest component, followed by C4 (18.9%), C1 (13.9%), C5 (13.0%) and C8 (7.1%). The largest component at 100µM was also C6 (62.6%), which was followed by C8 (12.1%), C5 (10.7%) and C4 (7.4%), with C1, C2, C3 and C7 also observed (each <5%).

RAT HEPATOCYTES

Six individual metabolites were observed following incubations of the substance with rat cryopreserved hepatocytes; C2 and C4 (hydroxylated acid), C5 (glucuronide conjugate), C6 (glucuronide conjugate of the alcohol), C7 (4-isopropylbenzoic acid) and C8 (acid).

Similar to the mouse, no metabolites were observed in the profiles of the control 0 h rat hepatocyte incubations at substance concentrations of 1 and 10μM, whilst the only component detected at the 100μM concentration was metabolite C8. Metabolite C6 was the only component observed in the profiles following hepatocyte incubations for 1 h at a substance concentration of 1μM. This metabolite was also the largest component (61.6% and 63.4%, respectively) for incubations at substance concentrations of 10 and 100μM. The second largest component was C5 (20.5% and 18.9%,respectively), followed by C8 (16.5% and 16.0%, respectively), with small quantities (<5%) attributed to C4 and C2 (100μM only).

The profiles were also similar for the 4 h hepatocyte incubations at 1, 10 and 100μM. The only component observed in the profiles following hepatocyte incubations for 1 h at a substance concentration of 1μM was metabolite C6. For 10 and 100μM incubations, the largest component was also C6 (60.9% and 66.3%, respectively), whilst the second largest component was C5 (22.0% and 20.6%, respectively). For the 10μM incubations, metabolites C4 and C8 each represented between 8% and 9%, whilst for the 100μM incubations, metabolite C8 accounted for 5.8% with small quantities (<5%) associated with C2, C4 and C7.

RABBIT HEPATOCYTES

The same six individual metabolites were observed following incubations of the substance with rabbit cryopreserved hepatocytes as were observed for rat incubations; C2 and C4 (hydroxylated acid), C5 (glucuronide conjugate), C6 (glucuronide conjugate of the alcohol), C7 (4-isopropylbenzoic acid) and C8 (acid). Only a single metabolite, C8, was observed in the control 0 h rabbit hepatocyte incubations at substance concentrations of 1, 10 and 100μM. For the 1 h hepatocyte incubations at a substance concentration of 1μM, no metabolites were observed. Metabolite C8 (44.3% of the total peak area) was the largest component in the 10μM incubation, followed by C6 (34.1%) and C3 (21.6%). For the 100μM incubation, C8 (63.7%) was also the largest component, followed by C6 (25.6%), with C2, C4 and C5 also detected (each <5%).

Metabolite C6 was the only component detected in the 4 h hepatocyte incubations at a concentration of 1μM and this was the largest component in the incubations at 10 and 100μM concentrations (62.8% and 44.6%, respectively). The second largest component in the 10μM incubations was C8 (17.5%), followed by C2 (10.9%) and C5 (8.8%). For the 100μM incubations, metabolite C8 (35.9%) was the second largest component, followed by C2 (7.3%) and C4 (6.3%), with small quantities (each <5%) associated with C5 and C7.

HUMAN HEPATOCYTES

A total of six metabolites were observed and identified by LC-MS following incubations of the substance with human cryopreserved hepatocytes. These were C1 (glucuronide conjugate of hydroxylated alcohol), C2 and C4 (hydroxylated acid), C5 (glucuronide conjugate), C6 (glucuronide conjugate of the alcohol) and C8 (acid). Metabolites C3 (hexose conjugate of alcohol) and C7 (4-isopropylbenzoic acid) were not detected in human hepatocytes incubations.

Consistent with the mouse and rat incubations, no metabolites were observed in the profiles of the control 0 h human hepatocyte incubations at substance concentrations of 1 and 10μM, whilst only metabolite C8 was detected in the profiles for the 100μM incubations. Similarly, no metabolites were observed in the hepatocyte incubations for 1 h at a substance concentration of 1μM. However, metabolites were observed in 1 h incubations at the higher concentrations. For the 10μM incubation, metabolite C6 (73.5%) was the largest in terms of total peak area, followed by C8 (17.3%), C4 (6.2%) and C5 (3.0%). The profile for the 100μM incubation was similar, with the largest component being C6 (68.5%), followed by C8 (21.2%) and C4 (7.2%).Metabolites C2 and C5 were also detected (each <5%).

Following a 4 h incubation of human hepatocytes at a substance concentration of 1μM, only a single metabolite, C6, was observed. At the 10μM concentration, the largest metabolite was also C6 (80.8%), followed by C8 (8.8%), C4 (6.9%) and C5 (3.6%). Metabolite C6 (64.6%) was again the largest component for the 100μM incubation, followed by C4 and C8 (each approximately 14%), with C1, C2 and C5 also detected (each <5%).

LDH leakage

The substance did not significantly increase the leakage of LDH from the hepatocytes under the conditions of the test.

Metabolite quantification

It was not possible to detect the unchanged test substances by mass spectrometry and it was considered likely that if present some intermediate metabolites would also have been undetected. It was therefore not possible to derive the actual percentage of total material represented by each metabolite observed, although the relative percentage of the total peak area detected within each component was calculated, with the assumption made that each component was detected with equal intensity.

## 2.6 Study 6: *p*-cymene

Basic toxicokinetics in vivo

Title: p-cymene metabolism in rats and guinea-pigs

Author: Walde A., Ve B., Scheline R. R. and Monge P.

Year: 2009

Bibliographic source: Xenobiotica, 13:8, 503-512, DOI: 10.3109/00498258309052290

Objective of study: metabolism

Qualifier: no guideline available

- Principle of test:

The study on metabolic fate of p-cymene using GLC-mass spectrometric techniques employing high-resolution capillary columns for metabolite identification.

The GLC system used for quantitative measurements was that described by Klungseyr and Scheline (1981) except that the OV-1 capillary column used previously was replaced in most cases with one coated with SE-54. A Hewlett Packard model 5992A g.l.c.-mass spectrometry system, as described in the above report but with several instrumental improvements, was used for metabolite detection and identification. Improvements included the fitting of an S.G.E. on-column-injector model OCI-3 (Scientific Glass Engineering, Melbourne, Australia) and the replacement of the standard interface to the mass spectrometer with an open-split type of our own design.

Quantitative measurements

p-Toluic acid and p-methylbenzyl alcohol were used as internal standards for quantification of acidic and neutral metabolites, respectively. An exception to this procedure was made with p-isopropylbenzoylglycine (XVII) for which a correction factor was derived (Klungseyr and Scheline 1981, Sporstel and Scheline 1982) to compensate for differences in extraction characteristics and g.l.c. response between it and the internal standard.

- Parameters analysed / observed: metabolites extracted from urine

Urine Sample preparation:

Urine samples were treated according to methods similar to those described by Klungseyr and Scheline (1981). Following hydrolysis by a glucuronidase + sulphatase preparation and ether extraction, fractionation was carried out to give ‘acidic’ and ‘neutral’ fractions. The former were dissolved in ethyl acetate and converted to their methyl esters with diazomethane. Following evaporation of the ethyl acetate the samples were dissolved in dichloromethane. Neutral components consisting of alcohols and phenols

were converted to their trimethylsilyl (TMS) derivatives and these, following removal of excess reagent, were dissolved in hexane.

GLP compliance:not specified

**Test material**

EC Number: 202-796-7

EC Name: p-cymene

Cas Number: 99-87-6

Molecular formula: C10H14

Radiolabelling: no

**Test animals**

Species: guinea pig

Strain: Dunkin-Hartley

Sex: male

TEST ANIMALS

- Source: Dunkin Hartley strain, Olac 1976 Ltd.

- Weight at study initiation: 300-350 g

- Diet: switched from standard pellet diets to a purified diet two days before dosing (Klungseyr and Scheline 1981).

**Administration / exposure**

Route of administration: oral and inhalation

Vehicle: propylene glycol

Remarks: 1 ml

Exposure: intragastric administration of p-cymene by stomach tube or given by inhalation as described by Walde and Scheline (1983)

Duration and frequency of treatment / exposure: 48 hrs

Dose / conc.:100 mg/kg bw/day

No. of animals per sex per dose / concentration: 3 animals

Control animals: not specified

Details on study design:

- Dose selection rationale: not specified

Details on dosing and sampling:

METABOLITE CHARACTERISATION STUDIES

- Tissues and body fluids sampled: urine

- Time and frequency of sampling: 48 hours

- From how many animals: (samples pooled or not) not speciifed

- Method type(s) for identification – GLC

**Results and discussion**

Metabolite characterisation studies

Metabolites identified: yes

Details on metabolites: The following metabolites have been identified (Table 1)

Table 1. Urinary metabolites of p-cymene in guinea pigs (n=3)

| **Metabolite** | **Intragastric** | **Inhalation** |
|---|---|---|
| 2-p-Tolylpropan-2-ol | 14 (12.7-17. 5) | 3 (2 .4, 3 .5) |
| 2-Hydroxy-4-isopropyl-1-methylbenzene (Carvacrol) | tr | tr |
| 2-p-Tolylpropan-1-ol | 8 (7.7-8.1) | 9 (8.1,9.6) |
| p-lsopropylbenzyl alcohol (Cuminyl alcohol) | 6 (3.7-8.8) | tr |
| 2-p-Tolylpropan-1,2-diol | 7 (5.3-9.0) | 2 (1.2, 2.0) |
| (Hydroxycarvacrol) | 1 (O.6-1.5) | tr |
| 2-p-Tolylpropan-1,3-diol | tr | tr |
| 2-p-(Hydroxymethyl)phenylpropan-2-ol | tr | tr |
| 2-p-(Hydroxymethyl)phenylpropan-1-ol | tr | tr |
| 2-p-Tolylpropionicacid | 4 (4.1-4.8) | 15 (10.6, 18.3) |
| p-Isopropylbenzoic acid (Cumic acid, cuminic acid) | tr | tr |
| p-lsopropenylbenzoic acid | - | - |
| 2-p-Carboxyphenylpropionic acid | tr | tr |
| 2-p-Carboxyphenylpropan-2-ol | tr | tr |
| 2-p (Hydroxymethyl)phenylpropionic acid | tr | tr |
| 2-p-Carboxyphenylpropan-1-ol | tr | tr |
| p- lsopropylbenzoylglycine (p-lsopropylhippuric acid, cuminuric acid) | 31 (2 5.0-40 5) | 31(24.9 -37.8) |
| p-lsopropenylbenzoylglycine | tr | tr |
| Total | 71 | 60 |

Values are given % dose (with range or individual values in parentheses) for a 48-h period.

tr - trace absent.

Conclusions:

The following metabolites have been identified: 2-p-tolylpropan-2-ol (14% and 3% of the dose after oral and inhalation exposure, respectively), 2-p-tolylpropan-1-ol (8% and 9% after oral and inhalation exposure, respectively), cuminyl alcohol (6% after oral exposure, traces - inhalation ), 2-p-tolylpropan-1,2-diol (7% and 2% after oral and inhalation exposure, respectively), Hydroxycarvacrol (1% after oral administration), 2-

p-tolylpropan-1,3-diol (traces after oral and inhalation exposure), 2-p-(hydroxymethyl)phenylpropan-2-ol (traces after oral and inhalation exposure), 2-p(hydroxymethyl)phenylpropan-1-ol (traces after oral and inhalation exposure), 2-p-tolylpropionic acid (4% and 15% after oral and inhalation exposure, respectively), cumic acid (traces after oral and inhalation exposure), p-isopropenylbenzoic acid (not detected), 2-p carboxylphenylpropionic acid (traces after oral and inhalation exposure), 2-p-carboxyphenylpropan-2-ol (traces after oral and inhalation exposure), 2-p(hydroxymethyl)phenylpropionic acid (traces after oral and inhalation exposure), 2-p-carboxyphenylpropan-1-ol (traces after oral and inhalation exposure), p-isopropylbenzoylglycine (31% after oral and inhalation exposure ) and p-isopropenylbenzoylglycine (traces after oral and inhalation exposure)

## 2.7 Study 7: *p*-cymene

Basic toxicokinetics in vivo

Title: p-cymene metabolism in rats and guinea-pigs

Author: Walde A., Ve B., Scheline R. R. and Monge P.

Year: 2009

Bibliographic source: Xenobiotica, 13:8, 503-512, DOI: 10.3109/00498258309052290

Objective of study: metabolism

Qualifier: no guideline available

- Principle of test:

The study on metabolic fate of p-cymene using GLC-mass spectrometric techniques employing high-resolution capillary columns for metabolite identification.

- Short description of test conditions:

Instrumental conditions

The GLC system used for quantitative measurements was that described by Klungseyr and Scheline (1981) except that the OV-1 capillary column used previously was replaced in most cases with one coated with SE-54. A Hewlett Packard model 5992A g.l.c.-mass spectrometry system, as described in the above report but with several instrumental improvements, was used for metabolite detection and identification. Improvements included the fitting of an S.G.E. on-column-injector model OCI-3 (Scientific Glass Engineering, Melbourne, Australia) and the replacement of the standard interface to the mass spectrometer with an open-split type of our own design.

Quantitative measurements
p-Toluic acid and p-methylbenzyl alcohol were used as internal standards for quantification of acidic and neutral metabolites, respectively. An exception to this procedure was made with p-isopropylbenzoylglycine (XVII) for which a correction factor was derived (Klungseyr and Scheline 1981, Sporstel and Scheline 1982) to compensate for differences in extraction characteristics and g.l.c. response between it and the internal standard.
- Parameters analysed / observed: metabolites extracted from urine

Urine Sample preparation:
Urine samples were treated according to methods similar to those described by Klungseyr and Scheline (1981). Following hydrolysis by a glucuronidase + sulphatase preparation and ether extraction, fractionation was carried out to give ‘acidic’ and ‘neutral’ fractions. The former were dissolved in ethyl acetate and converted to their methyl esters with diazomethane. Following evaporation of the ethyl acetate the samples were dissolved in dichloromethane. Neutral components consisting of alcohols and phenols
were converted to their trimethylsilyl (TMS) derivatives and these, following removal of excess reagent, were dissolved in hexane.

GLP compliance: not specified

**Test material**

Radiolabelling: no

**Test animals**

Species: rat

Strain: Wistar

Sex: male

TEST ANIMALS

- Weight at study initiation: 250 ± 20 g

- Diet: switched from standard pellet diets to a purified diet two days before dosing (Klungseyr and Scheline 1981).

**Administration / exposure**

Route of administration: oral and inhalation

Vehicle: propylene glycol

Remarks: 1 ml

Details on exposure:

Exposure: intragastric administration of p-cymene by stomach tube or given by inhalation as described by Walde and Scheline (1983)

Duration and frequency of treatment / exposure: 48 hrs

Dose / conc.: 100 mg/kg bw/day

No. of animals per sex per dose / concentration: 3 animals

Control animals: not specified

Details on study design:

- Dose selection rationale: not specified

Details on dosing and sampling:

METABOLITE CHARACTERISATION STUDIES
- Tissues and body fluids sampled: urine
- Time and frequency of sampling: 48 hours
- From how many animals: (samples pooled or not) not speciifed
- Method type(s) for identification - GLC

**Results and discussion**

Metabolite characterisation studies

Metabolites identified:yes

Details on metabolites: The following metabolites have been identified after intragastric exposure: 2-p-tolylpropan-2-ol (9% of the dose), 2-p-tolylpropan-1-ol (8%), cuminyl alcohol (1%), 2-p-tolylpropan-1,2-diol (tr), 2-p-tolylpropan-1,3-diol (tr), 2-p-(hydroxymethyl)phenylpropan-2-ol (tr), 2-p(hydroxymethyl)phenylpropan-1-ol (tr), 2-p-tolylpropionic acid (1%), cumic acid (19%), p-isopropenylbenzoic acid (tr), 2-p carboxylphenylpropionic acid (16%), 2-p-carboxyphenylpropan-2-ol (9%), 2-p(hydroxymethyl)phenylpropionic acid (4%), 2-p-carboxyphenylpropan-1-ol (11%), p-isopropylbenzoylglycine (2%) and p-isopropenylbenzoylglycine (tr).

Table 1. Urinary metabolites of p-cymene in rats (n=3)

| Metabolite | Intragastric | Inhalation |
|---|---|---|
| 2-p-Tolylpropan-2-ol | 9(6·8-11·6) | 9(8·8,9·2) |
| 2-Hydroxy-4-isopropyl-1-methylbenzene (Carvacrol) | - | - |
| 2-p-Tolylpropan-1-ol | 8(6·0-9·6) | 6(5·9,6·4) |
| p-lsopropylbenzyl alcohol (Cuminyl alcohol) | 1(0·9-1 ·2) | tr |
| 2-p-Tolylpropan-1,2-diol | tr | tr |
| (Hydroxycarvacrol) | - | - |
| 2-p-Tolylpropan-1,3-diol | tr | tr |
| 2-p-(Hydroxymethyl)phenylpropan-2-ol | tr | tr |
| 2-p-(Hydroxymethyl)phenylpropan-1-ol | tr | tr |
| 2-p-Tolylpropionicacid | 1(0·9-1·3) | 3(1·6,3-6) |
| p-Isopropylbenzoic acid (Cumic acid, cuminic acid) | 19(18·8-21·2) | 9(7·8,9·8) |
| p-lsopropenylbenzoic acid | tr | tr |
| 2-p-Carboxyphenylpropionic acid | 16(14-4-18·0) | 15(13·6,16-4) |
| 2-p-Carboxyphenylpropan-2-ol | 9(6·4-10·8) | 9(8·4,10·4) |
| 2-p (Hydroxymethyl)phenylpropionic acid | 4(3·4-4·7) | 7(5·6,7-6) |
| 2-p-Carboxyphenylpropan-1-ol | 11(8·8-12·8) | 9(8·4,10·4) |
| p- lsopropylbenzoylglycine (p-lsopropylhippuric acid, cuminuric acid) | 2(1·3-1·9) | 3(2·3,3-4) |
| p-lsopropenylbenzoylglycine | tr | tr |
| Total | 80 | 70 |

tr - total recovery
Values given are % dose (with range or individual values in parentheses) for a 48 h period.

Conclusions:
The following metabolites have been identified: 2-p-tolylpropan-2-ol (9% of the dose after oral and inhalation exposure, respectively), 2-p-tolylpropan-1-ol (8% and 6% after oral and inhalation exposure, respectively), cuminyl alcohol (1% after oral administration), 2-p-tolylpropan-1,2-diol (traces), 2-p-tolylpropan-1,3-diol (traces), 2-p-(hydroxymethyl)phenylpropan-2-ol (traces), 2-p(hydroxymethyl)phenylpropan-1-ol (traces), 2-p-tolylpropionic acid (1% and 3% of the dose after oral and inhalation exposure, respectively), cumic acid (19% and 9% of the dose after oral and inhalation exposure, respectively), p-isopropenylbenzoic acid (traces), 2-p carboxylphenylpropionic acid (16% and 15% after oral and inhalation exposure, respectively), 2-p-carboxyphenylpropan-2-ol (9% after oral and inhalation

exposure, respectively), 2-p(hydroxymethyl)phenylpropionic acid (4% and 7% after oral and inhalation exposure, respectively), 2-p-carboxyphenylpropan-1-ol (11% and 9% after oral and inhalation exposure, respectively), p-isopropylbenzoylglycine (2% and 3% after oral and inhalation exposure, respectively) and p-isopropenylbenzoylglycine (traces).

# 3 HEALTH HAZARDS

## 3.1 Acute toxicity - oral route

Not evaluated.

## 3.2 Acute toxicity - dermal route

Not evaluated.

## 3.3 Acute toxicity - inhalation route

Not evaluated.

## 3.4 Skin corrosion/irritation

Not evaluated.

## 3.5 Serious eye damage/eye irritation

Not evaluated.

## 3.6 Respiratory sensitisation

Not evaluated.

## 3.7 Skin sensitisation

Not evaluated.

## 3.8 Germ cell mutagenicity

Not evaluated.

## 3.9 Carcinogenicity

Not evaluated.

## 3.10 Reproductive toxicity

### 3.10.1 Animal data

#### 3.10.1.1 Study 1: 3-*p*-cumenyl-2-methylpropionaldehyde

**Reference:** Unnamed 2011a
**Test guideline**: OECD Guideline 415 [One-Generation Reproduction Toxicity Study (before 9 October 2017)]
Study period:19 Aug 2009 - 26 May 2011.
Deviations:no
GLP compliance:yes
Limit test:no
- Basis for dose level selection

The reproductive effects of 3-p-cumenyl-2-methylpropionaldehyde in corn oil were evaluated in TIF00041. In that study, dosages of 0 (Vehicle), 25, 75, and 150 mg/kg bw/day were administered orally (via gavage) to P generation male and female rats at a volume of 4 mL/kg. Treated rats were mated with untreated cohorts of male and female rats. Male rats were given the vehicle or test substance once daily beginning 14 days before cohabitation, through cohabitation, and continuing through the day before sacrifice, while female rats were given the vehicle or test substance once daily beginning 14 days before cohabitation, through cohabitation, and continuing through day 24 of presumed gestation (DG 24) (rats that did not deliver a litter), day 4 postpartum (DL 4) (rats that delivered a litter), or DS 44 (rats with no confirmed date of mating). In treated male rats, reproductive organ weights were reduced in the 75 and 150 mg/kg bw/day dosage group. Adverse effects on sperm analyses and histopathological changes to the epididymides were observed in the 150 mg/kg bw/day dosage group.
In treated female rats, reduced gestational body weights were observed at the 150 mg/kg bw/day dosage. At 75 and 150 mg/kg bw/day, reduced pup body weights were observed. In untreated females mated to the treated males (Cohort 2), a reduced number of implantation sites and a reduced fertility index were observed at 150 mg/kg bw/day. The dosages of 0 (Vehicle), 25, 75, and 150 mg/kg bw/day were selected for this study.
***Test material:***
Reference substance name:3-p-cumenyl-2-methylpropionaldehyde
EC Number:203-161-7
Cas Number:103-95-7
Name : 3-p-cumenyl-2-methylpropionaldehyde
CAS name : Benzenepropanal, α-methyl-4-(1-methylethyl)-
EC name : 3-p-cumenyl-2methylpropionaldehyde
IUPAC name : 2-methyl-3-[4-(propan-2-yl)phenyl]propanal
Batch No. : VE00051122
**Test animals:**
Species:rat
Strain:Sprague-Dawley
The Crl:CD(SD) rat was selected as the Test System because of known response to toxic effects on reproductive capacity and history of use as a rodent species in these evaluations.
Sex:male/female
Twenty-five Crl:CD(SD) strain rats/sex/dose were administered the test material at dosages of 0, 25, 75 or 150 mg/kg bw/day in corn oil. Dose volume was 4 ml/kg which was adjusted daily on the basis of the individual bodyweights.
These treated male and treated female rats were cohabitated (for a maximum of 21 days) with untreated cohort male or female rats. Treated P generation male rats were 38 days old upon arrival and weighed 131-166 grams at study start. The untreated P generation male rats were 122 days old upon arrival and weighed 437-550 grams at study start. Treated P generation female rats were 66 days old upon arrival and weighed 218-285 grams at study start. Untreated P generation female rats were 66 days old upon arrival and weighed 224-311 grams at study start.
Conditions

The study rooms were maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of 10 changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters. Room temperature was maintained at 64 F to 79 F (18 C to 26 C); relative humidity was targeted at 30% to 70 %. P generation rats were housed individually in stainless steel, wire-bottomed cages, except during the cohabitation and postpartum periods. During cohabitation, each pair of male and female rats was housed in the male rat's cage. Beginning no later than DG 20, P generation female rats were individually housed in nesting boxes until they either naturally delivered litters or were sacrificed on DG 25. Each dam and delivered litter were housed in a common nesting box during the postpartum period. After weaning, F1 generation pups selected for continued evaluation were individually housed in stainless steel, wire-bottomed cages. A 12 hour light/dark cycle was maintained. Cage pan liners were changed at least 3 times per week. Cages were changed approximately every other week. Bedding was changed as often as necessary to keep the rats dry and clean. Rats were given ad libitum access to Certified Rodent Diet #5002 meal in individual feeders. Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats ad libitum from an automatic watering access system and/or individual water bottles attached to the cages. Chlorine was added to the **processed** water as a bacteriostat. Bed-o'-cobs bedding was used as the nesting material. Chewable Nylabones were supplied to all rats during the study.

**Administration / exposure:**

Route of administration:oral: gavage

Vehicle:corn oil

Male P generation rats were gavaged once daily beginning 83 days prior to cohabitation, through cohabitation, continuing through the day before sacrifice. Female P generation rats were gavaged once daily beginning 14 days before cohabitation, through cohabitation and DG 25 (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). F1 generation rats were not directly dosed but may have been exposed to the test material in utero during gestation and through maternal milk postpartum.

Treated or untreated female rats wtih spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at DG 0 and assigned to individual housing. Treated female rats not mated within the first 14 days of cohabitation were assigned alternate male rats that had mated (same dosage group) and remained in cohabitation for a maximum of 7 additional days. Treated male rats that did not mate an untreated female rat within the first 14 days of cohabitation were assigned an alternate untreated female rat and remained in cohabitation for a maximum of 7 additional days. Treated or untreated female rats not mated after completing of the 21 day cohabitation period were considered at DG 0 on the last day of cohabitation and assigned to individual housing. Three untreated female rats not mated with a treated male rat within the first 14 days of cohabitation were considered DG 0. Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day of which all pups in a litter were individually weighed. All F1 generation rats were weaned on PPD 22, based on observed growth and viability of the pups. At weaning, 25 male and 25 female pups per group from treated dams (mated with untreated male rats) and 25 male and 25 female pups per group from untreated dams (mated with treated male rats) were selected resulting in a total of 350 F1 generation rats (175 per sex) chosen for continued evaluation.

Analytical verification of doses or concentrations:yes

Details on analytical verification of doses or concentrations:Concentration and homogeneity were assessed for all test article groups while only concentration was verified for the vehicle group (Group I). Quadruplicate samples were taken from the top, middle and bottom of each concentration on the first day of preparation. Two samples from each quadruplicate set were shipped for analysis; the remaining samples were retained at the Testing Facility as backup samples. Quadruplicate samples were taken from each concentration at the mid-point of the study period and on the last day of preparation. Two samples from each quadruplicate set were shipped to Charles River Laboratories Preclinical Services, Ohio for analysis; the remaining samples were retained as backup samples. Backup samples were stored at room temperature and protected from light. A quadruplicate set of samples was collected from the lowest concentration prepared on the first day of preparation for verification of formulation stability. All stability samples were shipped for analysis and storage as backups.

Duration of treatment / exposure:

Frequency of treatment:Once daily.

Dose / conc.:0 mg/kg bw/day, 25 mg/kg bw/day, 75 mg/kg bw/day, 150 mg/kg bw/day

No. of animals per sex per dose:Twenty-five Crl:CD(SD) strain rats/sex/dose were administered the test material at dosages of 0, 25, 75 or 150 mg/kg bw/day in corn oil. Dose volume was 4 ml/kg which was adjusted daily on the basis of the individual bodyweights.
These treated male and treated female rats were cohabitated (for a maximum of 21 days) with untreated cohort male or female rats. Treated P generation male rats were 38 days old upon arrival and weighed 131-166 grams at study start. The untreated P generation male rats were 122 days old upon arrival and weighed 437-550 grams at study start. Treated P generation female rats were 66 days old upon arrival and weighed 218-285 grams at study start. Untreated P generation female rats were 66 days old upon arrival and weighed 224-311 grams at study start.
Control animals:yes, concurrent vehicle
**Observations and examinations:**
The following parameters were evaluated in the P generation rats (treated or untreated): viability, clinical observations, body weights, feed consumption, estrous cycling, mating and fertility, natural delivery and litter observations, sperm assessments (motility and concentration), organ weights, histopathology and/or necropsy observations.
Oestrous cyclicity (parental animals):Estrous cycling was evaluated daily by examination of vaginal cytology beginning 28 days (treated female rats) or 14 days (untreated female rats) before scheduled cohabitation and continuing through cohabitation until mating was confirmed or until the end of the cohabitation period was reached.
Sperm parameters (parental animals):To assess the potential toxicity of the test material in P generation male rats on the male reproductive system, organ weights, sperm evaluation and histopathology were evaluated. Sperm concentration and motility were evaluated.
Litter observations:Treated rats were observed for clinical signs, abortions, premature deliveries and deaths daily before dosage administration, between one and two hours following dosage administration and at the end of the normal working day. Untreated rats were observed for clinical signs, abortions, premature deliveries and deaths weekly during the precohabitation and cohabitation periods, on DG 0, twice weekly during the gestation period and on days 1, 5, 8, 11, 15, 18 and 22 postpartum. In addition, treated and untreated female rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter size (all pups delivered) and pup viability at birth. Treated and untreated dams and their respective litters were observed for maternal behaviour on days 1, 5, 8, 14, 18 and 22 postpartum.
F1 generation pups were individually weighed and litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations. Anogenital distance was measured for all live F1 generation pups on days 1 and 22 postpartum. Nipple eruption was evaluated for all live F1 generation pups once on day 12 postpartum. F1 generation litters were observed at least twice daily for viability. The pups in each litter were counted once daily. Clinical observations were recorded once daily during he preweaning period. Pup body weights were recorded on days 1 (birth), 5, 8, 11, 15, 18 and 22 postpartum, and at least weekly during the postweaning period and on the day of sacrifice. During the postweaning period, rats were observed twice daily for viability and once daily for clinical observations and/or general appearance. Body weights and feed consumption values were recorded weekly during the postweaning period. Female F1 generation rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum and continuing until this developmental parameter is achieved, or until day 40 postpartum.
Postmortem examinations (parental animals):The body weight was recorded for each rat on the day the criterion was achieved/recorded. A terminal body weight was also recorded. Treated P generation male rats that died or were sacrificed before scheduled termination were examined for the cause of death or condition on the day the observation was made. The rats were examined for gross lesions and a complete necropsy was performed. Tissues were retained and/or microscopically evaluated, but not weighed. Surviving treated and untreated P generation male rats were sacrificed by CO2 asphyxiation after completion of the cohabitation period. Treated and untreated female rats that delivered a litter and their respective pups not selected for continued evaluation were sacrificed on day 22 of presumed lactation (DL 22). Female rats that did not deliver a litter were sacrificed on day 25 of gestation (DG 25). Male and female F1 generation rats weaned and selected for continued evaluation were sacrificed by CO2 asphyxiation on day 57, 58 or 60 postpartum. All surviving treated P generation male and female rats were subject to a complete necropsy examination which included:evaluation of the carcass and musculoskeletal system;all external surfaces and

orifices;cranial cavity and external surfaces of the brain and spinal column;the nasal cavity and neck with associated organs and tissues;and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.
To assess the potential toxicity of the test material in P generation male rats on the male reproductive system, organ weights, sperm evaluation and histopathology were evaluated. Sperm concentration and motility were evaluated. Treated dams with no surviving pus were sacrificed after the last pup was found dead or missing and presumed cannibalized. The number and distribution of implantation sites were recorded. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites, and retained in 10% Neutral buffered formalin for microscopic evaluation. Tissues identified for microsocpic evalution were embedded in paraffin, sectioned, mounted on glass slides and stained with hematoxylin and eosin. Histopathological evaluation was performed by a board-certified veterinary pathologist. Untreated P generation male rats were sacrificed via CO2 asphyxiation after completion of the cohabitation period and carcasses were discarded without evaluation. Untreated P generation female rats were sacrificed via CO2 asphyxiation after completion of the 22 day postpartum period. The number and distribution of implantation sites were recorded. Untreated female rats that did not deliver a litter were sacrificed on DG 25 and examined for pregnancy status. Uteri of apparently non-pregnant rats were examined to confirm the absence of implantation sites. Female rats without a confirmed mating date that did not deliver a litter were sacrificed on an estimated DG 25. The carcasses of *the* untreated female rats were discarded without further evaluation.
Postmortem examinations (offspring):Unscheduled deaths of F1 generation pups were evaluated as follows: pups that died before examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sunk were identified as stillborn; pups with lungs that floated were identified as liveborn and to have died shortly after birth. Pups with gross lesions were preserved in Bouin's solution for possible future evlaution. Pups that died or were sacrified before schedule termination were examined for gross lesions and the cuase of death or condition on the day the observation was made. Pups found on PPDs 2 to 5 were preserved in Bouin's solution for possible future evaluation. Pups found on days 6 to 22 postpartum were preserved in neutral buffered 10% formalin for possible future evaluation. Pups that died or were sacrified before scheduled termination from days 5 through 22 postpartum were examined for gross lesions and the cause of death or condition on the day the observation is made. All pups selected for continued evaluation were sacrified by CO2 asphyxiation on PPD 22 and examined for gross lesions; gross lesions were preserved in 10% NBF for possible future histopathological evaluation. Male and female F1 generation rats were sacrificed by CO2 asphyxiation on days 57, 58 and 60 postpartum. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were preserved in 10% NBF. Tissues selected were weighed and preserved in 10% NBF. Statistical analyses were conducted on data.
Statistics:Clinical observations and other proportional data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution.
Continuous data (e.g., body weights, feed consumption values, organ weights, and percent mortality per litter) were analyzed under the Parametric heading of the schematic. Bartlett’s Test of Homogeneity of Variances was used to estimate the probability that the dosage groups have different variances. A non-significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was not inappropriate, and the data were compared using the Analysis of Variance. If that test was significant ($p \leq 0.05$), the groups given the test substance was compared with the control group using Dunnett’s Test. If Bartlett’s Test was significant ($p \leq 0.001$), the Analysis of Variance Test was not appropriate, and the data were analyzed as described under the Nonparametric heading of the schematic. When 75% or fewer of the scores in all the groups were tied, the Kruskal-Wallis Test was used to analyze the data, and in the event of a significant result ($p \leq 0.05$), Dunn’s Test was used to compare the groups given the test substance with the control group. When more than 75% of the scores in any dosage group were tied, Fisher’s Exact Test was used to compare the proportion of ties in the dosage group. Anogenital distances for F1 generation pups (sired by untreated male rats mated with treated female rats) were analyzed as described above and were then subjected to an Analysis of Covariance (ANCOVA) using mean litter weight as the covariate.
Count data were evaluated using the procedures described above for the Kruskal-Wallis Test.

**Results and discussion**

Results: P0 (first parental generation)

Clinical signs:The number of male rats with slight or moderate excess salivation was significantly increased ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group, in comparison to the vehicle control group value. This observation occurred intermittently during the dosage period, and was not considered an adverse effect of 3-p-cumenyl-2-methylpropionaldehyde. All other clinical observations were considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because: 1) the incidences were not dose related; 2) the observations were common for this species and strain of laboratory rat; and/or 3) the number of rats affected was not statistically significant when compared to the vehicle control group.
Mortality:no mortality observed
Description (incidence):There were no treatment-related deaths.
Body weight and weight changes:effects observed, treatment-related
MALES:Reductions in body weight gain occurred in the 150 mg/kg bw/day dosage group intermittently during the dosage period prior to cohabitation, with statistically significant reductions ($p \leq 0.01$) occurring on DSs 50 to 57 and DSs 64 to 71, in comparison to the vehicle control group values. Body weight gains were also reduced in the 150 mg/kg bw/day dosage group on DSs 84 to 92 and at each tabulated interval between DS 99 and DS 134, in comparison to the vehicle control group values. Reflecting lower weight gains that occurred intermittently during the dosage period, the overall body weight gain in the 150 mg/kg bw/day dosage group was significantly reduced ($p \leq 0.05$) on DSs 1 to 134 a nd DS 1 to termination. Body weight gains in the 25, 75 and 150 mg/kg bw/day dosage groups were 98%, 95% and 92% of the vehicle control group value, respectively, on DSs 1 to 84 (the dosage period prior to cohabitation) and 100%, 96% and 90% of the vehicle control group value, respectively, for the cumulative dosage period (DSs 1 to 134).
The average body weight was minimally reduced (5% to 7% less than vehicle controls) in the 150 mg/kg bw/day dosage group beginning on DS 71 and continuing until scheduled euthanasia. These reductions reached statistical significance ($p \leq 0.05$) only on DS 120 when compared to the vehicle control group value. The average body weight on DS 134 % was 100%, 97% and 93% of the vehicle control group value in the three respective 3-p-cumenyl-2-methylpropionaldehyde-treated groups.
FEMALES:
Precohabitation:During the first week of the dosage period (DSs 1 to 8), body weight gains were reduced at 75 mg/kg bw/day (64% of the vehicle control group value) and signifcantly reduced ($p \leq 0.01$) at 150 mg/kg bw/day (44% of the vehicle control group value), as compared to the vehicle controls. These reductions were transient and did not persist during the second week of the dosage period (DSs 8 to 15). Despite the rebound during the second week of the dosage period, body weight gains in the 75 and 150 mg/kg bw/day dosage groups remained reduced (75 mg/kg bw/day) or significantly reduced ($p \leq 0.01$ at 150 mg/kg bw/day) for the entire premating dosage period (DSs 1 to 15), as compared to the vehicle control group value. Body weights and body weight gains during the premating period were unaffected by the 25 mg/kg bw/day dosage of 3-p-cumenyl-2-methylpropionaldehyde. The average body weight on DS 15 was 98%, 98% and 97% of the vehicle control group value in the 25, 75 and 150 mg/kg bw/day dosage groups, respectively.
Gestation:At 150 mg/kg bw/day, body weight gains remained reduced (by 5% to 21%) at each tabulated interval within the gestation dosage period relative to the vehicle control group values. Although the reductions within the gestation period did not reach statistical significance, the cumulative maternal body weight gains (DGs 0 to 21) were significantly reduced ($p \leq 0.01$), as compared to the vehicle control group value. Maternal body weight gains in the 25, 75 and 150 mg/kg bw/day dosage groups were 102%, 95% and 85% of the vehicle control group value, respectively, on DGs 0 to 21. The average maternal body weight on DG 18 and DG 21 was significantly reduced ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group, in comparison to the vehicle control group values. The average body weight on DG 21 was 99%, 97% and 92% of the vehicle control group value in the 25, 75 and 150 mg/kg bw/day dosage groups, respectively. Body weights and body weight gains during the gestation period were unaffected by the 25 and 75 mg/kg bw/day dosages of 3-p-cumenyl-2-methylpropionaldehyde.
Lactation:Body weight losses were observed in the 25, 75 and 150 mg/kg bw/day dosage groups at the beginning of the lactation period (DLs 1 to 5), compared to gains in the vehicle control group during this same period. The losses in body weight were more pronounced at 150 mg/kg bw/day, and also reached statistical significance ($p \leq 0.01$ on DLs 1 to 5) when compared to the vehicle control group value. Body weight gains rebounded in each 3-p-cumenyl-2-methylpropionaldehyde-treated during the next tabulated interval (DLs 5 to 8), but the weight gains at 150 mg/kg bw/day remained significantly reduced ($p \leq 0.05$) in comparison to the vehicle control group value. Thereafter, body weight gains were comparable to or significantly increased ($p \leq 0.05$ or $p \leq 0.01$) when compared to the vehicle control group values.

The average maternal body weight was significantly reduced ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group on DLs 5 through 11, in comparison to the vehicle control group values. Maternal body weight gains in the 25, 75 and 150 mg/kg bw/day dosage groups were 108%, 144% and 124% of the vehicle control group value, respectively, on DLs 1 to 22. The average body weight on DL 22 was 100%, 103% and 101% of the vehicle control group value in the 25, 75 and 150 mg/kg bw/day dosage groups, respectively. The statistically significant reduction ($p \leq 0.05$) in the average maternal body weight that occurred in the 75 mg/kg bw/day dosage group on DL 8 was considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because the reduction was transient and did not persist. In addition, the statistically significant increase ($p \leq 0.01$) in body weight gains that occurred at 75 mg/kg bw/day on DLs 8 to 11 and DLs 1 to 22 were considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because the increases were not dose related.

MALES:Absolute and relative feed consumption values were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 150 mg/kg bw/day. Absolute feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 100% of the vehicle control group value on DSs 1 to 84 (the dosage period prior to cohabitation) and 102%, 102% and 104% of the vehicle control group value, respectively, on DSs 106 to 134. Relative feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 101%, 102% and 103% of the vehicle control group value, respectively, on DSs 1 to 84, and 102%, 104% and 111% of the vehicle control group value, respectively, on DSs 106 to 134.

Relative to body weight, male rats in the 150 mg/kg bw/day dosage group consumed significantly more ($p \leq 0.05$ or $p \leq 0.01$) feed between DS 50 and DS 84 and between DS 106 and DS 134, in comparison to the vehicle control group values. In addition,relative feed consumption values were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 75 mg/kg bw/day dosage group on DSs 78 to 84 and DSs 127 to 134, in comparison to the vehicle control group value. These increases reflected the reductions in body weight and body weight gain that occurred in the 150 mg/kg bw/day dosage group during these same periods.

FEMALES:

Precohabitation: Absolute and relative feed consumption values in the 150 mg/kg bw/day dosage group were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) during the first week of the premating dosage period (DSs 1 to 8), in comparison to the vehicle control group values (8% and 6% less than the vehicle controls, respectively). These reductions correlated with the statistically significant reductions ($p \leq 0.01$) in body weight gain that occurred at 150 mg/kg bw/day during the same period.

Absolute and relative feed consumption values during the premating period were unaffected by the 25 and 75 mg/kg bw/day dosages of 3-p-cumenyl-2-methylpropionaldehyde. Absolute feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 98%, 96% and 95% of the vehicle control group value, respectively, for the premating dosage period (DSs 1 to 15). Relative feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 99%, 98% and 97% of the vehicle control group value, respectively, during the same period.

Gestation: Absolute and relative feed consumption values during the gestation period were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 150 mg/kg bw/day. All values were comparable among the four dosage groups and did not significantly differ. Absolute feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 101%, 99% and 97% of the vehicle control group value, respectively, for the entire gestation period (DGs 0 to 21). Relative feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 102%, 99% and 103% of the vehicle control group value, respectively, during the same period.

Lactation:Absolute and relative feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 75 and 150 mg/kg bw/day dosage groups during the lactation period (DSs 1 to 15), in comparison to the vehicle control group values. Within the lactation period, absolute and relative feed consumption values were reduced (often statistically significant at $p \leq 0.01$) in the 75 and 150 mg/kg bw/day dosage groups at each tabulated interval within the lactation period. Absolute feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 95%, 89% and 80% of the vehicle control group value, respectively, on DLs 1 to 15. Relative feed consumption values in the 25, 75 and 150 mg/kg bw/day dosage groups were 96%, 91% and 84% of the vehicle control group value, respectively,during the same period.

Absolute and relative feed consumption values during the lactation period were unaffected by the 25 mg/kg bw/day dosage of 3-p-cumenyl-2-methylpropionaldehyde.

3-p-cumenyl-2-methylpropionaldehyde increased the absolute and relative weights of the epididymides (left, right and cauda) at 150 mg/kg bw/day. The increased epididymal weights generally reflected the presence of

masses on the cauda epididymis, as previously described, and microscopic observations of moderate to marked sperm granulomas. Adrenal cortical atrophy, affecting the zona fasciculate and zona reticularis.
There were microscopic test substance-related pathology findings observed in the male rats evaluated. In male rats given 150 mg/kg bw/day of 3-p-cumenyl-2-methylpropionaldehyde the gross epididymal masses correlated microscopically with moderate to marked sperm granulomas, associated with mild to moderate epithelial degeneration. The testes were not affected. In addition, these male rats had decreased adrenal weights that correlated microscopically with minimal adrenal cortical atrophy, affecting the zona fasciculate and zona reticularis. These adrenal and epididymal gross or microscopic changes were not seen in the male rats in the lower dosage groups (25 and 75 mg/kg bw/day). Additional changes seen in these rats were considered to be incidental or spontaneous changes commonly observed in control Crl:CD(SD) rats.
*Reproductive function / performance (P0)*

Males:

Pregnancy occurred in 23, 24, 23 and 1 of the 25 to 28 untreated female rats that were assigned to mate with treated male rats in the 0, 25, 75 and 150 mg/kg bw/day dose groups, respectively. All pregnant dams in the 0, 25 and 75 mg/kg bw/day dosage groups delivered litters. The pregnant dam in the 150 mg/kg bw/day paternal dosage group did not deliver a litter. Natural delivery and litter observations were unaffected by doses up to 75 mg/kg bw/day.

Based on the individual data, motility of the sperm from the vas deferens could not be observed in 13 of 25 rats in the 75 mg/kg bw/day dosage group and any of the rats in the 150 mg/kg bw/day dosage group. The motility values in each of the samples generally reflected the presence of drifting debris, headless sperm, detached heads and/or less than the required number of sperm for evaluation. The effects observed at 150 mg/kg bw/day correlated with the infertility that was observed in the treated male rats that were mated with the untreated cohort female rats. Only 12 rats in the 75 mg/kg bw/day dosage group had enough viable sperm (at least 200 sperm in 20 fields) available for analysis. The sperm motility values from the 12 treated male rats in the 75 mg/kg bw/day were comparable to the vehicle control group values and were also within the ranges observed historically at the Testing Facility.
The 75 mg/kg bw/day dosage of 3-p-cumenyl-2-methylpropionaldehyde significantly reduced ($p \leq 0.05$) the sperm count and density from the cauda epididymis, as compared to the vehicle treated group values. Each of these average values were within the within the ranges observed historically at the Testing Facility.
All sperm parameters evaluated were unaffected by the 25 mg/kg bw/day dosage of 3-p-cumenyl-2-methylpropionaldehyde.
FEMALES:
The number of estrous stages per 14 days was comparable among the four dosage groups before the start of administration and during the precohabitation period.
All mating and fertility parameters [numbers of days in cohabitation, rats that mated, the fertility index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first or second week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 150 mg/kg bw/day. All values were comparable among the four dosage groups and did not significantly differ.
Pregnancy occurred in 25 (100.0%), 24 (96.0%), 24 (96.0%) and 24 (96.0%) of the 25 mated female rats in the 0 (Vehicle), 25, 75 and 150 mg/kg bw/day dosage groups, respectively. All pregnant dams delivered litters. At 150 mg/kg bw/day, the average number of implantation sites per delivered litter was significantly reduced ($p \leq 0.01$), in comparison to the vehicle control group value (13.8 implantation sites vs. 15.5 implantation sites in vehicle controls). However, the average value was within the range observed historically at the Testing Facility .
The number of dams with all pups dying between days 1 and 5 postpartum was significantly increased ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group, in comparison to the vehicle control group. Reflecting the reduction in implantation sites, the average number of pups delivered per litter as well as the average number of liveborn pups per litter was significant reduced ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group, in comparison to the vehicle control group values. In addition, there was a significant increase ($p \leq 0.01$) in the number of stillborn pups that were delivered in the 150 mg/kg bw/day dosage group (8 stillborn pups vs. 3 stillborn pups in the vehicle controls). However, the average values for the total number of delivered pups, the number of liveborn pups and the number of stillborn pups were within the range observed historically at

the Testing Facility. Pup mortality (i.e., found dead, presumed cannibalized or unscheduled sacrifice) was significantly increased ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group on days 1 to 5 postpartum, in comparison to the vehicle control group values. As a result of the increase in pup mortality, the overall viability index (i.e., number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) was significantly reduced ($p \leq 0.01$) in the 150 mg/kg bw/day dosage group, in comparison to the vehicle control group value (75.7% vs. 96.3% in the vehicle controls). At 150 mg/kg bw/day, the averages for the number of surviving pups per litter and the live litter size at weighing was significantly reduced ($p \leq 0.01$) on days 1 through 22 postpartum, in comparison to the vehicle control group values. In addition, the average pup body weight per litter was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 75 and 150 mg/kg bw/day dosage groups at each tabulated interval between days 1 and 22 postpartum, as compared to the vehicle control group values.

No other natural delivery and litter observations were affected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 150 mg/kg bw/day. Values for the numbers of dams delivering litters, the duration of gestation, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, lactation index, and percent male pups per number of pups sexed per litter were comparable among the four dosage groups.

The statistically significant increase ($p \leq 0.01$) in pup mortality observed on days 12 through 15 postpartum and the corresponding reduction (significant at p 0.01) in the lactation index that occurred at 25 mg/kg bw/day was considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because the changes were not dose related.

Ovarian Follicle Counts

Although the typical effect of a test substance upon ovarian follicle production and growth is depletive, the noted effect of an increase in the number of follicles may also be relevant. Significant decreases in individual (left and right) ovarian weights were observed at 150 mg/kg bw/day, as previously described. The increased number of primordial follicles may inversely correlate to these decreased organ weights in Group IV (150 mg/kg bw/day) rats because the smaller ovaries may cause a more concentrated dispersion of follicles than in the larger ovaries of the Control (Group I) rats. However, since no microscopic correlates were reported for the histopathology of these rat ovaries, biological relevance of the increased number of follicles could not be completely defined. Corpora lutea were present in all rats evaluated.

In treated male rats, reproductive organ weights were reduced at both 75 and 150mg/kg bw/day. Adverse effects on sperm analyses and histopathological changes to the epididymides were observed at 150mg/kg bw/day dose level. In treated female rats, reduced gestational body weights were observed at 150mg/kg bw/day dose level. Reduced pup body weights were also observed at both 150mg/kg bw/day dose levels. In untreated females mated to the treated males, a reduced number of implantation sites and a reduced fertility index were observed at th 150mg/kg bw/day dose level.

**Results: F1 generation**

F1 Generation Pups of Treated Male Rats Mated with Untreated Female Rats:

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day. None of the clinical signs that occurred in the F1 generation pups were attributed to treatment of P generation male rats with 3-p-cumenyl-2-methylpropionaldehyde at dosages as high as 75 mg/kg bw/day. According to study authors, all transient and persistent clinical observations were considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because: 1) the incidences were not dosage-dependent; and/or 2) the number of litters affected did not differ significantly from the vehicle control group values.

F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats:

At 150 mg/kg bw/day, 20 of 24 litters (significant at $p \leq 0.01$) had one or more pups with a lenticular opacity in one or both eyes. This observation only occurred in one pup from one litter in the vehicle control group on days 19 through 22 postpartum. In the 150 mg/kg bw/day dosage group, lenticular opacities were first observed on day 16 and generally persisted until day 22 postpartum. In addition, this clinical sign was confirmed during scheduled necropsy examination.

There were no other treatment-related clinical signs observed in the F1 generation pups following treatment of P generation female rats with 3-p-cumenyl-2-methylpropionaldehyde at dosages as high as 150 mg/kg bw/day. According to study authors, All transient and persistent clinical observations were considered unrelated to 3-p-cumenyl-2-methylpropionaldehyde because: 1) the incidences were not dosage-dependent; and/or 2) the number of litters affected did not differ significantly from the vehicle control group values.

Postweaning:
- F1 Generation Rats of Treated Male Rats Mated with Untreated Female Rats:
As previously described, there were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day. None of the clinical signs that occurred in the F1 generation male or female rats were attributed to treatment of P generation male rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested. Clinical signs that were observed in the F1 generation rats included mild dehydration (based on skin turgor), chromorhinorrhea, scabbing (neck, back, right forelimb or tail), an ulceration on the right axilla or back, chromodacryorrhea,urine-stained abdominal fur, slight excess salivation, bent tail, constrictions on the tail, and discoloration (purple) and an abrasion on the tail.
The significant increase ($p \leq 0.01$) in the number of F1 generation female rats in the 25 mg/kg bw/day paternal dosage group with a scab was considered unrelated to paternal treatment with 3-p-cumenyl-2-methylpropionaldehyde because the increase was not dose related.
- F1 Generation Rats of Treated Female Rats Mated with Untreated Male Rats:
At 150 mg/kg bw/day, the number of F1 generation male and female rats with a lenticular opacity in one or both eyes was significantly increased ($p \leq 0.01$), in comparison to the vehicle control group value. This observation was first observed in this dosage group during the preweaning period, as previously described. In addition, this observation was more prevalent in F1 generation male rats than in the female rats (18 male rats vs. 6 female rats).
Mortality / viability: no mortality observed
Body weight and weight changes:
F1 Generation Rats of Treated Male Rats Mated with Untreated Female Rats:
There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day. Body weight gains in the F1 generation male and female rats were unaffected by paternal treatment with 3-p-cumenyl-2-methylpropionaldehyde at 25 and 75 mg/kg bw/day. Body weight gains in the F1 generation male rats were 105% and 104% of the vehicle control group value at 25 and 75 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, body weight gains were 101% and 103% of the vehicle control group value at 25 and 75 mg/kg bw/day, respectively, during the same period.
The average body weight in the F1 generation male rats on day 57 postpartum was 105% and 104% in the 25 and 75 mg/kg bw/day paternal dosage group, respectively. In the F1 generation female rats, the average body weight on day 57 postpartum was 102% in the 25 and 75 mg/kg bw/day paternal dosage groups.
F1 Generation Rats of Treated Female Rats Mated with Untreated Male Rats:
In F1 generation male rats, body weight gains were significantly reduced ($p \leq 0.01$) in the 150 mg/kg bw/day maternal dosage group on days 23 to 30 postpartum and days 30 to 37 postpartum, in comparison to the vehicle control group values. Thereafter, body weight gains were comparable to the vehicle control group values during the remainder of the postweaning period. Reflecting the initial reductions in weight gain, body weight gains in the F1 generation male rats in the 150 mg/kg bw/day maternal dosage group were 94% (significant at $p \leq 0.05$) of the vehicle control group value for the entire postweaning period (days 23 to 57 postpartum). In F1 generation female rats, body weight gains were significantly reduced ($p \leq 0.05$) in the 150 mg/kg bw/day maternal dosage group on days 23 to 30 postpartum, in comparison to the vehicle control group value. However, this reduction was transient, and body weight gains were comparable to the vehicle control group values during the remainder of the postweaning period. Body weight gains in the 25, 75 and 150 mg/kg bw/day maternal dosage group were 101%, 98% and 100% of the vehicle control group value, respectively, on days 23 to 57 postpartum. Reflecting significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in the average pup body weight per litter that occurred prior to weaning, the average body weight were also significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the F1 generation male and female rats on days 23, 30, 37, 44, 51 and/or 57 postpartum in the 75 and 150 mg/kg bw/day dosage groups. All other statistically significant reductions were considered unrelated to maternal treatment with 3-p-cumenyl-2-methylpropionaldehyde because the reductions were transient and had no overall effect on the cumulative body weight gain.
- F1 Generation Rats of Treated Male Rats Mated with Untreated Female Rats:
There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day.Absolute and relative feed consumption values in the F1 generation male and female rats were unaffected by paternal treatment with 3-p-cumenyl-2-methylpropionaldehyde at 25 and 75 mg/kg bw/day. Absolute feed

consumption values in the F1 generation male rats were 107% and 103% of the vehicle control group value at 25 and 75 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, absolute feed consumption values were 101% of the vehicle control group value at 25 and 75 mg/kg bw/day during the same period.

- F1 Generation Rats of Treated Female Rats Mated with Untreated Male Rats:

Corresponding to significant reductions in body weight gains, absolute feed consumption values in the F1 generation male rats were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 150 mg/kg bw/day maternal dosage group on days 23 to 30 postpartum and days 30 to 37postpartum, in comparison to the vehicle control group values. Relative to body weight, F1 generation male rats consumed significantly more ($p \leq 0.05$ or $p \leq 0.01$) feed on days 30 to 37 and 37 to 44 postpartum at 75 and 150 mg/kg bw/day and days 44 to 51, 51 to 57 and 23 to 57 postpartum at 150 mg/kg bw/day.

Similar observations occurred in the F1 generation female rats, in that, absolute feed consumption values were significantly reduced ($p \leq 0.01$) in the 150 mg/kg bw/day maternal dosage group on days 23 to 30 postpartum, in comparison to the vehicle control group value. Relative to body weight, F1 generation female rats consumed significantly more ($p \leq 0.05$ or $p \leq 0.01$) feed on days 37 to 44 and 23 to 57 postpartum at 75 and 150 mg/kg bw/day and days 44 to 51 and 51 to 57 postpartum at 150 mg/kg bw/day.

Absolute feed consumption values in the F1 generation male rats were 98%, 97% and 93% of the vehicle control group value at 25, 75 and 150 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, absolute feed consumption values were 98%, 99% and 96% of the vehicle control group value at 25, 75 and 150 mg/kg bw/day, respectively, during the same period.

Sexual maturation: no effects observed

- F1 Generation Rats of Treated Male Rats Mated with Untreated Female Rats:

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day. There were no effects on sexual maturation (preputial separation or vaginal opening) at any paternal dosage level tested. The average day on which sexual maturation was achieved and the average body weight on the day criterion was met was comparable among the dosage group and within the ranges observed historically at the Testing Facility.

- F1 Generation Rats of Treated Female Rats Mated with Untreated Male Rats:

There were no effects on sexual maturation (preputial separation or vaginal opening) at any maternal dosage level tested. The average day on which sexual maturation was achieved was comparable among the dosage group and within the ranges observed historically at the Testing Facilitya. The average body weight of male rats on the day preputial separation occurred was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 75 and 150 mg/kg bw/day dosage groups, in comparison to the vehicle control group value. These reductions in body weight reflect significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in the average pup body weight per litter that occurred prior to weaning. Each of the average values for body weight on the day of sexual maturation was within the ranges observed historically at the Testing Facility. Therefore, this finding was not considered an adverse effect of 3-p-cumenyl-2-methylpropionaldehyde.

Anogenital distance (AGD):

- F1 Generation Pups of Treated Male Rats Mated with Untreated Female Rats:

Anogenital distance on days 1 or 22 postpartum in F1 male and female pups was not affected by treatment of P generation male rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested.

Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum. There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day.

- F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats:

In male pups, anogenital distance on day 1 postpartum was not affected by treatment of P generation female rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested. On day 22 postpartum, there was a significant reduction ($p \leq 0.05$) in the anogenital distance of male pups in the 75 and 150 mg/kg bw/day dosage groups, in comparison to the vehicle control group value. When covaried with fetal body weights per litter, the statistically significant reduction ($p \leq 0.05$) in anogenital distance was not apparent in the 75 and 150 mg/kg bw/day dosage groups, in comparison to the vehicle control group value. This developmental delay correlated with an overall reduction in pup body weights on day 22 postpartum, as previously described. In female pups, anogenital distance on day 1 postpartum was not initially affected by treatment of P generation female rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested. However,

when covaried with fetal body weights per litter, there was a statistically significant increase ($p \leq 0.01$) in anogenital distance at 150 mg/kg bw/day, in comparison to the vehicle control group value. This increase in anogenital distance was no longer apparent by day 22 postpartum. Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

Nipple retention in male pups: no effects observed

F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats

In male pups, anogenital distance on day 1 postpartum was not affected by treatment of P generation female rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested. On day 22 postpartum, there was a significant reduction ($p \leq 0.05$) in the anogenital distance of male pups in the 75 and 150 mg/kg bw/day dosage groups, in comparison to the vehicle control group value. When covaried with fetal body weights per litter, the statistically significant reduction ($p \leq 0.05$) in anogenital distance was not apparent in the 75 and 150 mg/kg bw/day dosage groups, in comparison to the vehicle control group value. This developmental delay correlated with an overall reduction in pup body weights on day 22 postpartum, as previously described.

In female pups, anogenital distance on day 1 postpartum was not initially affected by treatment of P generation female rats with 3-p-cumenyl-2-methylpropionaldehyde at any dosage level tested. However, when covaried with fetal body weights per litter, there was a statistically significant increase ($p \leq 0.01$) in anogenital distance at 150 mg/kg bw/day, in comparison to the vehicle control group value. This increase in anogenital distance was no longer apparent by day 22 postpartum.

Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

- F1 Generation Rats of Treated Male Rats Mated with Untreated Female Rats:

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day.

Terminal body weights in the F1 generation male and female rats were comparable among the three remaining dosage groups and did not significantly differ. According to study authors, there were no test substance-related changes in the absolute or relative (% terminal body weight) weight of the reproductive organs or the pituitary, brain or adrenal glands of the F1 generation male or female rats at any dosage level tested. In the full study report additional information is provided: The relative brain weight was reduced at 25 and 75 mg/kg bw/day in male pups (-6% compared to controls).

- F1 Generation Rats of Treated Female Rats Mated with Untreated Male Rats:

According to study authors, there were no test substance-related changes in the absolute or relative (% terminal body weight) weight of the reproductive organs or the pituitary, brain or adrenal glands of the F1 generation male or female rats at any dosage level tested. In the full study report additional information is provided: The absolute weight of (left) epididymis was statically significantly reduced (-10%) in male pups at 150 mg/kg bw/day, as were absolute weights of the pituitary (-17%), brain (-4%) and adrenals (-15% at 75 and -13% at 150 mg/kg bw/day). Relative weights of testis were statistically significantly increased at 75 and 150 mg/kg bw/day. In female offspring, absolute brain weights were significantly reduced at 75 (-3%) and 150 mg/kg bw/day (-6%), as were absolute ovary weights at 150 mg/kg bw/day (-17%).

Gross pathological findings: no effects observed

F1 Generation Pups of Treated Male Rats Mated with Untreated Female Rats:

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, no data is available at 150 mg/kg bw/day.

There were no gross lesions observed in the F1 generation pups that were stillborn or found dead or in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum.

F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats:

The only gross lesion observed in the F1 generation pups was a tan area on the left kidney of one male pup (19694-3) in the 150 mg/kg bw/day. There were no other gross lesions observed in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum. This gross lesion was not attributed to 3-p-cumenyl-2-methylpropionaldehyde because it was limited to a single pup in the high dosage group. In the pups that were stillborn, found dead or humanely euthanized, no milk was present in the stomach of 1, 5, 5

and 14 F1 generation pups in the 0 (Vehicle), 25, 75 and 150 mg/kg bw/day dosage groups, respectively. The remaining pups that were stillborn, found dead or humanely euthanized appeared normal.
Developmental neurotoxicity (F1)Behaviour (functional findings): not examined
Developmental immunotoxicity (F1): not examined

#### 3.10.1.2 Study 2: 3-*p*-cumenyl-2-methylpropionaldehyde

Reference: Unnamed 2021
Study guideline:OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Study period:2020-2021
GLP compliance:yes
Remarks:Exceptions to GLPs: characterization of the test substance performed by the Sponsor according to established SOPs, controls, and approved test methodologies to ensure integrity and validity of the results generated
Limit test:no

**Test material**

Reference substance name:3-p-cumenyl-2-methylpropionaldehyde
EC Number:203-161-7
EC Name:3-p-cumenyl-2-methylpropionaldehyde
Cas Number:103-95-7

**Test animals**

Species:rat
Strain:Wistar Han
Test animals: time-mated female Crl:WI(Han) rats were received from the lab on Gestation Day 1, 2, 3, or 4. The animals were approximately 12–13 weeks old and weighed between 186 and 240 g at the initiation of dosing on Gestation Day 6.
Housing: single/individual housing in solid-bottom cages containing appropriate bedding material. Cages were arranged on the racks in group order. Where possible, control group animals were housed on a separate rack from the test substance-treated animals.
Food:
Type: Meal
Frequency: Ad libitum.
Analysis: Results of analysis for nutritional components and environmental contaminants were provided by the supplier and are on file at the Testing Facility. It was considered that there were no known contaminants in the feed that would interfere with the objectives of the study.
Water:
Type: Municipal tap water, treated by reverse osmosis and ultraviolet irradiation.
Frequency: Ad libitum, via an automatic watering system. Water bottles were provided, if required.
Analysis: Periodic analysis of the water was performed, and results of these analyses are on file at the Testing Facility. It was considered that there were no known contaminants in the water that could interfere with the outcome of the study.
Acclimation period: After receipt at the Testing Facility, the Crl:WI(Han) rats were acclimated prior to the initiation of dosing.
Environmental conditions:
Temperature: 68°F to 77°F (20°C to 25°C)
Humidity: 30% to 70%
Light Cycle: 12 hours light and 12 hours dark.

**Administration / exposure**

Route of administration:oral: gavage
Vehicle:corn oil
Details on exposure:Dose formulations were divided into aliquots where required to allow them to be dispensed on each dosing occasion.

Vehicle was used as received and test substance was prepared daily. For both, the storage conditions were set to maintain 18°C to 24°C, protected from light, purged with nitrogen.
Dosing formulations were prepared at appropriate concentrations to meet dose level requirements. The prepared formulations were not adjusted for purity.
Analytical verification of doses or concentrations:yes
Details on analytical verification of doses or concentrations:Dose formulation samples were collected for analysis.
Concentration analyses:
Concentrations: all groups
Stratum: middle
Number of samples per concentration: 4 collected samples, 2 analyzed samples, 2 backup samples
Intervals: first and last preparation
Storage conditions: temperature set to maintain 18°C to 24°C.
Acceptance criteria: mean sample concentration within 100% ± 10% of theoretical concentration. Individual sample concentration of ± 15%.
Analyses were performed by a high performance liquid chromatography method with ultraviolet absorbance detection using a validated analytical procedure.
Details on mating procedure:Time-mated rats were received by the lab on Gestation Day 1, 2, 3 and 4. The day on which confirmation of mating is made will be designated Gestation Day 0.
Duration of treatment / exposure:Gestation Days 6–20
Frequency of treatment:Once daily
Duration of test:Gestation Days 0-21
Doses / concentrations: 25 mg/kg bw/day, 75 mg/kg bw/day, 150 mg/kg bw/day
No. of animals per sex per dose:22 females/dose group
Control animals:yes, concurrent vehicle
The oral route of exposure was selected because this a possible route of human exposure. The dose levels were selected based on information provided by the Sponsor. In a previous 1 generation reproduction toxicity study, gestating rats given 150 mg/kg bw/day of 3-p-cumenyl-2-methylpropionaldehyde were observed with reduced mean body weight and body weight gains compared to the control group generally throughout gestation. Based on these results, 150 mg/kg bw/day was selected as the high dose for the current study. It was expected that this high dose would induce maternal toxicity while not resulting in death or severe suffering. Lower doses were selected to assess dose response.

**Examinations**
Maternal examinations:
Mortality: checked at least twice daily (morning and afternoon), beginning upon arrival through termination. Animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Clinical observations: checked once daily, beginning with the day of animal arrival and continuing through (and including) the day of euthanasia. Animals were removed from the cage. Mortality and all signs of overt toxicity were recorded on the day observed. The observations included, but were not limited to, evaluations for changes in appearance of skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous systems, somatomotor activity, and behavior. On days of scheduled dosing, the observations were collected prior to dosing.
Postdose observations (with animal handling): checked approximately 1 hour postdose. Animals were removed from the cage. Animals were observed for findings that were potentially related to treatment or that might change before the next scheduled observation.
Individual Body Weights: checked at Gestation Days 0 (by supplier) and 5–21 (daily). Gestation Day 0 bodyweight collected under Non-GLP conditions. Not collected from animals found dead.
Food Consumption: checked at Gestation Days 5–21 (daily). Quantitatively measured. Reported as g/animal/day for each corresponding body weight interval during gestation.
Examinations after necropsy: liver and thyroid gland (organ weights, histology, histopathology)
Ovaries and uterine content:The uterus of each dam was excised, and its adnexa trimmed. Corpora lutea were also counted and recorded. Gravid uterine weights were obtained and recorded. The uterus of each dam was opened, and the number of viable and nonviable fetuses, early and late resorptions, and total number of

implantation sites were recorded, and the placentae were examined. The individual uterine distribution was documented using the following procedure: all implantation sites, including early and late resorptions, were numbered in consecutive fashion beginning with the left distal uterine horn, noting the position of the cervix and continuing from the proximal to the distal right uterine horn. Uteri which appear nongravid by macroscopic examination were opened and placed in a 10% ammonium sulfide solution for detection of early implantation loss. Representative sections of corresponding organs from a sufficient number of controls were retained for comparison, if possible.
Blood sampling:
Thyroid Hormone Analyses:
Time: Gestation Day 21.
Special requirements: Collected prior to noon on each day of collection, around the same time each day, and within a 2-hour window on each collection day, to reduce variability due to normal diurnal variations in physiological levels of thyroid hormones. Blood collections were performed in an animal ante room, the necropsy laboratory, or as far away from live animals as possible to minimize stress-induced hormone fluctuations.
Method: Venipuncture from a jugular vein using the hand-held restraint method.
Target volume: approximately 1.0 mL/time point collected.
Anticoagulant: none
Processing: serum
Number of aliquots: 2
Analyses: for total T3 and T4 analyses, hormone samples were analyzed using validated ultra-high performance liquid chromatography with dual mass spectroscopy (UHPLC/MS/MS) assays. Analysis of serum samples to determine TSH concentrations was conducted using a validated Luminex Bead Based (TSH) assay.
Fetal examinations: Fetal examinations were conducted without knowledge of treatment group. External, internal, and skeletal fetal findings were recorded as either developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal), malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life), or incidental (minor changes in coloration, mechanical damage to specimen, etc.).
External: Each viable fetus was examined in detail, sexed, weighed, tagged, and euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region. Following euthanasia, anogenital distance was measured for all viable fetuses. The absolute and relative values (to the cube root of fetal body weight) were reported. The crown-rump length of late resorptions (advanced degree of autolysis) was measured, the degree of autolysis recorded, a gross external examination performed (if possible), and the tissue was discarded.
Internal (visceral): The sex of all fetuses was confirmed by internal examination. Approximately one-half of the fetuses in each litter were examined for visceral anomalies by dissection in the fresh (non-fixed) state. The thoracic and abdominal cavities were opened and dissected. This examination included the heart and major vessels. Fetal kidneys were examined and graded for renal papillae development. The heads from these fetuses were removed and placed in Harrison's fixative for subsequent processing and soft-tissue examination.
Skeletal: The remaining fetuses (approximately one-half from each litter, excluding any carcasses without heads) were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, fetuses were stained with Alizarin Red S and Alcian Blue. The skeletal examination was made following this procedure.
Statistics:Levene's test was used to assess the homogeneity of group variances. The groups were compared using an overall one-way ANOVA F-test if Levene's test was not significant or the Kruskal-Wallis test if it was significant. If the overall F-test or Kruskal-Wallis test was found to be significant, then pairwise comparisons were conducted using Dunnett's or Dunn's test, respectively.
Indices:The following parental indices and litter calculations were included, where applicable:
Pre-Implantation Loss = (No. of corpora lutea – no. of implants) x 100/No. of corpora lutea
Post-Implantation Loss = (No. of implants – no. of live fetuses) x 100/No. of implants
Sex Ratio (% males) = No. male fetuses x 100/Total no. of fetuses
Litter % of Fetuses with Abnormalities = No. of fetuses in litter with a given finding x 100/No. of fetuses in litter examined

Historical control data: Historical control data were available from the testing laboratory.

**Results and discussion**

Results: maternal animals

General toxicity (maternal animals)
There were no test substance-related clinical observations noted at any dose level at the daily examinations or approximately 1 hour postdose.
Mortality: no mortality observed
All females in the control, 25, 75, and 150 mg/kg bw/day groups survived to the scheduled necropsy.
Body weight and weight changes:
In the 150 mg/kg bw/day group, a slight mean body weight loss with correspondingly lower mean food consumption were noted following the initiation of dosing (Gestation Days 6–7) compared to the control group. Mean body weight gains and food consumption were generally comparable to the control group for the remainder of the study and when the entire treatment period (Gestation Days 6–21) was evaluated and the mean absolute body weights in this group were unaffected. Therefore, the initial decrements in the 150 mg/kg bw/day group were considered test substance-related, but nonadverse.
Mean body weights and body weight gains in the 25 and 75 mg/kg bw/day groups and mean corrected body weights, corrected body weight gains in the 25, 75, and 150 mg/kg bw/day groups were unaffected by test substance administration.
Food consumption and compound intake:
In the 150 mg/kg bw/day group, a slight mean body weight loss with correspondingly lower mean food consumption were noted following the initiation of dosing (Gestation Days 6–7) compared to the control group. Mean body weight gains and food consumption were generally comparable to the control group for the remainder of the study and when the entire treatment period (Gestation Days 6–21) was evaluated and the mean absolute body weights in this group were unaffected. Therefore, the initial decrements in the 150 mg/kg bw/day group were considered test substance-related, but nonadverse.
Food consumption in the 25 and 75 mg/kg bw/day groups was unaffected by test substance administration.
Food efficiency: not examined
Water consumption and compound intake (if drinking water study): not examined
Ophthalmological findings:not examined
Haematological findings:not examined
Clinical biochemistry findings:not examined
Endocrine findings:
Lower mean total T3 and T4 levels were observed in the 75 (-34.0% and -23.4% respectively) and 150 (-44.3% and -45.3% respectively) mg/kg bw/day groups compared to the control group; differences were statistically significant. Mean total T3 and T4 levels in the 25 mg/kg bw/day group were comparable to the control group mean.
Mean TSH levels in the 25, 75 and 150 mg/kg bw/day groups were higher than the control group mean; differences were non-dose responsive and statistically significant only for the 25 and 75 mg/kg bw/day groups. In addition, TSH mean values across all treated groups were within the range of the lab historical control data for Sprague Dawley rats and the control group mean was atypically low, and outside the range of the historical control data; when evaluated on an individual animal basis, more than 50% of the animals in the control group had TSH levels that fell outside of the range of the historical control data.
There were no differences in thyroid gland weights or any corresponding changes in thyroid histopathology in any of the treated groups when compared to the control group. However, test article-related higher mean (absolute) liver weights were observed in the 75 and 150 mg/kg bw/day group females.
Based on these data, the lower T3 and T4 levels noted in the 75 and 150 mg/kg bw/day groups were likely attributable to increased hormone metabolism in the liver, rather than a direct effect on the thyroid gland, and hence considered nonadverse.
Urinalysis findings:not examined
Behaviour (functional findings):not examined
Immunological findings:not examined
Organ weight findings including organ / body weight ratios:effects observed, treatment-related

Test article-related higher mean (absolute) liver weights were observed in the 75 and 150 mg/kg bw/day group females. Higher mean liver weights were not dose-responsive, but multiple individual absolute liver values in the 75 and 150 mg/kg bw/day group females were out of the concurrent control group range. There were no microscopic correlates.
No test substance-related organ weight changes were noted in the thyroid gland.
Gross pathological findings: No test substance-related gross pathology findings were noted. The gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of 3-p-cumenyl-2-methylpropionaldehyde.
Neuropathological findings:not examined
Histopathological findings: non-neoplastic: Test substance-related, microscopic liver changes were present in the 25, 75, and 150 mg/kg bw/day group females and consisted of minimal hepatocellular single-cell necrosis. Single-cell necrosis was perivascular and/or random, and was characterized as individual eosinophilic rounded hepatocytes with pyknotic nuclei. Single-cell necrosis was dose-responsive in incidence rate.
Of note, a marginal increased incidence of focal or multifocal hepatocellular necrosis (n=4), characterized as random groups (vs. single-cell/individual) of necrotic hepatocytes with associated inflammatory infiltrates, was also observed in the 150 mg/kg bw/day group females, but was considered incidental given the observation of this change in the concurrent control group females (n=2), and frequent occurrence of this change in both the lab historical control database and in untreated rats used in toxicity studies.
There were no test substance-related microscopic changes in the thyroid gland. Remaining changes were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of 3-p-cumenyl-2-methylpropionaldehyde.
Histopathological findings: neoplastic:not examined
There were no adverse effects on maternal parameters observed at any dosage level.
Maternal developmental toxicity
Number of abortions:Intrauterine survival was unaffected by test substance administration at dose levels of 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of postimplantation loss,mean number of live fetuses, and fetal sex ratios.
Pre- and post-implantation loss:Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups.
Intrauterine survival was unaffected by test substance administration at dose levels of 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of postimplantation loss,mean number of live fetuses, and fetal sex ratios.
Total litter losses by resorption: Intrauterine survival was unaffected by test substance administration at dose levels of 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of postimplantation loss,mean number of live fetuses, and fetal sex ratios.
Early or late resorptions:Intrauterine survival was unaffected by test substance administration at dose levels of 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of postimplantation loss,mean number of live fetuses, and fetal sex ratios.
Dead fetuses:Intrauterine survival was unaffected by test substance administration at dose levels of 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of postimplantation loss,mean number of live fetuses, and fetal sex ratios.
Changes in pregnancy duration:not specified
Changes in number of pregnant:not specified
There were no adverse effects on maternal parameters observed at any dosage level.
*Results (fetuses)*
Fetal body weight changes:Mean fetal body weights (males, females, and combined) in the 75 and 150 mg/kg bw/day groups were lower than the concurrent control group (4.0%–5.9% and 8.5%–9.5%, respectively) and below the mean values in the lab historical control data. The lower mean fetal body weights in these groups were considered test substance-related and adverse at 150 mg/kg bw/day based on the magnitude of change from the control group, but nonadverse at 75 mg/kg bw/day due to the low magnitude of change from the control group and lack of any fetal morphological effects.
Intrauterine growth at 25 mg/kg bw/day was unaffected by test substance administration.

Reduction in number of live offspring:no effects observed
Changes in sex ratio:no effects observed
Changes in litter size and weights:not specified
Anogenital distance of all rodent fetuses:Mean anogenital distances (absolute and relative to the cube root of the fetal weight) were unaffected by test substance administration at all dose levels. Differences from the control group were slight and not statistically significant.
Changes in postnatal survival:not examined
External malformations:No external developmental malformations were observed for fetuses in the test substance-treated groups. In the control group, Fetus No. 1505-01 was noted with a thread-like tail which consisted skeletally of absent sacral and caudal vertebrae. No external developmental variations were observed in fetuses in this study.
Skeletal malformations:No test substance-related skeletal developmental malformations were noted. In the 150 mg/kg bw/day group, a malformation of bent humerus was observed for a single fetus (Fetus No. 4501-12); variations of bent scapula, wavy ribs, thoracolumbar supernumerary ribs, and incomplete ossification of the frontal, parietal, squamosal, and supraoccipital bones of the skull were also observed for this fetus.
No test substance-related skeletal developmental variations were noted. Findings observed in the test substance-treated groups were noted infrequently, similarly in the control group, were not observed in a dose-related manner, the differences in the mean litter proportions were not statistically significant compared to the concurrent control group, and/or the values were within the ranges of the lab historical control data.
Visceral malformations:No visceral developmental malformations were observed in fetuses in this study.
No test substance-related visceral developmental variations were noted. Findings observed in the test substance-treated groups were noted infrequently, similarly in the control group, were not observed in a dose-related manner, the differences in the mean litter proportions were not statistically significant compared to the concurrent control group, and/or the values were within the ranges of the lab historical control data.
A discolored liver lobe was noted for Fetus No. 2503-04 in the 25 mg/kg bw/day group and was not classified as either a malformation or variation. This finding was considered incidental and was not considered to be test substance-related because it occurred infrequently and/or in a manner hat was not dose-related.
Details on embryotoxic / teratogenic effects:In the 75 and 150 mg/kg bw/day groups, test substance-related lower (up to 5.913% and 9.499%, respectively) mean fetal body weights (males, females, and combined) were noted compared to the control group. Based on the magnitude of change versus the control group, the effects on mean fetal body weights were considered nonadverse at 75 mg/kg bw/day but adverse at 150 mg/kg bw/day. There were no test substance-related effects on intrauterine growth at 25 mg/kg bw/day or intrauterine survival and fetal morphology (external, visceral, and skeletal) at 25, 75, and 150 mg/kg bw/day.
The numbers of fetuses (litters) available for morphological evaluation were 177(20), 186(19), 202(20), and 209(22) in the control, 25, 75, and 150 mg/kg bw/day groups, respectively.
Malformations were observed in 1 fetus each in the control and 150 mg/kg bw/day groups and were considered spontaneous in origin. When the total malformations and developmental variations were evaluated on a proportional basis, no statistically significant differences from the control group were noted.
Fetal malformations and developmental variations, when observed in the test substance-treated groups, occurred infrequently or at a frequency similar to that in the control group, did not occur in a dose-related manner, and/or were within the lab historical control data ranges. Based on these data, no fetal malformations or developmental variations were attributed to the test substance.
In the 150 mg/kg bw/day group, a malformation of bent humerus was observed for a single fetus (Fetus No. 4501-12); variations of bent scapula, wavy ribs, thoracolumbar supernumerary ribs, and incomplete ossification of the frontal, parietal, squamosal, and supraoccipital bones of the skull were also observed for this fetus.
Relation to maternal toxicity:developmental effects occurring together with maternal toxicity effects, but not as a secondary non-specific consequence of maternal toxicity effects.

#### 3.10.1.3 Study 3: 3-*p*-cumenyl-2-methylpropionaldehyde

Reference: Unnamed 2020a

short-term repeated dose toxicity: oral

Study period:1 Mar 2019 7 May 2019

Test guideline: no guideline followed

The objectives of this study were:

a) Determine the potential toxicity of 3-p-cumenyl-2-methylpropionaldehyde mainly reproductive organs of Male Wistar Han rats treated for 28 consecutive days by daily oral gavage at dose levels of 0, 30, 100 and 300 mg/kg bw/day;

b) Determine the circulating blood concentration of metabolites of 3-p-cumenyl-2-methylpropionaldehyde in plasma sampled by GC-MS.

c) Determine the CoA-conjugate formation in tissue samples of both the testes and the liver after necropsy by LC-MS;

d) Determine the metabolite profile in tissue samples of both the testes and the liver and in plasma samples obtained on day 28 using high-resolution LC-MS analysis.

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, sperm analysis, gross necropsy findings and histopathologic examinations (testis only), plasma concentration of 3-p-cumenyl-2-methylpropionaldehyde; CoA-conjugate formation in testes and liver, metabolite profile in the testes, liver and plasma samples obtained on day 28 of treatment.

GLP compliance:no

**Test material**

Reference substance name:3-p-cumenyl-2-methylpropionaldehyde

EC Number:203-161-7

EC Name:3-p-cumenyl-2-methylpropionaldehyde

Cas Number:103-95-7

Molecular formula:C13H18O

IUPAC Name:2-methyl-3-[4-(propan-2-yl)phenyl]propanal

Test material form:liquid

**Test animals**

Species:rat

Strain:Wistar

Remarks:WI(Han)

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.

Sex:male

Details on test animals or test system and environmental conditions:

Crl: WI(Han) rats were received from Charles River Deutschland, Sulzfeld, Germany. The animals were 7 weeks old at initiation of dosing and weighed between 205 and 235 g. A health inspection was performed before the initiation of dosing.

The animals were allowed to acclimate to the Test Facility toxicology accommodation for 6 days before the commencement of dosing.

On arrival and following randomization, animals were group housed (up to 5 animals of the same sex and same dosing group together) in polycarbonate cages (Makrolon type IV, height 18 cm) containing appropriate bedding (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. Animals were separated during designated procedures/activities.

Environmental Conditions

Target temperatures of 18 to 24°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 21°C with an actual daily mean relative humidity of 39 to 54%.

Food:Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) was provided ad libitum throughout the study, except during designated procedures.

Water: Municipal tap water was freely available to each animal via water bottles.

**Administration / exposure**

Route of administration:oral: gavage

Details on route of administration: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.

Vehicle:corn oil

Remarks:Supplier: Sigma-Aldrich

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 weekly, filled out in daily portions and stored at room temperature. Formulations were stirred for at least 30 minutes before use. If practically possible, the dosing formulations and vehicle were continuously stirred during dosing. Adjustment was made for specific gravity of the vehicle and test item. Any residual volumes were discarded.

Sample Collection and Analysis

The Sponsor provided data that demonstrated that the test article was stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data provided by the Sponsor have been retained in the study records.

Analytical verification of doses or concentrations:no

Duration of treatment / exposure:Rats dosed for 28 days.

Frequency of treatment:Once daily.

Doses / concentrations: 0,30, 100, 300  mg/kg bw/day (nominal)

No. of animals per sex per dose:5

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test item. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives. At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models which do not use live animals currently do not exist. The study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD2360020172866 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

Control animals:yes, concurrent vehicle

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. The total number of animals used in this study (5 males/group) was considered to be the minimum required to properly characterize the effects of the test item.

Male Wistar Han rats, approximately 7 weeks of age on treatment Day 1 were administered 3-p-cumenyl-2-methylpropionaldehyde extra via oral gavage daily for at least 28 consecutive days at dose levels of 30, 100 and 300 mg/kg bw/d.

Animals were assigned to groups by a stratified randomization scheme to achieve similar group mean body weights, with all animals within ± 20% of the sex mean. The dose levels were selected based on information provided by a 14-day oral gavage study in rabbits.

Positive control:No

**Examinations**

Observations and examinations performed and frequency:5 males/group

CAGE SIDE OBSERVATIONS: once daily throughout the Dosing Period. During the Dosing Period, these observations were performed after dosing. Animals were not removed from the cage during observation, unless necessary for identification or confirmation of possible findings.

DETAILED CLINICAL OBSERVATIONS: Time schedule: twice daily (in the morning and at the end of the working day).

BODY WEIGHT: Weekly, starting on Day 1. A fasted weight was recorded on the day of necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE: Weekly, starting on Day 1. A fasted weight was recorded on the day of necropsy.

BIOANALYSIS:

Bioanalytical Sample Collection:

Prior to necropsy, blood was collected from the jugular vein. Blood samples at a target volume of 0.5 mL were collected into tubes containing K2-EDTA as anticoagulant. 4.9.1.2. Bioanalytical Sample Processing Samples were centrifuged within 2 hours after blood sampling at approximately 2000g for 10 minutes at 4-8 °C. Immediately after centrifugation, plasma was stored in labeled polypropylene tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) at ≤ -75 C until shipped on dry ice to the Sponsor. Samples were used to determine the circulating blood concentration of metabolites of 3-p-cumenyl-2-methylpropionaldehyde in plasma sampled at the end of a 28 days range finder gavage study in rats by GC-MS. Collection on ice. Theoretical number of samples 21

Others: Testis, epididymis and liver were collected for metabolite analysis to determine the CoA-conjugate formation in tissue samples of both the testes and the liver, and to determine the metabolite profile in tissue samples of both the testes and the liver and in plasma samples obtained on day 28 using high-resolution LC-MS analysis.

Sacrifice and pathology:

Necropsy

All animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

At necropsy, the left testis, right epididymis and liver were collected, stored in plastic bags and snap frozen into liquid nitrogen. Samples were stored at ≤ -75 C until shipment on dry ice to the Sponsor for metabolite analysis. The left epididymis was used for sperm analysis. The right testis was used for histopathology.

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.

Tissue Collection and Preservation

Epididymis, Liver, Testis, Gross lesions/masses 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.

Histology

Tissues were embedded in paraffin (Klinipath, Duiven, The Netherlands), sectioned, mounted on glass slides, and stained with hematoxylin and eosin (Klinipath, Duiven, The Netherlands).

Histopathology

The right testis of each animal was examined by a board-certified toxicological pathologist with training and experience in laboratory animal pathology. A peer review on the histopathology data was performed by a second pathologist.

Other examinations:

From all males, sperm samples were taken from the proximal part of the vas deferens (right) at necropsy. Sperm motility was assessed from all samples and sperm smears were fixed for morphological evaluation. Abnormal forms of sperm from a differential count of 200 spermatozoa (if possible) per animal were recorded. Evaluation was performed for all males. One epididymis (left) from all males was removed, placed in labeled bags, and kept in the freezer at ≤-15°C. After thawing the left epididymis were weighed, homogenized and evaluated for sperm numbers. Evaluation was performed for all males.

Plasma was collected to determine circulating blood concentration of metabolites of 3-p-cumenyl-2-methylpropionaldehyde at day 28. Testes, liver and plasma were collected to determine the CoA-conjugate formation. Metabolite profile were evaluated for plasma, testes and liver.

Statistics:

Body weight gain, food consumption were were summarized and statistically analyzed as indicated below according to sex and occasion.

**Results and discussion**

Clinical signs:No clinical signs of toxicity were noted during the observation period.

Salivation was seen after dosing in all males at 100 and 300 mg/kg bw/day on most occasions. This was not considered toxicologically relevant, taking into account the nature and minor severity of the effect, its time of occurrence (i.e. after dosing) and as it was also seen in some control animals. This sign was considered to be a physiological response related to the taste of the test item rather than a sign of systemic toxicity.

Mortality:No mortality occurred during the study period.

Body weight and weight changes: No test item-related effects on body weight and body weight gain were observed in males at 30 mg/kg bw/day. Slightly lower body weight and body weight gain was observed in males at 100 mg/kg bw/day starting on Day 15, with body weight being 0.93x of controls at the end of treatment (Day 28). Body weight and body weight gain in males at 300 mg/kg bw/day were moderately decreased starting on Day 8 (mean body weight was 0.89x of controls on Day 28), achieving statistical significance for body weight on Day 29.

Food consumption and compound intake :No clear test item-related effects on food consumption were noted. Food consumption was minimally lower at 100 and 300 mg/kg bw/day in Week 1, but lacked a dose-related effect and was therefore considered not to be toxicologically relevant.

Food efficiency:not examined

Water consumption and compound intake (if drinking water study):not specified

Ophthalmological findings:not examined

Haematological findings:not examined

Clinical biochemistry findings:not examined

Urinalysis findings:not examined

Behaviour (functional findings):not examined

Immunological findings:not examined

Organ weight findings including organ / body weight ratios:not examined

Gross pathological findings:Test item-related gross lesions were observed in the epididymis in males at 300 mg/kg bw/day. A focal nodule (soft, yellow) was noted unilaterally in the tail of the epididymis in 3 out of 5 males at 300 mg/kg bw/day (No. 16 (right) and Nos. 18 and 19 (left)).

The remainder of the recorded macroscopic findings (prominent lobular architecture of the liver in one male (No. 17) and red foci in the kidney of one male (No. 18) at 300 mg/kg bw/day were within the range of background gross observations encountered in rats of this age and strain and were interpreted as likely to be unrelated to treatment with the test item.

Sperm Analysis: No effects on sperm motility, concentration and morphology were observed in males at 30 mg/kg bw/day. The insufficient number of cells present for sperm cell morphology determination in one male at 30 mg/kg bw/day (No. 7) was considered to be an incidental finding and, in absence of any findings in the other 30 mg/kg bw/day animals, considered not to be toxicologically relevant.

At 100 mg/kg bw/day, a lower percentage of motile sperm (0.76x of control), progressive sperm (0.70x of control) and number of cells with a normal morphology (0.87x of control) was recorded. In addition, an increased number of cells with a detached head (4.43x of control) and abnormal neck (7.0x of control), and decreased number of cells with a coiled tail (0.39x of control) were observed. At 300 mg/kg bw/day, severe effects on the sperm motility, concentration and morphology were observed. These consisted of decreased total sperm count in the epididymis (0.61x of control), percentage of motile sperm (0.22x of control), percentage of progressive sperm (0.10x of control) and number of sperm cells with a normal morphology (0.04x of control).

The change in percentage of motile sperm and progressive sperm were statistically significant. The sperm cell morphology from 3 out of 5 males could not be determined as the sperm cell count for morphology was below the required 200 cells, which was considered to be caused by the test item. In addition, a lower number of cells with a coiled tail (0.13x of control), accompanied by an increase in number of cells with detached head (24.6x of control), abnormal head (2.0x of control) and/or neck (9.0x of control) and combined cells were observed in the remaining 2 out of 5 animals.

Neuropathological findings:not examined

Histopathological findings: The right testis was evaluated histologically from all males. Test item-related microscopic findings were noted at 300 mg/kg bw/day group males and are summarized below. Reference to stages or steps in the description of the histologic changes refer to those described by Russel et al. (1990).

Testis Right (males treated with 300 mg/kg/d):

- Degeneration, elongated spermatids: Minimal (1); Moderate (4)

-Spermatid retention: Mild (1); Moderate (4)

-Degeneration, round spermatids: Moderate (1);

- Depletion, spermatid: Mild (1)

Degeneration of elongating spermatids was noted in all animals at 300 mg/kg bw/day, up to moderate degree. This was most readily observed in early tubular stages, approximately IVIII (corresponding to Step 15 to Step 19 spermatids), and was characterized by both an abnormal shape and abnormal location within the seminiferous tubules for the given stage. Abnormal shape was variable and consisted of either condensed, round, darkly basophilic nucleus with a bent/squiggled ‘neck’ giving a tadpole-like appearance to the nucleus, large cytoplasmic droplets extending into the lumen, and/or round shape with pale eosinophilic cytoplasm as a small condensed darkly basophilic nucleus which was often dissociated from the adjacent cells near the luminal border or sloughing into the lumen. Normally shaped elongated spermatids were not uncommonly present in the same tubular profile.

Spermatid retention was noted in all animals at 300 mg/kg bw/day, up to moderate degree. This was characterized by the presence of elongated spermatids (bothnormal and abnormal shaped) at the luminal surface of the seminiferous epithelium beyond the expected point of release (i.e. Stage VIII), and affected of primarily Stage IX-XII tubules. Less often, elongated spermatids were observed in low numbers at the base of the seminiferous tubules in all tubular stages.

Degeneration of round spermatids was prominent in one male at 300 mg/kg bw/day (No 18) and was characterized by condensed and hyper-eosinophilic round spermatids, often dissociated from the surrounding cells in the seminiferous tubule and sloughing into the lumen. Concurrent depletion of spermatocytes (round and elongated) and degeneration of elongated spermatids (as described above) were noted in this animal. The few remaining histologic changes noted in the testis, including minimal Sertoli cell vacuolation, were considered to be incidental findings and/or were within the range of background pathology encountered in the testis of 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.

The combination of histologic changes noted in the testis of 3-p-cumenyl-2-methylpropionaldehyde-treated male rats is suggestive of a test item-related abnormality in spermiogenesis (transformation of round spermatids to mature, elongated spermatids) and spermiation (release of mature spermatids from the seminiferous epithelium) (O'Donnell, 2014).

Increased vacuolation of Sertoli cells can be a test item-related change. However, in the present study vacuolation was observed in the controls as well and severity was minimal in all groups. Therefore, there was insufficient evidence to suggest a test item-related effect in the context of this study.

The changes noted by light microscopy correlate with the changes on sperm analysis including lower sperm concentrations, and morphologic abnormalities.

The nodules noted macroscopically in the epididymis of 3 of 5 males at 300 mg/kg bw/day are suggestive of sperm granulomas, however, the exact nature of these macroscopic changes requires histologic evaluation.

CONCLUSIONS

Adverse test item-related morphologic alterations following the administration of 3-p-cumenyl-2-methylpropionaldehyde extra for 28 days to Wistar Han rats, were present in the testis of males treated at 300 mg/kg bw/day. These adverse test item-related morphologic alterations consisted of microscopic spermatid degeneration, spermatid retention, and spermatid depletion in the testis, and macroscopic focal nodules in the tail of the epididymis.

Histopathological findings: neoplastic:no effects observed

Levels of 3-p-cumenyl-2-methylpropionaldehyde and Cyclamen alcohol were below detection limit in all plasma samples including the non-diluted plasma samples. 4-iPBA plasma concentrations were below detection limit in plasma samples collected from the control group and at 30 mg/kg bw/day. 4-iPBA was detected in all plasma samples at 100 and 300 mg/kg bw/day, ranging from 13.2 to 26.6 µM (100 mg/kg bw/day) and 151.1 to 385.0 µM (300 mg/kg bw/day). Average 4-iPBA concentrations were 18.8 ± 5.1 µM and 264.6 ± 85.4 µM at 100 and 300 mg/kg bw/day, respectively, corresponding to a 14-fold difference between the animals receiving the medium and the highest dose.

Cyclamen acid concentrations were below detection limit in plasma samples collected from the control group. Cyclamen acid was detected in all plasma samples of all test item groups and ranged from 0.1 to 0.3 µM (30 mg/kg bw/day), 0.3 to 1.2 µM (100 mg/kg bw/day) and 1.4 to 6.4 µM (300 mg/kg bw/day). Average Cyclamen acid concentrations were 0.2 ± 0.1 µM, 0.7 ± 0.4 µM and 3.2 ± 2.1 µM at 30, 100 and 300 mg/kg bw/day, respectively. Plasma concentrations of Cyclamen acid were 3.5-fold higher at 100 mg/kg bw/day compared to 30 mg/kg bw/day, whereas concentrations were 4.6-fold higher at 300 mg/kg bw/day compared to 100 mg/kg bw/day. However, plasma levels of Cyclamen acid were 27-fold lower (100 mg/kg bw/day) and 83-fold lower (300 mg/kg bw/day), respectively, compared to 4-iPBA.

Testes and liver:

In animals dosed with 30 mg/kg bw/day, trace amounts 4-iPBA-CoA were detected in the testes of only one individual. At 100 mg/kg bw/day, the conjugate was detectable at low levels in testes samples from all animals. At 300 mg/kg bw/day, 5-6 times higher levels (0.724 ± 0.222 nmol/gtissue) than at 100 mg/kg bw/day were observed, indicating that at the toxic dose this metabolite is significantly formed in the reproductive tissue. The concentration in the liver is clearly higher (> 500 fold), and strong accumulation in the liver of this metabolite as previously shown in the in vitro studies.

Metabolites

The major metabolite based on peak area observed by LC-MS analysis in the tissue samples and in the blood plasma is the acyl-glucuronide conjugate of iPBA (M25). The second most abundant peak is U1, an unknown metabolite with a mass of 208.1099 which was detected as the most abundant peak in plasma at 30 and 100 mg/kg bw/day and the second most abundant metabolite at 300 mg/kg bw/day. Further abundant metabolites were 4-iPBA (M3), hydroxylated 4-iPBA (M8), hydroxylated 4-iPBA-acylglucuronide (M37) and the glycine conjugate of 4-iPBA (M36).

3-p-cumenyl-2-methylpropionaldehyde is easily oxidized to the corresponding acid (M2), but this is only a minor intermediate as shown before by GC-MS analysis of plasma samples and it was not detectable by LC-MS, neither in tissue nor in plasma samples. The acid is either directly degraded to iPBA or it is hydroxylated, putatively at the isopropyl-side chain. Hence the hydroxylated Cyclamen acid (M5) is a further important metabolite in this analysis especially in the plasma samples, where also the product of a further oxidation step is observed (di-acid, M6, detected in plasma mainly). M5 can be degraded to the hydroxylated iPBA (M8), which is found in tissue and plasma samples. However, M5 could be also formed from 3-p-cumenyl-2-methylpropionaldehyde by side chain degradation to 4-iPBA followed by hydroxylation. In both cases, the hydroxylated metabolites (M5 and M8) are then again conjugated, especially to glucuronic acid and esp. the glucuronide of M8 (M37) is quite abundant.

Next to the glucuronide, different iPBA conjugates are detected in the plasma and in testes and liver (glycine-, taurine-, carnitine- and glutamic acid-conjugates; M36, M28, M30 and M34). Thus in summary, the key metabolic pathways observed are formation of iPBA and subsequent conjugation mainly with glucuronic acid and glycine and/or hydroxylation and further oxidation.

#### 3.10.1.4 Study 4: 3-*p*-cumenyl-2-methylpropionaldehyde

Reference: Unnamed 2011b

short-term repeated dose toxicity: oral

Study period:06 July 2011

Test guideline:no guideline available

Rabbits were administered the test substance, and the vehicle control (corn oil) formulations orally via gavage once daily from Day 1 to Day 14.

This study was performed in 2011 in a GLP facility in accordance with OECD guidelines and good scientific practice. It was reported, however, as being non-GLP and cannot be verified as being conducted to full GLP standards. Based on the evidence available the study is considered to be acceptable as a key study as it has been conducted in a GLP facility and in accordance with principles essentially equivalent to GLP standards.

GLP compliance:no

Remarks:2011 GLP facility Study in accordance with OECD guidelines and good scientific practice. Study reported as non-GLP, full GLP standards not verified. Acceptable as key study conducted in GLP facility in accordance with principles equivalent to GLP

Limit test:no

**Test material**

Reference substance name:3-p-cumenyl-2-methylpropionaldehyde

IUPAC Name:3-p-cumenyl-2-methylpropionaldehyde

Reference substance name:3-p-cumenyl-2-methylpropionaldehyde

EC Number:203-161-7

EC Name:3-p-cumenyl-2-methylpropionaldehyde

Cas Number:103-95-7

Molecular formula:C13H18O

IUPAC Name:2-methyl-3-[4-(propan-2-yl)phenyl]propanal

Test material form:other: liquid

Identification: 3-p-cumenyl-2-methylpropionaldehyde

Batch (Lot) No.: VE00130506

Receipt Date: 27 Jan 2011

Expiration Date: 04 Mar 2012

Physical Description: Clear, colorless liquid

Purity: Purity assumed to be 100% for dose calculation purposes.

Storage Conditions: Room temperature, protected from light

Supplier: Givaudan Suisse SA 5, Chemin de la Parfumerie, Vernier, Switzerland

**Test animals**

Species:rabbit

Strain:New Zealand White

The New Zealand White [Hra:(NZW)SPF] rabbit was selected as the Test System because it is one nonrodent mammalian species accepted and widely used throughout the industry. The total number of animals used in this study was the minimum required to properly characterize the effects of the test substance. In addition, this study did not duplicate any previous work.

Sex:male

Twenty-one male Hra:(NZW)SPF rabbits were received from Covance Research Products, Inc., Swampbridge Road, Box 7200, Denver, PA. Body weight range was 2.3 to 3.0 kg on the day ofarrival, and was 2.4 to 3.1 kg at randomization. The rabbits were approximately 5 months of age at arrival to the Testing Facility.

Animal Identification

Rabbits were permanently identified using Monel® self-piercing ear tags. Male rabbits were given unique permanent identification numbers when assigned to the study.

Environmental Acclimation

After receipt at the Testing Facility, the rabbits were acclimated for at least 1 day prior to predose sperm sample collection.

Selection, Assignment, and Replacement of Animals

Upon arrival, rabbits were assigned to individual housing on the basis of computer-generated random units. After acclimation, rabbits were selected for study on the basis of physical appearance and body weights recorded during acclimation. The rabbits were assigned to 4 dose groups (Groups 1 through 4), 5 rabbits per dose group based on computer-generated (weightordered) randomization procedures.

Disposition

The remaining rabbit not assigned to study was humanely euthanized.

Husbandry

Housing

The rabbits were individually housed in units of six to eight stainless steel cages. All cage sizes and housing conditions were in compliance with the Guide for the Care and Use of Laboratory Animals. Cage pan liners were changed at least three times weekly. Cages were changed approximately every other week.

Environmental Conditions

The study rooms were maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of 10 changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 61°F to 72°F (16°C to 22°C); relative humidity was targeted at 30% to 70%.

An automatically controlled 12-hours light:12-hours dark fluorescent light cycle was maintained. Each dark period began at 1900 hours (± 30 minutes).

Food

Approximately 150 g of Certified Rabbit Chow® #5322 (PMI® Nutrition International, St. Louis, MO) was available to each rabbit each day. The certified food was available from individual, stainless steel, "J-type" feeders attached to each cage.

Analyses were routinely performed by the food supplier. No contaminants at levels exceeding the maximum concentration limits for certified food or deviations from expected nutritional requirements were detected by these analyses. Copies of the results of the food analyses are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the food that would have interfered with the results of this study.

Water

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rabbits ad libitum from an automatic watering access system. Chlorine was added to the processed water as a bacteriostat.

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, PA) and monthly for possible bacterial contamination (QC Laboratories, Southampton, PA). Copies of the results of the water analyses are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the water that would have interfered with the results of this study.

Animal Enrichment

For psychological enrichment, rabbits were provided with items such as a bell or rattle. Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the chewable enrichment devices at levels that would have interfered with the results of this study.

Veterinary Care

Upon animal arrival and twice during the course of the study, rabbits were examined by the veterinary staff. Records of examinations are maintained with the raw data. No medical treatments were administered. None of the medical examinations had an adverse impact on the integrity of the study data or on the interpretation of the study results.

**Administration / exposure**

Route of administration:oral: gavage

The oral gavage route was selected for use to maximize systemic absorption.

Dosage levels were chosen based on a comparative study made on 4-tert butyl priopionaldehyde, a structurally similar substance, which was tolerated with no acute toxicity at up to 300 mg/kg in the rabbit and shows equivalent acute toxicity potential to 3-p-cumenyl-2-methylpropionaldehyde in rats.

Vehicle:corn oil

Details on oral exposure:

Rabbits were administered the test substance and/or the control article formulations orally (stomach tube) once daily on Day 1 of study (DS 1) to 14. The dose volume for each animal was based on the most recent

body weight measurement. The first day of dosing for each animal was designated as DS 1. The dosing formulations were stirred continuously during dose administration.

The control article, corn oil, was dispensed once weekly for administration to Group 1 control rabbits. An adequate amount of the control article was dispensed into daily aliquots, which were stored at room temperature until used. The control article was stirred continuously during dosing. Details of the preparation and dispensing of the control article have been retained in the Study Records. Any residual volumes were discarded daily after the completion of dosage administration.

Test substance dosing formulations were prepared in accordance with Sponsor instructions at appropriate concentrations to meet dosage level requirements. The dosing formulations were prepared once weekly and dispensed into daily aliquots which were stored at room temperature, protected from light until used. The dosing formulations were stirred for at least 30 minutes before dosing and continuously during dosing. Details of the preparation and dispensing of the test substance have been retained in the Study Records. The bulk test substance was handled in a chemical fume hood. Any residual volumes were discarded daily after the completion of dosage administration

Analytical verification of doses or concentrations:no

Remarks:Samples for concentration, homogeneity and/or stability were not collected or analyzed.

Duration of treatment / exposure:14 consecutive days.

Frequency of treatment:Once daily doses.

Doses / concentrations. 0, 30, 100, 300 mg/kg bw/day (nominal)

No. of animals per sex per dose:5 males per dose

Control animals:yes, concurrent vehicle

Dosage levels were chosen based on a comparative study made on 4-tert butyl priopionaldehyde, a structurally similar substance, which was tolerated with no acute toxicity at up to 300 mg/kg in the rabbit and shows equivalent acute toxicity potential to 3-p-cumenyl-2-methylpropionaldehyde in rats.

Rabbits were administered the test substance and/or the control article formulations orally (stomach tube) once daily on Day 1 of study (DS 1) to 14. The dose volume for each animal was based on the most recent body weight measurement. The first day of dosing for each animal was designated as DS 1. The dosing formulations were stirred continuously during dose administration.

Positive control:No.

**Examinations**

Observations and examinations performed and frequency: In-life Procedures, Observations, and Measurements

Clinical observations, mortality, general appearance, body weight, food consumption.

Viability Checks

The rabbits were assessed for viability at least twice daily during the study.

General Appearance

The rabbits were observed for general appearance daily during the predose period, before each dose was administered and on the day of scheduled euthanasia.

Postdose Observations

For the first 4 days of dosing, postdose observations were conducted immediately after each animal was administered the test substance or control article, then at hourly intervals for the first four hours after dose administration and again at the end of the normal working day. Beginning on the fifth day of dosing, postdose observations were conducted between 1 to 2 hours after dose

administration and at the end of the normal working day.

Body Weights

Body weights were recorded on the day of arrival at the Testing Facility, at least once during the predose period, daily during the dose period, and on the day of scheduled euthanasia.

Food Consumption

Food consumption values were recorded daily after arrival at the Testing Facility, daily during the dose period, and prior to placement of the rabbits in metabolism cages (food left value).

Laboratory Evaluations

Urinalysis

Urine samples (as much as possible) were collected overnight from all rabbits once on DS 14. Food was withheld during the collection interval. Overnight urine samples were collected over cold packs. For 1, 2, 2, and 1 in each of the 4 respective dose groups, urine samples were unable to be obtained overnight; therefore, a urine sample was collected from the urinary bladder following euthanasia using a 21 G needle. For these rabbits (8582 in the 0 mg/kg bw/day dose group, 8584 and 8586 in the 30 mg/kg bw/day dose group, 8588 and 8589 in the 100 mg/kg bw/day dose group and 8596 in the 300 mg/kg bw/day dose group) , the 21 G needle was inserted into the urinary bladder and as much urine as possible was collected into an appropriately sized syringe. Urine samples were stored in appropriately sized, labeled polypropylene tubes and then stored in a freezer set to maintain -20°C or colder for possible future analysis. Storage tubes were labeled at minimum with the Testing Facility study number, rabbit number, group number, dosage level, day of study, date of collection, species, biological matrix and storage conditions.

Male Reproductive Assessments

Sperm Evaluation

Semen samples were collected from each rabbit prior to initiation of dose administration and on the day of scheduled euthanasia (DS 15). Predose sample collections were attempted on four days prior to initiation of dosage administration. Not all rabbits yielded a sample or a yielded a viable sample at each predose collection day. Predose data will be retained in the raw data and will not be summarized in this report. Samples were collected utilizing an artificial vagina (and a teaser female rabbit of the same source and strain), and analyzed for motility and sperm count utilizing the Hamilton Thorne IVOS for computer-assisted sperm analysis (CASA) or manually for sperm morphology.

Sperm motility was evaluated following dispersion, into an appropriate medium, of semen ejaculate.

Sperm count was evaluated following dispersion, into an appropriate medium, of semen ejaculate.

Sperm morphology was evaluated from a stained sperm smear prepared from a semen ejaculate. Due to an insufficient volume of ejaculate collected prior to euthanasia, the sperm morphology was evaluated from the left cauda epididymis collected after euthanasia. Four slides were prepared, and approximately 200 sperm cells were evaluated from each animal. Images of motility and concentration samples were taken, retained as electronic images and archived with the raw data.

Sacrifice and pathology:

Scheduled Euthanasia and Necropsy

On DS 15 (the day following the completion of the 14-day dose period), rabbits were euthanized via an intravenous injection of a euthanasia solution (390 mg pentobarbital sodium and 50 mg phenytoin sodium) and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. See Section 6.12.3. (Tissue Collection and Preservation) for tissues retained, weighed and evaluated microscopically. For 6 rabbits, urine samples were collected from the urinary bladder following euthanasia using a 21 G needle as described in Section Urinalysis.

Organ Weights

The organs identified below were weighed at necropsy for all scheduled euthanasia animals. Paired organs were weighed together, where required. Organ to body weight ratio (using the terminal body weight) were calculated.

Organs weighed at necropsy:

Epididymis - Individually weighted.

Gland, prostate

Gland, seminal vesicle (with and without fluid)

Liver (gallbladder drained prior to weighing)

Kidney - Paired organ weight.

Testis - Paired organ weight. Fixed in Bouin's solution for 48 to 96 hours, rinsed per Testing Facility Standard Operating Procedures, and then preserved in Modified Davidson's fixative; individually weighed.

Tissue Collection and Preservation

Representative samples of the tissues identified below were collected from all rabbits and preserved in 10% neutral buffered formalin, unless otherwise indicated.

Epididymides

Epididymis, left cauda - Retained due to an insufficient volume of semen ejaculate collected for the preparation of sperm morphology sample analysis

Gland, prostate

Gland, seminal vesicle

Kidney

Liver

Testes - Fixed in Bouin's solution for 48 to 96 hours, rinsed per Testing Facility Standard Operating Procedures, and then preserved in Modified Davidson's fixative.

Histology

Tissues were processed at Charles River Laboratories Pathology Associates - Maryland. Tissue trimming was performed at the Testing Facility. All other histology procedures were performed by Charles River Laboratories Pathology Associates - Maryland. The following tissues were embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

• The testes and epididymides from each rabbit in Groups 1 and 4

Histopathological evaluation was performed by a board-certified veterinary pathologist. The following tissues were evaluated microscopically:

• The testes and epididymides from each rabbit in Groups 1 and 4

Statistics:Means and standard deviations were calculated for body weights, food consumption, sperm count and motility and organ weights.

**Results and discussion**

Clinical signs:no effects observed

None of the clinical signs that occurred were attributed to administration of 3-p-cumenyl-2-methylpropionaldehyde because they were transient and were limited to one rabbit in the 100 or 300 mg/kg bw/day dosage groups. These clinical signs included scant feces and no feces in the cage pan. No other clinical signs occurred.

Mortality:no mortality observed

All rabbits survived to scheduled euthanasia.

Body weight and weight changes:no effects observed

Body weights and body weight gains were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. Overall, body weights and body weight gains were comparable among the

dosage groups for the cumulative dosage period (DSs 1 to 14). All groups, including the control group, lost body weight between DS 14 and DS 15 because of overnight fasting for urine collection.

Body weight gains in the 30, 100 and 300 mg//kg/day dosage groups were 108%, 119% and 115% of the control group value, respectively, for the cumulative dosage period (DSs 1 to 14). The average body weight on DS 14 (day of last dosage) was 101%, 99% and 100% of the control group value in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively.

Food consumption and compound intake:no effects observed

Absolute (g/day) and relative (g/kg/day) food consumption values were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. Food consumption values were also comparable among the dosage groups for the cumulative dosage period. Absolute food consumption values in the 30, 100 and 300 mg//kg/day dosage groups were 102%, 100% and 103% of the control group value, respectively, for the cumulative dosage period (DSs 1 to 14). Relative food consumption values were 101%, 102% and 103% of the control group value in the 3 respective 3-p-cumenyl-2-methylpropionaldehyde-treated groups during the same period.

Food efficiency:not specified

Water consumption and compound intake:not specified

Ophthalmological findings:not examined

Haematological findings:not examined

Clinical biochemistry findings:not examined

Urinalysis findings:not examined

Urine samples were collected from all rabbits on Day 15 prior to euthanasia for possible future evaluation.

Behaviour (functional findings):not specified

Immunological findings:not specified

Organ weight findings including organ / body weight ratios:no effects observed

Terminal body weights were comparable among the four dosage groups.

The weights of the epididymides, left cauda epididymis, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organ weights to terminal body weight were unaffected by dosages 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. In addition, there were no test substance-related changes in the non-reproductive organs (i.e., liver or paired kidneys) at any dosage level. There were no patterns, trends, or correlating data to suggest these values were toxicologically relevant. The apparent increase in the absolute and relative (% body weight) weights of the liver that occurred at 300 mg/kg bw/day (18% and 21% over controls, respectively) was largely attributed to one rabbit (no. 8596) in the 300 mg/kg bw/day dosage group with an absolute liver weight of 111.1 g (range: 70.1 to 92.3 for other rabbits in the group). Exclusion of this rabbit from summarization would still result in a higher group mean weight in comparison to the control group value. The absolute and relative (% body weight) weights of the paired kidneys were also increased in the 300 mg/kg bw/day dosage group (11% and 13% over controls, respectively), again attributed to one rabbit (no. 8595) in this dosage group. Overall, the individual organ weight differences observed were considered incidental and unrelated to administration of 3-p-cumenyl-2-methylpropionaldehyde.

Gross pathological findings:no effects observed

There were no gross lesions observed at necropsy examination. All rabbits appeared normal.

Neuropathological findings:not specified

Histopathological findings: non-neoplastic:no effects observed

No test substance-related microscopic changes were observed in the testes or epididymides examined.

Histopathological findings: neoplastic:no effects observed

Sperm Evaluations:The values for the number of motile sperm and total sperm count from ejaculated semen samples were highly variable across the dosage groups, including the control group. Whilst a slight trend in the mean number of motile sperm (596.6, 543.3 and 431.0 in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively, vs. 627.0 in controls) and total sperm count (616.6, 595.5 and 496.2 in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively, vs. 679.0 in controls) from ejaculated samples in the 3-p-cumenyl-2-methylpropionaldehyde-treated groups was observed, individual values were highly variable and the lowest reported individual values were within the range of the control group values. In general, all values across all treated groups were within the range of the concurrent control group values and/or the historical control range at the Testing Facility.

Values for percent motile sperm, number of nonmotile sperm from the semen ejaculate sample and cauda epididymal sperm count and density were comparable among the four dosage groups.

Mortality

All rabbits survived to scheduled euthanasia.

Clinical Observations

None of the clinical signs that occurred were attributed to administration of 3-p-cumenyl-2-methylpropionaldehyde because they were transient and were limited to one rabbit in the 100 or 300 mg/kg bw/day dosage groups. These clinical signs included scant feces and no feces in the cage pan. No other clinical signs occurred.

Body Weights and Body Weight Changes

Body weights and body weight gains were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. Overall, body weights and body weight gains were comparable among the dosage groups for the cumulative dosage period (DSs 1 to 14). All groups, including the control group, lost body weight between DS 14 and DS 15 because of overnight fasting for urine collection.

Body weight gains in the 30, 100 and 300 mg//kg/day dosage groups were 108%, 119% and 115% of the control group value, respectively, for the cumulative dosage period (DSs 1 to 14). The average body weight on DS 14 (day of last dosage) was 101%, 99% and 100% of the control group value in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively.

Food Consumption

Absolute (g/day) and relative (g/kg/day) food consumption values were unaffected by dosages of 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. Food consumption values were also comparable among the dosage groups for the cumulative dosage period. Absolute food consumption values in the 30, 100 and 300 mg//kg/day dosage groups were 102%, 100% and 103% of the control group value, respectively, for the cumulative dosage period (DSs 1 to 14). Relative food consumption values were 101%, 102% and 103% of the control group value in the 3 respective 3-p-cumenyl-2-methylpropionaldehyde-treated groups during the same period.

Gross Pathology

There were no gross lesions observed at necropsy examination. All rabbits appeared normal.

Organ Weights

Terminal body weights were comparable among the four dosage groups. The weights of the epididymides, left cauda epididymis, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organ weights to terminal body weight were unaffected by dosages 3-p-cumenyl-2-methylpropionaldehyde as high as 300 mg/kg bw/day. In addition, there were no test substance-related changes in the non-reproductive organs (i.e., liver or paired kidneys) at any dosage level. There were no patterns, trends, or correlating data to suggest these values were toxicologically relevant. The apparent increase in the absolute and relative (% body weight) weights of the liver that occurred at 300 mg/kg bw/day (18% and 21% over controls, respectively) was largely attributed to one rabbit (no. 8596) in the 300 mg/kg bw/day dosage group with an absolute liver weight of 111.1 g (range: 70.1 to 92.3 for other rabbits in the group). Exclusion of this rabbit from summarization would still result in a higher group mean weight in comparison to the control

group value. The absolute and relative (% body weight) weights of the paired kidneys were also increased in the 300 mg/kg bw/day dosage group (11% and 13% over controls, respectively), again attributed to one rabbit (no. 8595) in this dosage group. Overall, the individual organ weight differences observed were considered incidental and unrelated to administration of 3-p-cumenyl-2-methylpropionaldehyde.

Sperm Evaluations

The values for the number of motile sperm and total sperm count from ejaculated semen samples were highly variable across the dosage groups, including the control group. Whilst a slight trend in the mean number of motile sperm (596.6, 543.3 and 431.0 in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively, vs. 627.0 in controls) and total sperm count (616.6, 595.5 and 496.2 in the 30, 100 and 300 mg/kg bw/day dosage groups, respectively, vs. 679.0 in controls) from ejaculated samples in the 3-p-cumenyl-2-methylpropionaldehyde-treated groups was observed, individual values were highly variable and the lowest reported individual values were within the range of the control group values. In general, all values across all treated groups were within the range of the concurrent control group values and/or the historical control range at the Testing Facility. The observed trend in sperm motility and total sperm count was not considered an adverse finding of 3-p-cumenyl-2-methylpropionaldehyde. Values for percent motile sperm, number of nonmotile sperm from the semen ejaculate sample and cauda epididymal sperm count and density were comparable among the four dosage groups. In addition, there were no patterns or trends in the morphology data to suggest any toxicological relevance.

Histopathology

No test substance-related microscopic changes were observed in the testes or epididymides examined.

Table 1: Summary of sperm motility, count and density (adapted from registration dossier).

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Dose material | Corn oil | 3-*p*-cumenyl-2-methylpropionaldehyde | 3-*p*-cumenyl-2-methylpropionaldehyde | 3-*p*-cumenyl-2-methylpropionaldehyde |
| Dose level (mg/kg/day)[a] | 0 | 30 | 100 | 300 |
| Rabbits tested N | 5 | 5 | 5 | 5 |
| Sperm motility | | | | |
| Number motile (mean±SD) | 627.0±425.6 | 596.6±449.6 | 543.3±352.2 | 431.0±351.5 |
| Motile precent (mean±SD) | 83.2±25.7 | 95.4±6.5 | 88.3±13.1 | 87.6±8.0 |
| Static count | | | | |
| Nonmotile (mean±SD) | 52.0±45.1 | 20.0±22.1 | 52.3±37.3 | 38.2±17.2 |
| Total count[b] (mean±SD) | 679.0±387.3 | 616.6±444.0 | 595.5±362.3 | 469.2±339.2 |
| Cauda epididymal sperm count | | | | |
| Sperm count[c] (mean±SD) | 150.6±77.7 | 112.4±58.4 | 114.6±81.0 | 150.0±79.4 |
| Sperm denisty[d] (mean±SD) | 434.56±165.83 | 353.00±124.28 | 262.64±177.50 | 389.90±189.66 |

[a]Excludes values for one rabbit, which 200 sperm were not located in twenty fields. [b]Sum of number of motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achived or 20 fields were evaluated. [c]Sperm count used in the calculation of sperm density. Twenty fields were evaluated. [d]The sperm density was calculated by dividing the sperm count by the volume in the image area multiplying by 2 and multiplying by $10^{-6}$ to obtain the sperm concentraton. The calculated sperm concentration value was multiplied by 50 (volume) and divided by the weight of the left cause epididymis.

Table 2: Summary of cauda epididymal sperm morphology (adapted from registration dossier).

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Dose material | Corn oil | 3-*p*-cumenyl-2-methylpropionaldehyde | 3-*p*-cumenyl-2-methylpropionaldehyde | 3-*p*-cumenyl-2-methylpropionaldehyde |

| Dose level (mg/kg/day)[a] | 0 | 30 | 100 | 300 |
|---|---|---|---|---|
| Rabbits examined N | 5 | 5 | 5 | 5 |
| Normal (mean±SD) | 141.6±17.2 | 121.4±30.5 | 102.2±25.6 | 128.2±31.3 |
| Percent abnormal (mean±SD) | 30.4±9.5 | 31.1±9.1 | 48.9±12.8 | 35.9±15.7 |
| Detached head (mean±SD) | 39.0±19.9 | 35.0±13.5 | 58.8±15.1 | 52.2±23.0 |
| No head (mean±SD) | 13.8±6.3 | 15.2±11.9 | 26.8±14.9 | 10.4±5.7 |
| Amorphous (mean±SD) | 1.0±1.0 | 0.2±0.4 | 0.8±1.8 | 0.0±0.0 |
| Macrohead (mean±SD) | 0.0±0.0 | 0.0±0.0 | 0.2±0.4 | 0.2±0.4 |
| Microhead (mean±SD) | 0.0±0.0 | 0.0±0.0 | 0.2±0.4 | 0.0±0.0 |
| Broken flagellum (mean±SD) | 4.3±2.3 | 2.2±1.1 | 2.4±1.7 | 3.8±4.8 |
| Coiled flagellum (mean±SD) | 3.6±2.1 | 5.0±5.8 | 8.2±4.1 | 4.8±3.0 |
| Bent flagellum (mean±SD) | 0.8±0.8 | 0.0±0.0 | 0.4±0.5 | 0.6±0.9 |

[a]dosage occurred on days 1 through 14 of study.

### 3.10.1.5 Study 5: 3-*p*-cumenyl-2-methylpropionaldehyde

**Reference**: Unnamed 2020b

Study period:02 Jul 2019 - 9 Nov 2019

Test Guideline: OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents)

Version / remarks:June 2018

GLP compliance:yes

**Test material**

Reference substance name:3-p-cumenyl-2-methylpropionaldehyde

EC Number:203-161-7

EC Name:3-p-cumenyl-2-methylpropionaldehyde

Cas Number:103-95-7

Molecular formula:C13H18O

IUPAC Name:2-methyl-3-[4-(propan-2-yl)phenyl]propanal

Test material form:liquid

**Test animals**

Species:rat

Strain:Wistar Han Rats

The rat was chosen as the animal model for this study as it is an accepted rodent species for preclinical toxicity testing by regulatory agencies.

The total number of animals used in this study was considered to be the minimum required to properly characterize the effects of the test item. This study has been designed such that it does not require an unnecessary number of animals to accomplish its objectives.

Sex:male/female

TEST ANIMALS:

Animals (rats) received on 03 Jul 2019, Crl: WI(Han) from Charles River Deutschland, Sulzfeld, Germany. The animals were 6-7 weeks old at initiation of dosing. Males weighed between 157 and 187 g and females weighed between 125 and 144 g.

- Housing:

On arrival and following randomization, animals were group housed (up to 5 animals of the same sex and same dosing group together) in polycarbonate cages (Makrolon type IV, height 18 cm or Makrolon type 2000P, height 21.5 cm) containing appropriate bedding (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. Animals were separated during designated procedures/activities. The room(s) in which the animals were kept was documented in the study records.

During locomotor activity monitoring, animals were housed individually in a Hi-temp polycarbonate cage (Ancare corp., USA; dimensions: 48.3 x 26.7 x 20.3 cm) without cageenrichment, bedding material, food and water.

ENVIRONMENTAL CONDITIONS:

Target temperatures of 18 to 24°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 20 to 21°C with an actual daily mean relative humidity of 46 to 72%. The values that were outside the targeted range occurred for 11 out of 100 days with a maximum of 72% and were without a noticeable effect on the clinical condition of the animals or on the outcome of the study.

12-hour light/12-hour dark cycle was maintained (except during designated procedures). Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

DETAILS OF FOOD AND WATER QUALITY:

Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) was provided ad libitum throughout the study, except during designated procedures. During motor activity measurements, animals did not have access to food for a maximum of 2 hours.

Municipal tap water was freely available to each animal via water bottles.

During motor activity measurements, animals had no access to water for a maximum of 2 hours.

**Administration / exposure**

Route of administration: oral: gavage

Details on route of administration:

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.

Vehicle:Corn oil, specific gravity 0.92

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 (test item was not pre-weighed in the formulation container). Test item dosing formulations were kept at room temperature until dosing. If practically possible, the dosing formulations and vehicle were continuously stirred until and during dosing. Adjustment was made for specific gravity of the vehicle and test item. No correction was made for the purity/composition of the test item.

Sample Collection and Analysis:

Dose formulation samples were collected for concentration analysis for all groups on Week 1 (days 1, 2 and 3), weeks 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13.

Dose formulation samples were collected for homogeneity analysis for groups 2 and 4 on Week 1 (days 1, 2 and 3), weeks 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13. 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.

All samples to be analyzed were transferred (at room temperature protected from light) to the analytical laboratory at the Test Facility.

Analytical Method: Analysis was performed using a validated analytical procedure (Test Facility Study No. 20185929).

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 ± 10% for solutions 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: Stability analyses performed previously in conjunction with the method development and validation study (Test Facility Study No. 20185929) demonstrated that the test item is stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data have been retained in the study records for Test Facility Study No. 20185929.

Batch: PE00245593

Expiry date: 18 Jul 2020

Purity >= 98%

Analytical verification of doses or concentrations:yes

Chemical analyses of formulations were conducted weekly to assess accuracy and homogeneity.

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 (test item was not pre-weighed in the formulation container).

Concentration Analysis, Homogeneity Analysis and Stabiility Analysis were performed.

Analytical Method: Analysis was performed using a validated analytical procedure (Test Facility Study No. 20185929).

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 ± 10% for solutions 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: Stability analyses performed previously in conjunction with the method development and validation study (Test Facility Study No. 20185929) demonstrated that the test item is stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data have been retained in the study records for Test Facility Study No. 20185929.

Duration of treatment / exposure:A minimum of 90 days.

Frequency of treatment:The test item and vehicle were administered to the appropriate animals by once daily oral gavage 7 days a week for a minimum of 90 days, up to and including the day before scheduled necropsy. The dose volume for each animal was based on the most recent body weight measurement. The doses were given using a plastic feeding tube. The first day of dosing was designated as Day 1.

Doses / concentrations: 0, 15, 30, 120 mg/kg bw/day (nominal)

No. of animals per sex per dose:10 animals per sex per dose.

Control animals:yes, concurrent vehicle

- Dose selection rationale:

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 of 15, 30 and 120 mg/kg/d were selected based on 28-day range finder oral gavage rat study to determine the potential toxicity of 3-p-cumenyl-2-methylpropionaldehyde to male. Administration of 3-p-cumenyl-2-methylpropionaldehyde by once daily oral gavage for 28 days was well tolerated in male rats at 30 mg/kg bw/day. Test item-related lower body weight and body weight gain were observed at 100 and 300 mg/kg bw/day, which was considered to be adverse at 300 mg/kg bw/day. The high-dose level should produce some toxic effects, but not excessive lethality that would prevent meaningful evaluation. The mid-dose level is expected to produce minimal to moderate toxic effects. The low-dose level should produce no observable indications of toxicity.

Positive control:There is no positive control.

**Examinations**

Observations and examinations performed and frequency:

Mortality/Moribundity Checks: Throughout the study, animals were observed for general health/mortality and moribundity twice daily.

Detailed Clinical Observations: The animals were removed from the cage, and a detailed clinical observation was performed weekly, starting on Day 1 (prior to dosing), and on the day of necropsy.

Cage Side Observations: once daily, 0 to 1h after dosing.

Body Weights: Animals were weighed individually weekly, starting on Day 1. A fasted weight was recorded on the day of necropsy.

Food Consumption: quantitatively measured weekly starting on Day 1.

Water Consumption: Subjective appraisal was maintained during the study, but no quantitative investigation introduced as no effect was suspected.

Ophthalmic Examinations: The eyes were examined using an ophthalmoscope after application of a mydriatic agent (Tropicol 5 mg/ml solution, THEA Pharma, Wetteren, Belgium) during Pretreatment in all animals, and at the end of the Dosing Period in Week 13 in all Group 1 and 4 animals.

Functional Tests: were performed on the first 5 animals per sex per group during Week 12-13. These tests were performed after completion of clinical observations.

 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 = hzabnormal/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.

Estrous Stage Determination: Estrous cycles were evaluated by examining the vaginal cytology of samples obtained by vaginal lavage for evaluation of extreme thyroid hormone effects in females

Haematology:Blood samples at a target volume of 0.5 mL were collected into tubes containing K3-EDTA as anticoagulant. Samples were analyzed for the parameters specified in the following table. A blood smear was

prepared from each hematology sample. Blood smears were labeled, stained, and stored. Blood smears were evaluated when required to confirm analyzer results.

Coagulation: Blood samples at a target volume of 0.45 mL were collected into tubes containing citrate as anticoagulant. Samples were processed for plasma, and plasma was analyzed for the parameters listed in the following table.

Blood samples at a target volume of 0.5 mL were collected into tubes containing Li-heparin as anticoagulant. Samples were processed for plasma.

Sacrifice and pathology:

All animals surviving until scheduled euthanasia were weighed, and euthanized using isoflurane, followed by exsanguination. Animals were fasted (overnight with a maximum of 24 hours) before their scheduled necropsy.

Necropsy: All animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

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 .

Organ Weights: The organs identified in the following table 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. In the event of gross abnormalities, in addition to the combined weight, the weight of the aberrant organ was taken and recorded in the raw data. Organ to body weight ratio (using the terminal body weight) were calculated. Organs Weighed at Necropsy: Brain,Cervix, Epididymisa, Gland, adrenal, Gland, pituitary, Gland, prostate,Gland, seminal vesiclea,Gland, thyroid, Heart, Kidneya, Liver, Ovary, Spleen, Testisa, Thymus, Uterus.

Tissue Collection and Preservation: Representative samples of the tissues identified in the following table 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.

Sperm Analysis: Sperm samples were taken from the proximal part of the vas deferens (right) at necropsy. Sperm motility and progressive motility were assessed from all samples. Sperm smears for morphological evaluation were fixed from all samples and stained with haematoxylin and eosin for evaluation (see Appendix 1 for the deviation). One epididymis (left side) was removed, placed in labeled bags, and kept in the freezer at ≤ - 15°C. After thawing the left epididymis was weighed, homogenized and evaluated for sperm numbers. Evaluation was performed for all samples. In the case of any abnormalities in the epididymis, the left side organ was fixed in modified Davidson's, and the right side organ was used for evaluation of sperm numbers. If abnormalities were found in epididymides (both sides), both these organs were fixed in modified Davidson's solution, and no evaluation of sperm numbers was performed.

Histology: Tissue Collection and Preservation: Artery, aorta, Body cavity, nasopharynx, Bone, femur, Bone marrow, Bone, sternum, Brain, Cervix, Epididymisa

Esophagus, Eyea, Gland, adrenal, Gland, clitoral, Gland, harderiana, Gland, lacrimal, Gland, mammary, Gland, parathyroid, Gland, pituitary, Gland, preputial,Gland, prostate, Gland, salivary,Gland, seminal vesicle, Gland, thyroid, Gross lesions/masses,Gut-associated lymphoid tissue, heart, Kidney, Large intestine, cecum Large intestine, colon, Large intestine, rectum, Larynx, Liver, Lung, Lymph node, mandibular, Lymph node, mesenteric, Muscle, skeletal, Nerve, optica, Nerve, sciatic, Ovary, Pancreas, Skin, Small intestine, duodenum, Small intestine, ileum, Small intestine, jejunum, Spinal cord, Spleen, Stomach,Testis,Thymus, Tongue, Trachea, Urinary bladder, Uterus, vagina, Vas deferensb (except animal identification, nasopharynx, bone marrow smears, clitoral gland, lacrimal gland, preputial gland, arynx, tongue and vas deferens) were embedded in paraffin (Klinipath, Duiven, The Netherlands), sectioned, mounted on glass slides, and stained with hematoxylin and eosin (Klinipath, Duiven, The Netherlands).

Histopathology: All tissues as defined above 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. A peer review on the histopathology data was performed by a second pathologist.

Statistics:Any data collected during the predose period are tabulated, summarized or statistically analyzed. All statistical analyses were performed within the respective study phase, unless otherwise noted. Numerical data collected on scheduled occasions were summarized in the main report and statistically analyzed as per the appropriate statistical method.

Variables analyzed:Body Weight, Body Weight Gains, Food Consumption, Hematology Variables, Coagulation Variables, Clinical Chemistry Variables, Sperm Variables, Organ Weights, Organ Weight relative to Body Weight

**Results and discussion**

No clinical signs were observed at 15 mg/kg bw/day.

Salivation seen after dosing in three females at 120 mg/kg bw/day between Days 17 and 55 and in a single male and female at 30 mg/kg bw/day was considered not toxicologically relevant, taking into account the nature and minor severity of the effect and its time of occurrence (i.e. after dosing). This sign was considered to be a physiological response related to taste of the test item rather than a sign of systemic toxicity.

Other clinical signs noted during the Dosing Period included fur loss, scabs and incidental erected fur, which occurred within the range of background findings to be expected for rats of this age and strain 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 with the test item.

Mortality:no mortality observed

No effects on body weight and body weight gain were noted in males and females up to 30 mg/kg bw/day.

Slightly lower body weight gain was seen in males and females at 120 mg/kg bw/day starting at respectively Days 29 and 36 (statistically significant ($p \leq 0.05$) in females). At the severity observed, these body weight effects were considered to be not adverse. Food consumption did not reveal any test item-related effects.

Food consumption and compound intake:no effects observed

Food consumption of treated animals was similar to the control level over the study period.

Food efficiency:not examined

Water consumption and compound intake:not examined

Ophthalmological findings:no effects observed

There were no ophthalmology findings that were related to treatment with the test item.

The nature and incidence of ophthalmology findings noted during the Pretreatment Period and in Week 13 was similar among the groups, and occurred within the range considered normal for rats of this age and strain. These findings were therefore considered to be unrelated to treatment with the test item.

Haematological findings:no effects observed

No test item-related effects on hematology parameters were observed.

A statistically significantly ($p \leq 0.01$) lower platelets count was observed in females of all test item groups (up to 0.76x of controls). In absence of a dose-related response and based on the low magnitude of change, this finding was considered to be not test item related.

Other values in treated males and females achieving a level of statistical significance, when compared to controls, were considered to have arisen as a result of slightly high or low control values, occurred in the absence of a dose-related distribution and/or were, given the magnitude of change, considered to be of no toxicological significance.

Coagulation:No test item-related effects on coagulation parameters were observed in females up to 120 mg/kg bw/day.

The statistically significant ($p \leq 0.01$) changes in prothrombin time (PT) and activated partial thromboplastin (APTT) in treated males were, in absence of a dose-related response and based on the low magnitude of change, considered to be not test item related.

Clinical biochemistry findings:No test item-related effects on clinical chemistry parameters were observed in males and females up to 30 mg/kg bw/day.

A statistically significantly lower total protein concentration was observed in males ($p \leq 0.05$) and females ($p \leq 0.01$) at 120 mg/kg bw/day (0.96x and 0.93x of controls respectively).

Furthermore, a statistically significantly ($p \leq 0.01$) lower total cholesterol and HDL cholesterol was observed in males at 120 mg/kg bw/day (0.72x and 0.69x of controls, respectively).

Other values in treated males and females achieving a level of statistical significance when compared to controls, were considered to have arisen as a result of slightly high or low control values, occurred in the absence of a dose-related distribution and/or were, given the magnitude of change, considered to be of no toxicological significance.

Urinalysis findings:not specified

Behaviour (functional findings):no effects observed

Hearing ability, pupillary reflex and static righting reflex were normal in all examined animals.

The hind grip strength was statistically significantly ($p \leq 0.01$) reduced in males at 120 mg/kg bw/day (0.69x of controls), but was still within the historical control range1.

Therefore, this finding was, in absence of any related clinical sign, considered to be not toxicologically relevant. Statistically significantly ($p \leq 0.01$) higher total movements and ambulations were seen in females at 15 mg/kg bw/day (1.58x and 1.64x of controls respectively). With values remaining within historical control range2 and in the absence of a dose-related response, this finding was considered to be not test item-related. No effects were seen in the other test item groups.

All groups showed a similar motor activity habituation profile with a decreasing trend in activity over the duration of the test period.

Immunological findings:not specified

Organ weight findings including organ / body weight ratios:No test item-related effects were seen in males at 15 mg/kg bw/day.

Test item-related organ weight differences were noted in the liver (males and females), kidney and heart (females), and the epididymis and seminal vesicle (males).

Higher liver weight was noted in males at 120 mg/kg bw/day, which was only statistically significant ($p \leq 0.01$) relative to body weight. Higher liver weight was also noted in females starting at 15 mg/kg bw/day and was statistically significant ($p \leq 0.01$) as absolute value and relative to body weight at all doses. Higher liver weights correlated with microscopic hepatocellular hypertrophy.

Higher kidney weight was noted in females only, starting at 30 mg/kg bw/day, and was statistically significant as absolute ($p \leq 0.05$) value and relative to body weight ($p \leq 0.01$) at 30 and 120 mg/kg bw/day. There was no microscopic correlate for this increased kidney weight.

In males, a statistically significantly ($p \leq 0.05$) higher kidney weight was noted at 120 mg/kg bw/day only relative to body weight and was interpreted to be secondary to the difference in body weight.

A statistically significantly higher heart weight was noted in females only, starting at 30 mg/kg bw/day ($p \leq 0.01$ at 30 mg/kg bw/day and $p \leq 0.05$ at 120 mg/kg bw/day) when expressed relative to body weight. The differences in relative heart weight were slightly larger than proportional to the differences in body weight. The relevance of this finding is unclear, since was not followed by any microscopic changes.

A statistically significantly lower seminal vesicle gland weight was noted in males at 120 mg/kg bw/day (absolute value $p \leq 0.01$ and relative to body weight $p \leq 0.05$) and 30 mg/kg bw/day (relative to body weight only, $p \leq 0.05$). There was no microscopic correlate. A statistically significantly ($p \leq 0.01$) lower epididymis weight was noted in males at 120 mg/kg bw/day (absolute value only), which was without a microscopic correlate. There was no difference in the absolute testis weight. A statistically significantly ($p \leq 0.01$) higher weight of the testis relative to body weight was noted in males at 120 mg/kg bw/day, which was secondary to the lower terminal body weights in that group.

There were no other test item-related organ weight changes. Any differences that reached statistical significance were either a reflection of the differences in terminal body weight, or were without a dose-relationship.

Gross pathological findings:No test item-related macroscopic findings were noted in males up to 30 mg/kg bw/day and females up to 120 mg/kg bw/day.

A nodule was noted on the tail of the epididymis (unilateral, left) of one male treated at 120 mg/kg bw/day (No. 35). This correlated microscopically to a sperm granuloma.

The few remaining recorded macroscopic findings were within the range of background gross observations encountered in rats of this age and strain.

Sperm Analysis: No effects on sperm motility, concentration and morphology were observed in males up 30 mg/kg bw/day. At 120 mg/kg bw/day, statistically significantly ($p \leq 0.01$) lower percentage of motile sperm (0.45x of control), progressive sperm (0.24x of control) and number of cells with a normal morphology (0.27x of control) was recorded. In addition, a statistically significant ($p \leq 0.01$) increase in number of cells with a detached head (34.3x of control) and abnormal neck (23.0x of control) were observed. The sperm count in the epididymides was not statistically significantly reduced. A statistically significant ($p \leq 0.01$) decrease in number of cells with a coiled tail was observed at 120 mg/kg bw/day (0.15x of control).

Neuropathological findings:no effects observed

Histopathological findings: non-neoplastic:Test item-related microscopic findings were noted in the liver, epididymis, and urinary bladder and are described below.

Hepatocellular liver hypertrophy up to mild degree was noted in males starting at 30 mg/kg bw/day, and in females starting at 15 mg/kg bw/day, up to mild degree, with a dose-related increase in incidence and severity. The hypertrophy was located predominantly in the centrilobular area, and occasionally extending to panlobular distribution (generally associated with mild severity grade). This finding correlated to higher liver weights.

Pigment in the liver was noted in females starting at 15 mg/kg bw/day, concurrent with hypertrophy, at minimal degree. Microscopically, the pigment was brown-green, located in the centrilobular region, and located in the sinusoidal lining cells. Single cell necrosis of hepatocytes was noted in one male at 120 mg/kg bw/day and one female at 30 mg/kg bw/day.

Vacuolation of the urothelium in the urinary bladder was noted in males and females at 120 mg/kg bw/day only, up to mild degree. This was characterized by multiple small, variably sized, well-delineated, round, clear vacuoles in the cytoplasm of umbrella cells.

Sperm granulomas in the epididymis (examined unilaterally) were noted in 3/10 males at 120 mg/kg bw/day only, at mild degree. In one male (Animal No. 35) this correlated to a nodule macroscopically.

Vacuolation of the pineal gland (when present in section) was noted in test item-treated males at a higher incidence and severity than that observed in the control males, however potential relationship of this finding to the test item is uncertain. The vacuolation was observed in 6 out of 8 males at 120 mg/kg bw/day (up to mild degree), 3 out of 8 males (up to moderate degree) and 2 out of 9 females (minimal) at 30 mg/kg bw/day, 5 out of 7 males at 150 mg/kg bw/day (up to mild degree) and 1 out of 4 control males (minimal). Due to the infrequent evaluation of this gland in routine toxicology studies, thus lack of historical control data and the difference in number of available glands for evaluation between groups, the assessment of this finding in relationship to the test item is difficult. Microscopically, pineal gland vacuolation was characterized by

clusters of pinealocytes, with small to medium-sized, well-delineated, clear cytoplasmic vacuoles which did not cause notable increase in the size of the cells.

Remaining histologic changes were considered to be incidental findings or 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.

Histopathological findings: neoplastic:no effects observed

Thyroid Hormones:No test item-related effects on thyroid hormones were observed in males and females up to 30 mg/kg bw/day.

A decrease in triiodothyronine (T3) and thyroxine (T4) levels was seen in males at 120 mg/kg bw/day (0.69x and 0.45x of controls respectively), reaching statistical significance ($p \leq 0.01$) for T4. T3 and T4 levels in females at 120 mg/kg bw/day were not statistically different from controls.

TSH remained within the historical control data range at these dose level.

Table 1: Mean percent organ weight differences from control groups (adapted from registration dossier).

| | **Males** | | | **Females** | | |
|---|---|---|---|---|---|---|
| **Dose level (mg/kg/day)** | 15 | 30 | 120 | 15 | 30 | 120 |
| **Body weight** | -3.5 | -0.8 | -9.3* | 0.4 | -2.7 | -7.1** |
| **Liver** | | | | | | |
| **absolute** | 1.9 | 1.8 | 10.4 | 22.4** | 23.1** | 33.4** |
| **relative** | 5.5 | 2.4 | 21.4** | 22.0** | 26.5** | 43.8** |
| **Kidney** | | | | | | |
| **absolute** | 0.5 | 0.4 | -1.6 | 6.0 | 7.5* | 8.2* |
| **relative** | 4.0 | 1.1 | 8.4* | 5.7 | 10.5** | 16.4** |
| **Heart** | | | | | | |
| **absolute** | -3.9 | -1.3 | -6.0 | 5.1 | 9.7 | 4.0 |
| **relative** | -0.3 | -0.4 | 3.6 | 4.9 | 12.8** | 12.1* |
| **Epididymis** | | | | | | |
| **absolute** | -4.9 | -6.7 | -12.5** | | | |
| **relative** | -1.4 | -5.7 | -3.6 | | | |
| **Seminal vesicle** | | | | | | |
| **absolute** | -14.5 | -19.6 | -27.7** | | | |
| **relative** | -11.0 | -18.6* | -20.1* | | | |

*p<0.05, **p<0.01.

Table 2: Summary test item-related microscopic findings – scheduled euthanasia animals (day 91-92) (adapted from registration dossier).

| | **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|---|
| **Dose level** | 0 | 15 | 30 | 120 | 0 | 15 | 30 | 120 |

| **(mg/kg/day)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Liver**[a] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hypertrophy, hepatocellular minimal | - | - | 3 | 3 | - | 5 | 8 | 6 |
| Mild | - | - | - | 5 | - | - | - | 4 |
| Pigment minimal | - | - | - | - | - | 1 | 3 | 4 |
| Single cell necrosis minimal | - | - | - | 1 | - | - | 1 | - |
| **Urinary bladder**[a] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Vacuolation, urothelial minimal | - | - | - | 4 | - | - | - | 7 |
| mild | - | - | - | 1 | - | - | - | 1 |
| **Epididymis**[b] | 10 | 10 | 10 | 10 | | | | |
| Sperm granuloma mild | - | - | - | 3 | | | | |

[a]Number of tissues examined from each group; [b]=unilateral examination.

### 3.10.1.6 Study 6: 3-(*p*-cumenyl)propionaldehyde

Unnamed A
Oral (gavage) 14 -day toxicity study in rat.
In an oral (gavage) study in male Crl:CD(SD) rats, the animals were dosed once daily at 25, 75 and 250 mg/kg bw for 14 consecutive days according to GLP principles. 10 animals were included in each dose group and a vehicle control group (corn oil) was included.
Rats were observed for viability at least twice each day throughout the study and were examined daily (and the first 4 hours after dosing) for clinical observations during the dosing period. Body weights were recorded daily during the dosage period and prior to sacrifice. Rats were fasted following the final dosage on day 14 to facilitate urine sample collection overnight, beginning on the evening of day 14. Fecal samples were collected following dosage administration on day 14. Surviving rats were sacrificed by carbon dioxide asphyxiation following the last administration (day 15).
All rats were subjected to a complete necropsy examination. For the surviving animals, weights of the epididymides, epididymis left cauda, kidneys, liver, prostate, seminal vesicles and testes were recorded. In addition, microscopic evaluation of the adrenal glands, gross lesions, kidney, liver, prostate, seminal vesicles and testes was performed. In addition, sperm concentration, motility and morphology were assessed in each male.
**Results**
Systemic effects: All rats survived until scheduled sacrifice and no treatment related clinical observations were recorded. At 250 mg/kg bw significant decreased body weight was observed in the first week of treatment, which was corresponding to the reduced food intake in this period. There were no biologically important differences between the vehicle and treated groups in the urinalysis and there were no substance-related necropsy observations. In the 250 mg/kg bw terminal body weights were significantly reduced in the

high dose group. In the 250 and 75 mg/kg bw statistically significant increase in absolute and relative weights of the liver were seen, without related microscopic changes.
Reproductive Organ effects: At 250 mg/kg bw the weights of the epididymides, caudal epididymis, testes and paired kidneys and the ratios of these organ weights to terminal body weight were unaffected. The weights of the seminal vesicle and prostate were reduced in this group. At this dose microscopic examination showed microscopic lesions in testes, epididymides and seminal vesicles including atrophy in the latter. At 250 mg/kg bw/day, values for the number and percent motile sperm, the number of non-motile sperm and the total sperm count from the vas deferens were unable to be determined.
Reproductive-Sperm effects: At 250 and 75 mg/kg bw/day, the average sperm count and sperm density from the cauda epididymis was reduced or significantly reduced. In addition, the percentage of abnormal sperm, specifically sperm with detached heads or no heads, was significantly increased in the 75 and 250 mg/kg bw/day dosage groups. In these groups, also a slight increase in the number of sperm that had a broken flagellum.

### 3.10.1.7 Study 7: 3-(*p*-cumenyl)propionaldehyde

Study report B.

Oral (gavage) 14 -day toxicity study in rabbit

In a non-GLP range finder study the substance was administered once daily via a stomach tube for 14 consecutive days to male New Zealand White (Hra:(NZW)SPF) rabbits. Initially the study was performed at dose levels of 10, 30 and 100 mg/kg bw/day, however since no toxicity was observed, a dose of 300 mg/kg bw/day was assessed in a study extension as well. 5 animals were included in each dose group and concurrent vehicle control groups (corn oil) were included. The rabbits were assessed for viability at least twice daily during the study. The animals were observed for clinical signs before each dose was administered and on the day of scheduled euthanasia. For the first 4 days of dosing in the initial study and the first 2 days in the extension study, post dose observations were conducted at hourly intervals. Body weight and food consumption were checked daily during the dose period, and on the day of scheduled euthanasia (body weight) or prior to placement in the metabolism cages (food consumption). Urine samples (as much as possible) were collected overnight from all rabbits. On day 14 semen samples were collected from each rabbit prior to initiation of dose administration and prior to euthanasia. Sperm motility, count and morphology were evaluated. On day 15, the animals were euthanized by an intravenous injection of a phenobarbital and phenytoin solution. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Microscopic evaluation of the Epididymis and Testis were performed and organ weights of the Epididymis, prostate gland, seminal vesicle gland, kidney, liver and testis were recorded.

**Results**

Systemic effects: No signs of toxicity were observed in the initial study (up to 100 mg/kg bw/day) in any of the assessments. In the extended study (300 mg/kg bw/day) all animals survived until scheduled euthanasia on Day 15 of study. No treatment related clinical observations were recorded and no gross lesions were revealed. Body weights and body weight gains were unaffected by the 300 mg/kg bw/day dosage. Absolute and relative food consumption values were unaffected and terminal body weights and organ weights were comparable to the control group. Relative liver and kidney weights were slightly increased at 300 mg/kg bw, 13 and 9%, respectively without reaching statistical significance.

Reproductive effects: Test article-related microscopic findings were noted in the testes and epididymis of rabbits given 300 mg/kg bw/day. All five rabbits in this group had minimal or mild increases in residual bodies in the testes. Three of these rabbits also had mild or minimal depletion of spermatozoa in the epididymides and of these three rabbits, two also had detachment of the seminiferous tubules of the testes. The detachment in the seminiferous tubules was noted when large clear areas were present within the seminiferous epithelium separating the germ cells. The minimal to mild nature of these changes wouldn't likely have biologic significance. Values for number and percent motile sperm, number of non-motile sperm

and total sperm count were comparable to the control group. The values for sperm count and concentration from ejaculated semen samples were highly variable across the dose groups. The percentage of abnormal sperm on DS 15 was highest in the 300 mg/kg bw/day dose group (38.0%), as compared to the concurrent control value as well as the values in the initial study (ranged from 15.4% to 29.2%). The abnormal sperm consisted primarily of sperm with detached heads. The increase for the group average could be attributed to two rabbits, which had 49.5% and 60.0% abnormal sperm, respectively. The significance of this effect is somewhat inconclusive because the percentage of abnormal sperm before dose administration was also highest in the 300 mg/kg bw/day dose group (23.7%), as compared to the concurrent control (17.8%), and as compared to the pre-dose values for the rabbits in the other groups (ranged from 12.9% to 17.7%).

#### 3.10.1.8 Study 8: *p*-cymene

Reference: Unnamed 2019

Study guideline:OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test)

Study period:2018-05-16 to 2019-03-20

Deviations:yes

Remarks:Deviations were not considered to have compromised the validity or integrity of the study

GLP compliance:yes

Limit test:no

**Test material**

Reference substance name:p-cymene

EC Number:202-796-7

Cas Number:99-87-6

**Test animals**

Species:rat

Strain:Sprague-Dawley

Remarks:Crl:CD®(SD)

OECD Test Guideline 422 was designed for use with the rat. Dosing studies in a rodent species are required by chemical regulatory agencies such as ECHA and US EPA. In addition, a historical control data base is available for comparative evaluation.

Sex:male/female

Details on test animals or test system and environmental conditions:

- Source: Charles River Laboratories (Raleigh, North Carolina 27610)

- Females nulliparous and non-pregnant: Not Specified

- Age at study initiation: Approximately 11 to 13 weeks

- Weight at study initiation: Males: 332 - 434 grams; Females: 235 - 299 grams

- Fasting period before study: Not specified

- Housing: Polycarbonate cages with a stainless steel mesh lid. From arrival until one day prior to treatment, animals were pair or group housed (2 or 3 rats of the same sex per cage, respectively) in solid bottom cages with cellulose-based contact bedding. From the initiation of treatment (pre-cohabitation phase until termination of the study), the P0 males and females were pairhoused [same sex and treatment group per cage (except during the cohabitation phase)] in suspended, solid bottom cages with cellulose-based contact bedding. Per the OECD 422 guideline, each P0 female wasindividually housed during presumed gestation

and housed with her litter after delivery. During cohabitation, the male and female rats were co-housed (1:1) within each treatment group in suspended, solid bottom cages with cellulose-based contact bedding.

- Diet (e.g. ad libitum): Teklad Global 18% Protein Rodent Diet (Certified), 2018C (Envigo, Madison, Wisconsin) ad libitum. Fresh feed was presented weekly in the home cage of each animal.

- Water (e.g. ad libitum): Potable water from the public supply (New Jersey-American Water Company, Cherry Hill, New Jersey) via an automated watering system. ad libitum.

- Acclimation period: 19 days (The pre-test period was 19 days. All animals were examined during the pre-test period to confirm suitability for study. Pre-test procedures other than routine husbandry care and identification procedures were not performed until animals had been allowed to stabilize for at least 5 days. Prior to assignment to study, all animals were examined to ascertain suitability for study).

DETAILS OF FOOD AND WATER QUALITY:

Teklad Global 18% Protein Rodent Diet (Certified), 2018C (Envigo, Madison, Wisconsin) was provided without restriction. Fresh feed was presented weekly in the home cage of each animal. Analysis of each feed lot used during this study was performed by the manufacturer. There were no known contaminants in the feed that were expected to interfere with the results of this study. Potable water from the public supply (New Jersey-American Water Company, Cherry Hill, New Jersey) via an automated watering system was provided without restriction. Water analyses are conducted by New Jersey-American Water Company, Cherry Hill, New Jersey (Raritan-Millstone Plant) to ensure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). In addition, water samples are collected biannually from representative rooms in the Testing Facility; chemical and microbiological water analyses are conducted on these samples by a subcontract laboratory. There were no known contaminants in the water which were expected to interfere with the results of this study.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20 to 26°C

- Humidity (%): 30 to 70%

- Air changes (per hr): Not specified

- Photoperiod (hrs dark / hrs light): A twelve hour light/dark cycle was provided and controlled via an automatic timer

IN-LIFE DATES: From: 2018-05-17 & 2018-05-24 To: 2018-08-04

**Administration / exposure**

Route of administration:oral: gavage

Vehicle:corn oil

p-cymene was prepared for administration by mixing appropriate amounts of the test item with the vehicle to achieve the desired concentrations (details provided in Table 1). The test item was used as supplied when calculating quantities to be used during dose preparation. Fresh formulations were prepared once weekly and stored at room temperat ure (20± 5 °C).

- Justification for use and choice of vehicle (if other than water): Corn oil (Justification not specified)

- Concentration in vehicle: 0, 10, 20, & 40 mg/L for control, low-, mid-, and high dose levels, respectively.

- Amount of vehicle (if gavage): 5 mL/Kg

- Lot/batch no. (if required): Spectrum Chemical Mfg. Corp (755 Jersey Avenue, New Brunswick, New Jersey 08901); Lot# 2HB0068

- Purity: Assume 100%

Details on mating procedure:

- M/F ratio per cage: 1:1

- Length of cohabitation: up to 2 weeks

- Proof of pregnancy: vaginal plug or sperm in the vaginal smear referred to as day 0 of pregnancy

- After 14 days of unsuccessful pairing replacement of first male by another male with proven fertility.

- Further matings after two unsuccessful attempts: No

In the absence of positive signs of mating, the morning of the last day of cohabitation was considered "presumed GD0" for that female. If the presumed pregnant dam did not appear visibly/palpably pregnant, she was euthanized on presumed GD 25. If a female showed no positive evidence of mating, but appearedvisibly/palpably pregnant, it was allowed to deliver, and the estimated GD 0 calculated.

- After successful mating each pregnant female was caged (how): Once mated, the female rat was removed from the mating cage and housed individually until delivery

Analytical verification of doses or concentrations:yes

Details on analytical verification of doses or concentrations:

Homogeneity:Prior to initiation of dosing, homogeneity of dose formulations was demonstrated by taking two samples each from the top, middle and bottom portion (2.5 mL/sample) of the low and high concentration formulations prepared for use on the study.

Stability: Stability of the low- and high-concentration dose formulations under the storage conditions used in this study was determined at time points of 4 and 8 days from preparation under the method validation Study No. TN40YX.

Dose Concentration:Samples collected from the middle of the dose formulations for homogeneity analysis were used for the dose confirmation results of the low and high concentrations. Two samples (2.5 mL each) were taken from the middle region of each formulation (including control) on the day of the first and last dose preparation. One sample was analyzed, in duplicate, for dose confirmation analysis and one sample was retained at room temperature (20 ± 5°C). Retained samples were discarded after valid analytical results are obtained.

Method of Analysis: Analyses were performed by the Department of Formulation and Inhalation Analysis at the Testing Facility.

Duration of treatment / exposure:P0 males: 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days).

P0 females: 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).

Frequency of treatment:Once Daily

Doses: 0, 50, 100, 200 mg/kg bw/day (nominal)

No. of animals per sex per dose:10 animals/sex/group

Control animals:yes, concurrent vehicle

The doses selected for the study were based on a 14-day repeat dose oral (gavage) range-finding toxicity study. One 500 mg/kg bw/day female was sacrificed as moribund onStudy Day 13. This animal was thin and exhibited rapid breathing, decreased activity and hunched posture. There were no macroscopic findings indicative of a gavage accident. There were no adverse clinical signs at any dose level for both genders except for the moribund 500 mg/kg bw/day female animal. Body weights, body weight changes and food consumption were significantly reduced in the 500 mg/kg bw/day group. Body weights, body weight changes and food consumption were also reduce d in the 150 mg/kg bw/day group. Necropsy and gross pathology revealed one 150 mg/kg bw/day male with discoloured lungs and bronchi (dark red area on the right caudal lobe [

The high dose (200 mg/kg bw/day) used in this OECD TG 422 study is 40% of the high dose level in the 14-day oral gavage range-finding toxicity study. It was anticipated that the 200 mg/kg bw/day dose level would not cause mortality but might cause some adverse toxicity. The low dose (50mg/kg bw/day) is 25% of the

high dose in the 14-day oral gavage range-finding toxicity study and was anticipated to cause little or no toxicity. The mid-dose (100 mg/kg bw/day) is 50% of the high dose.

Males considered suitable for study on the basis of pre-test physical examinations, body weight data and any other pre-test evaluations, were randomly assigned to control, treatment or spare groups, using a computerized program, in an attempt to equalize mean group body weights. Individual weights of male animals placed on test were within ± 20% of the mean weight. Females considered suitable for study on the basis of pre-test physical examinations, body weight data, regular estrous cycles and any other pre-testevaluations, were randomly assigned to control, treatment or spare groups, using a computerized program, in an attempt to equalize mean group body weights. Normally cycling P0 females (demonstrating 4 to 5 days cyclicity) were selected for each dose group. Individual weights of female animals placed on test were within ± 20% of the mean weight.

- Fasting period before blood sampling for clinical biochemistry: Yes

**Examinations**

Parental animals:

- Time schedule: Animals were observed in their cages at least twice daily for mortality and general condition

- Time schedule: P0 male rats were removed from their cages and observed once weekly (prior to dosing) from the initiation of treatment until termination. P0 female rats were removed from their cages and observed once weekly (prior to dosing) from the initiation of treatment through mating. Once mating was confirmed, animals were observed on GD 0, 7, 14 and 20 and female rats that delivered a litter were observed on LD 1, 4, 7 and 14. Maternal behavior was observed daily from GD 18 to LD 13. Female rats without evidence of mating from the male pairing were moved into the gestation phase at the end of the cohabitation phase and upon delivery were moved into the lactation phase and examined similarly as other females with evidence of mating. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluation of respiration. During the treatment period, all animals were observed for signs of toxic effects once daily within 2 hours (± 30 minutes) after test material administration

BODY WEIGHT:

- Time schedule for examinations:

P0 male rats: recorded weekly (prior to dosing), from the initiation of treatment and weekly thereafter throughout the study and at termination.

P0 female rats: recorded weekly (prior to dosing), from the initiation of treatment and weekly during the pre-cohabitation and cohabitation phases until mated. Mated female rats were weighed on GD0, 7, 14 and 20 and female rats that delivered litters were weighed on LD 1, 4, 7 and 13 and at termination on Day 14. Female rats without evidence of mating from the male pairing were moved into the gestation phase and upon delivery were moved into the lactation phase and weighed similarly as other females with evidence of mating.

FOOD CONSUMPTION AND COMPOUND INTAKE:

Food consumption for the male and female rats was measured (weighed) weekly prior to initiation of treatment (pre-test) and during the pre-cohabitation phase. Food consumption was not measured during the cohabitation phase when male rats were being co-housed with female rats. For male rats, food consumption was measured weekly during the post-cohabitation phase. For female rats, gestation and lactation food consumption was measured on GDs 0-7, 7-14 and 14-20 and on LDs 1-7 and 7-14, respectively.Food consumption for the female rats without evidence of mating was measured similarly as other females with evidence of mating.

HAEMATOLOGY:

- Time schedule for collection of blood: Blood was obtained from anesthetized P0 rats as a terminal procedure via puncture of the vena cava. P0 males were terminated on Day 35 (approximately) while P0 females were terminated on Day 63 (approximately).

- Anaesthetic used for blood collection: Yes (isoflurane)

- Animals fasted: Yes (Animals were fasted overnight prior to collection)

- How many animals: Hematology samples were collected from the first 5 surviving animals/sex/group. Coagulation samples were collected from upto the second

5 surviving animals/sex/group.

CLINICAL CHEMISTRY:

- Time schedule for collection of blood: Blood was obtained from anesthetized P0 rats as a terminal procedure via puncture of the vena cava. P0 males were terminated on Day 35 (approximately) while P0 females were terminated on Day 63 (approximately).

- Animals fasted: Yes (Animals were fasted overnight prior to collection)

- How many animals: Clinical chemistry samples were collected from the first 5 surviving animals/sex/group.

NEUROBEHAVIOURAL EXAMINATION:

- Time schedule for examinations: Sensory reactivity, grip strength and locomotor activity were performed prior to dosing during Week 5 for the first five P0 males in each group and on LD 8 ± 1 for the first five P0 females.

- Dose groups that were examined: All dose groups

- Battery of functions tested: sensory activity, grip strength, motor activity

OTHER:

Thyroid Hormone Analysis: P0 males and females at termination (Up to 10 animals/sex/group). Blood for thyroid hormone analysiswas collected into polypropylene serum gel tubes with no anticoagulant and allowed to clot for at least 30 minutes at room temperature. Serum was harvested within 1 hour after collection of each blood sample. Serum was separated by centrifugation (for 10 minutes at approximately 2000 g, at approximately 2 to 8°C). Approximately 255 µL or more of serum was harvested and stored frozen (-80 ° ± 10 °C) at the Testing Facility until shipped for analysis. Serum samples from P0 males were assessed for serum levels of Thyroxine (T4) and Thyroid Stimulating Hormone (TSH) by AntechGLP. Serum samples (T4) from P0 females were maintained frozen (-80ºC ± 10 ºC) at the Testing Facility pending sample analysis or final disposition of samples.

Oestrous cyclicity (parental animals):

Vaginal smears were obtained daily from each P0 female pre-test(to determine suitability for study)and during the pre-cohabitation and cohabitation phases until mating was confirmed and the stage of estrous was determined.

Sperm parameters (parental animals):

During the microscopic examination of the testes, special emphasis was placed on the stage of spermatogenesis and the interstitial testicular cell structure. Any cell- or stage-specificity of testicular findings was noted.

Litter observations:

STANDARDISATION OF LITTERS

- Performed on day 4 postpartum: Not specified

PARAMETERS EXAMINED

The following parameters were examined in [F1] offspring:

Litters were observed as soon as possible after completion of parturition for the number of live and dead pups, runts and pup abnormalities and the sex of each pup. Thereafter, litters were observed twice daily (morning and afternoon); all pups in the litter were uniquely identified by tattoo after parturition was completed. Because the litters were not culled, the full number of pups remained in each litter. The presence of dead pups was recorded and these were removed from the litter as found and necropsied. Unusual observations and the absence of milk in the stomach were noted.

Each pup was given a physical examination daily from PND 1 through PND 13. Observations of dead or missing pups were noted. Individual pup body weights were recorded on PND 1, 4, 7, 11 and 13. The sex of each pup was identified on PND 1 and verified on PND 4, 7 and 13.

Anogenital distance was measured in millimeters using a caliper for all pups on PND 4. Nipple retention was assessed for all F1male pups on PND 13.

GROSS EXAMINATION OF DEAD PUPS:

The presence of dead pups was recorded and these were removed from the litter as found and necropsied.

Thyroid Hormone Analysis – F1 Pups

F1 pups at PND 4 - Up to 2 females per litter(when possible) pooled sample by litter

Pooled blood (~0.6 mL) was obtained from two (when possible) non-fasted but anesthetized (intraperitoneal injection of sodium pentobarbital) female PND 4 F1pups per litter via the greater vessels as a terminal procedure immediately after the appropriate level of anesthesia was reached.

F1pups at PND 13 - Up to 2 males and 2 females(when possible) per litter

Blood (~0.6 mL) was obtained from non-fasted but anesthetized (intraperitoneal injection of sodium pentobarbital) PND 13 F1pups via the greater vessels as a terminal procedure immediately after the appropriate level of anesthesia was reached.

Blood for thyroid hormone analysiswas collected into polypropylene serum gel tubes with no anticoagulant and allowed to clot for at least 30 minutes at room temperature. Serum was harvestedwithin 1 hour after collection of each blood sample. Serum was separated by centrifugation (for 10 minutes at approximately 2000 g, at approximately 2 to 8°C). Approximately 255 µL or more of serum was harvested and stored frozen (-80 ° ± 10 °C) at the Testing Facility until shipped for analysis.

Serum samples for T4 and TSH analysis from PND 13 F1pups were shipped frozen, on dry ice to AntechGLP for analysis. Serum samples (T4) from PND 4

F1 pups were maintained frozen (-80ºC ± 10 ºC) at the Testing Facility pending sample analysis or final disposition of samples.

Postmortem examinations (parental animals):

SACRIFICE

Adult males and females were euthanized by exsanguination following isoflurane inhalation. Necropsy was performed on 10 P0 males/group after males had been treated for 5 weeks. Necropsy schedules were established to ensure that approximately equal numbers of males from each group were examined at similar times of the day. Necropsy was performed on up to 10 P0 females in Groups 1, 2 and 3 on LD 14. Female rats that failed to deliver a litter were euthanized on GD 25.

GROSS PATHOLOGY

Macroscopic postmortem examinations were performed on all P0 rats. Postmortem examinations included an external examination as well as a detailed internal examination. Special attention was paid to the organs of the reproductive system. The number of implantation sites, scars and corpora lutea was recorded for each female rat. For apparently non-pregnant animals, and for apparently empty horns, the number of uterine implantation sites were checked after staining with ammonium sulfide.

Organs were weighed from the first and second set of five surviving adult males and lactating females that delivered a litter in each group. Organs were not weighed for any female that failed to deliver a litter. Prior to

weighing, the organs were carefully dissected and properly trimmed to remove adipose and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection in order to avoid drying. Paired reproductive organs were weighed separately; all other paired organs were weighed together.

HISTOPATHOLOGY:

Tissues were preserved for the first set of 5 P0 animals that survived until termination of dosing (Week 5 for males; LD 14 for females) as well as all females that failed to deliver a litter (GD 25). Slides of the indicated tissues were prepared and examined microscopically for all animals.

Tissues were preserved for all remaining P0 animals that survived until termination of dosing (Week 5 for males; LD 14 for females). Slides of the indicated tissues were prepared and examined microscopically for all animals.

During the microscopic examination of the testes, special emphasis was placed on the stage of spermatogenesis and the interstitial testicular cell structure. Any cell- or stage-specificity of testicular findings was noted. During the microscopic examination of the ovary, qualitative depletion of the primordial follicle population was noted when present.

Eyes and testes were placed in Modified Davidson's solution initially and then retained in 10% neutral buffered formalin (NBF). Lungs were infused with 10% NBF prior to their immersion into a larger volume of the same fixative. All other tissues were preserved in 10% NBF. After fixation, the tissues and organs from all animals were routinely processed, embedded in paraffin, sectioned at approximately 5microns, mounted on glass slides, stained with hematoxylin and eosin. All animals in Groups 1 and 4 were examined by light microscopy including animals in the intermediate dose groups where target organs were identified, animals that died prior to scheduled necropsy and females that failed to litter (GD 25).

Organ Weights:

The first and second set of five surviving adult males and lactating females that delivered a litter in each group had organs weighed. Organs were not weighed for any female that failed to deliver a litter. Prior to weighing, the organs were carefully dissected and properly trimmed to remove adipose and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection in order to avoid drying. Paired reproductive organs were weighed separately; all other paired organs were weighed together.

Postmortem examinations (offspring):

Pups were euthanized using an intraperitoneal injection of sodium pentobarbital. Macroscopic post-mortem examinations (external only) were performed on all surviving F1 pups on PND 13. Particular attention was paid to the external reproductive genitals which were examined for signs of altered development. Unusual observations, including gross abnormalities were noted and then the carcasses were discarded.

F1pups that died during the study were given a macroscopic postmortem examination. Particular attention was paid to the external reproductive genitals which were examined for signs of altered development. Unusual observations and the absence of milk in the stomach (up to PND 2), including gross abnormalities were noted. All carcasses were discarded following examination.

F1 pups selected for thyroid hormone blood collections were euthanized on PND 4 and discarded without further examination. The thyroid gland from one randomly selected male and female F1pup per litter was removed, fixed, weighed and stored in 10% neutral buffered formalin and processed for histopathology.

Reproductive indices:

1) Female Mating Index (%):

(Number of females with confirmed mating (sperm and/or vaginal plug) + Number of pregnant females without evidence of mating (no sperm or vaginal plug) / (Number of females placed with males)) x 100

2) Female Fertility Index (%): ((Number pregnant) / (Number copulated)) x 100

3) Female Gestation Index (%): ((Number of females with live-born) / (Number of females with confirmed pregnancy)) x 100

4) Male Mating Index (%):

((Number of males with confirmed mating with a female or pregnancy for females without evidence of mating) / (Number of males placed with females)) x 100

5) Male Fertility Index (%):

((Number of males mating and impregnating a female + Number of males with a pregnant female without evidence of mating) / (Number of males with confirmed mating + number of males with a pregnant female without evidence of mating)) x 100.

Offspring viability indices:

1) Sex Ratio (% males): ((Number of male pups) / (number of total pups)) x 100

2) Viability Index (%): ((Number of pups alive on the specified day) / (Total number of live pups on Day 1)) x 100

3) Live Birth Index (%): ((Total number of live pups on Day 1) / (total number of pups born)) x 100

4) Post Implantation Survival (%): ((Number of live pups delivered) / (number of implantations)) x 100

5) Pre-Implantation Loss (%): ((Number of corpora lutea – Number of implantation sites) / (Number of corpora lutea)) x 100

6) Post-Implantation Loss (%): ((Number of implantation sites – Total number of live pups on Day 1) / (Number of implantation sites)) x 100

**Results and discussion**

Results: P0 (first parental generation)

There were no test item-related reductions in the body weights and body weight changes at ≤200 mg/ kg/day p-cymene.

All animals during Pre-cohabitation (males and females), Cohabitation (males and females), Post-mating (males only) and Lactation (females only) with the exception of Animal Number 4572 either “Within Normal Limits”or had observations that were common to this strain of rodent in laboratory conditions. During the Gestation period, Animal Number 4572 was observed as being in poor condition and was sacrificed on presumed GD 24 for welfare reasons.

Eighteen females across all groups, including controls, were euthanized early. Seventeen of these females (1/10 control, 1/10 at 50 mg/kg bw/day, 6/10 at 100 mg/kg bw/day, and 9/10 at 200 mg/kg bw/day) were euthanized on GD 25 due to failure to become pregnant. At the 200 mg/kg bw/day dose level, females that failed to become pregnant was likely the result of germ cell degeneration/depletion and/or sperm retention in the testes of males in the 200 mg/kg bw/day group. A minimal degree of sperm retention was present in some males at 100 mg/kg bw/day which may have contributed to the reduced incidence of pregnancy in females at 100 mg/kg bw/day.

One female at 200 mg/kg bw/day was euthanized for welfare reasons on GD 24; this female was not pregnant. Microscopic findings in this female were present in the liver, adrenal, and kidney and were considered the source of morbidity. Moderate, diffuse, micro/macrovesicular hepatocellular vacuolation was present in the liver, along with similar vacuolation in the cortex of the adrenal glands. In the kidney, bilateral tubular dilation and vacuolation (slight) were present in the cortex and bilateral tubular necrosis with degeneration/regeneration (slight) and neutrophilic inflammation were present in the papilla. No changes were present in the urinary bladder. The mechanism/cause of these findings in this one female were not determined by light microscopy. Although these findings were isolated to this one female, a test item effect could not be excluded, given that this was a high dose animal. Additional findings in this animal were considered stress-related and included lymphoid depletion (decreased cellularity) or necrosis/apoptosis in lymphoid tissues (thymus, mesenteric lymph node, splenic white pulp, Peyer’s patch/gut-associated lymphoid tissue) and atrophy of the splenic red pulp.

Body weights and body weight changes in the 50, 100 and 200 mg/kg bw/day p-cymene male and female groups were comparable with control group values during Pre-cohabitation (males and females), Cohabitation (males and females), and Post-mating (males only) phases. The 200 mg/kg bw/day p-cymene

female group body weights and body weight changes could not be evaluated during the gestation and lactation phases because none of the females were pregnant.

Body weights and body weight changes during the gestation and lactation phases in the 50 and 100 mg/kg bw/day p-cymene female groups were comparable with control group values.

Food consumption and compound intake (if feeding study):no effects observed

Food consumption in the 50, 100 and 200 mg/kg bw/day p-cymene male and female groups were comparable with control group values during Pre-cohabitation (males and females), Cohabitation (males and females), and Post-mating (males only) phases. The 200 mg/kg bw/day p-cymene female group food consumption could not be evaluated during the gestation and lactation phases due to none of the females being pregnant. Food consumption during the gestation and lactation phases in the 50 and 100 mg/kg bw/day p-cymene female groups were comparable with control group values.

Food efficiency:not examined

Water consumption and compound intake (if drinking water study):not examined

Ophthalmological findings:not examined

Haematological findings:There were no p-cymene-related hematology changes at ≤ 100 mg/kg bw/day.

p-cymene-related hematology changes at 200 mg/kg bw/day included increases in reticulocytes (1.30 × control; males only) with corresponding increases in red cell distribution width (1.07 × control; males only). These changes were considered non-adverse due to their relatively small magnitude.

There were no p-cymene-related coagulation changes at any dose. All differences between control and treated mean values, including those that were statistically significant, were not considered related top-cymene as there was general overlap between individual control and treated values, and a lack of concordance between PT and APTT.

Clinical biochemistry findings:

There were no p-cymene-related clinical chemistry changes at 50 mg/kg bw/day. p-cymene-related clinical chemistry changes at ≥ 100 mg/kg bw/day included: decreases in triglycerides (0.50 to 0.52 × control; males only); increases in alkaline phosphatase activity (1.79 × control in females at 100 mg/kg bw/day and 1.45 × control in males at 200 mg/kg bw/day); and decreases in albumin (0.91 × control in females at 100 mg/kg bw/day only). At 200 mg/kg bw/day, increases in blood urea nitrogen (1.50× control; males only) were considered p-cymene related. All of these changes were considered non-adverse due to their relatively small magnitude.

All other differences between control and treated mean values, including those that were statistically significant, were considered to be unrelated to p-cymene exposure as they lacked a dose relationship and/or there was general overlap between individual control and treated values.

Urinalysis findings:not examined

Behaviour (functional findings):

Functional Observational Battery:There were test item-related reductions in the 200 mg/kg bw/day p-cymene male group. The week 5 hindlimb grip strength was significantly reduced in the 200 mg/kg bw/day p-cymene male group. The LD 8 fore limb and the hindlimb grip strength in the 50 and 100 mg/kg bw/day p-cymene female groups and the Week 5 male fore limb grip strength in the 50, 100 and 200 mg/kg bw/day p-cymene were comparable to control group values.

Motor Activity:There were no p-cymene-related effects on male (Week 5) or female (LD 8) horizontal or vertical motor activity. All males in the 0, 50, 100 and 200 mg/kg bw/day p-cymene groups and all females in the 0, 50 and 100 mg/kg bw/day p-cymene groups showed habituation.

Immunological findings:not examined

Organ weight findings including organ / body weight ratios:

Histopathological findings: non-neoplastic:

p-cymene-related microscopic findings were present in the reproductive tract of P0 males at ≥100 mg/kg bw/day and in the liver of P0 males and/or females at 50 and 200 mg/kg bw/day.

Testes and Epididymides:

Varying degrees of germ cell degeneration/depletion, depletion, and/or sperm retention were present in the testis at 200 mg/kg bw/day. In some animals, this bilateral change consisted of multifocal drop out of round spermatids in early stage tubules (stages I to VIII), with germ cell degeneration, multinucleated giant cells, and vacuolation; general to multifocal depletion of elongated/mature spermatids in early stage tubules; germ cell drop out and disorganization in late stage tubules (stages X to XIII); bizarre mitotic figures in stage XIV tubules. In some animals, the findings consisted of multifocal depletion of round spermatids or depletion/degeneration of elongated spermatids in early stage tubules and occasional vacuolation. Most males also had sperm retention (retention of sperm in late stage tubules beyond stage VIII, stage at which sperm are released) along the apical surface of the tubules. In concert with the testicular findings, varying degrees of luminal cell debris and reduced sperm with or without cribriform change were present bilaterally in the epididymis at 200 mg/kg bw/day.

At 100 mg/kg bw/day, some males had marginal sperm retention bilaterally in the testis with 2 of these males having decreased sperm in the epididymis with or without cribriform change. Unilateral or bilateral seminiferous tubular atrophy, with or without luminal cell debris and reduced sperm in the epididymis, was observed in 1 male at 50 mg/kg bw/day and 1 male at 100 mg/kg bw/day and was considered an incidental background finding and unrelated to findings attributed to p-cymene. Isolated incidences of seminiferous tubular atrophy in rodents are not uncommon.

Liver: Hepatocellular hypertrophy was minimally present in 2/5 males and 1/10 females at 200 mg/kg bw/day and in 1/6 females at 50 mg/kg bw/day. The distribution pattern of hepatocellular hypertrophy was diffuse at 200 mg/kg bw/day and centrilobular at 50 mg/kg bw/day. There were no degenerative changes associated with this finding. Focal hepatocellular necrosis and inflammatory cell infiltrates, observed sporadically or at a similar incidence in controls, were considered incidental background findings unrelated to hepatocellular hypertrophy and were not considered test article-related.

Kidney: There was a marginal increase in incidence and severity of hyaline droplet accumulation in males at 200 mg/kg bw/day compared to control males. Given the degree of increase was marginal, this change was likely incidental.

Minimal tubular epithelial vacuolation was present in the renal medulla in 2/5 males at 200 mg/kg bw/day. The relationship of the medullary epithelial vacuolation to the test item was unclear.

All other microscopic findings occurred sporadically or at similar incidence and severity in control and p-cymene-treated groups and were considered incidental background findings. Evaluation of primordial follicles in the ovary sections was limited because few primordial follicles were present in the sections for either controls or treated animals. Based on the tissues available for evaluation, there was no apparent differences between the controls and the 200 mg/kg bw/day females.

Histopathological findings: neoplastic:not examined

Other effects:

Thyroid Analysis: At study termination, T4 values in P0 males were lower (0.44 to 0.63 × control) at 100 and 200 mg/kg bw/day. Also, most or all of the individual animal TSH values were below the level of detection in P0 males at all doses and in P0 females at 50 and 100 mg/kg bw/day. As there were no thyroid weight changes and/or microscopic findings in the thyroid glands in these animals the significance of these changes was considered unclear and likely not the result of test article administration.

Reproductive function: oestrous cycle:

There were treatment-related alterations of Estrous Cyclicity at 200 mg/kg bw/day. The number of animals with all regular cycles (cycles that have 4 day, 4/5 day and 5 day durations) during pre-cohabitation were reduced in the 100 and 200 mg/kg bw/day dose groups but was considered adverse in the 200 mg/kg bw/day dose group due to the magnitude. The number of animals with at least one irregular cycle (cycles < 4 days or > 5days duration) during the pre-cohabitation period were increased in the 200 mg/kg bw/day dose group.

The number of cycles and the cycle duration of the 50, 100 and 200 mg/kg bw/day groups were comparable with the control values.

Reproductive function: sperm measures:

Lower mean testes, epididymides, and levator ani-bulbocavernosus muscle weights were present in P0 males at 200 mg/kg bw/day compared to controls. In the testes and epididymides, these weight differences, respectively, correlated microscopically with germ cell degeneration/depletion and decreased sperm, respectively.

Reproductive performance:

There were treatment-related reductions in fertility at ≥ 100 mg/kg bw/day. The number of pregnant females was significantly reduced (4 and 0 females pregnant and the male and female fertility indices of 40% and 0%) in the 100 and 200 mg/kg bw/day dose groups. The number of pregnant females were 9, 9, 4 and 0 in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively. The male and female fertility indices were 90%, 90%, 40% and 0% in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively.

The number of females paired with males were 10, 10, 10 and 10 in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively. The number of females that mated with males were 10, 10, 10 and 9 in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively. The number of females that had a defined GD 0 were 10, 10, 9 and 8 in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively. The mean number of days to mating were 2.5, 2.4, 3.2 and 2.6, respectively, was considered within normal limits. These mating values were comparable with the concurrent control values and were within normal historical data. Mating and fertility data of the 50 mg/kg bw/day dose group were comparable with the concurrent control values.

Analytical Chemistry: The homogeneity results and concentration results for p-cymene dose formulations of all groups analyzed during the course of the study met the study plan specified acceptance criteria. No test item was detected in the control samples. Therefore, all dose formulations used for dosing in this study met the acceptance criteria required by the study plan.

Haematological Findings: p-cymene-related hematology changes at 200 mg/kg bw/day included increases in reticulocytes (1.30 × control; males only) with corresponding increases in red cell distribution width (1.07 × control; males only). These changes were considered non-adverse due to their relatively small magnitude.

Clinical Chemistry Findings: There were no p-cymene-related clinical chemistry changes at 50 mg/kg bw/day. p-cymene-related clinical chemistry changes at ≥ 100 mg/kg bw/day included: decreases in triglycerides (0.50 to 0.52 × control; males only); increases in alkaline phosphatase activity (1.79 × control in females at 100 mg/kg bw/day and 1.45 × control in males at 200 mg/kg bw/day); and decreases in albumin (0.91 × control in females at 100 mg/kg bw/day only). At 200 mg/kg bw/day, increases in blood urea nitrogen (1.50× control; males only) were considered p-cymene related. All of these changes were considered non-adverse due to their relatively small magnitude.

Functional Observational Battery: There were test item-related reductions in the 200 mg/kg bw/day p-cymene male group. The Week 5 hindlimb grip strength was significantly reduced in the 200 mg/kg bw/day p-cymene male group.

Organ weight findings: p-cymene-related organ weight differences were present in the testes, epididymides, and levator anibulbocavernosus muscle in adult P0 males and in the liver in adult P0 males and females. Possible test material-related differences were present in the seminal vesicles and prostate in adult P0 males.

Gross pathology findings: p-cymene-related macroscopic findings were limited to adult (P0) males at 200 mg/kg bw/day. Two males had unilateral (Male No. 4068) or bilateral (Male No. 4065) small testis. These findings correlated with microscopic findings of germ cell degeneration/depletion. One of these males (Male No. 4065) also had a small prostate; there was no correlating microscopic finding. One additional male (Male No. 4069) at 200 mg/kg bw/day had a small levator ani-bulbocavernosus muscle complex; this tissue was not examined microscopically.

Histopathology findings (non-neoplastic): p-cymene-related microscopic findings were present in the reproductive tract of P0 males at ≥100 mg/kg bw/day and in the liver of P0 males and/or females at 50 and 200 mg/kg bw/day.

Reproductive Function/Performance:

There were treatment-related alterations of Estrous Cyclicity at 200 mg/kg bw/day. The number of animals with all regular cycles (cycles that have 4 day, 4/5 day and 5 day durations) during pre-cohabitation were reduced in the 100 and 200 mg/kg bw/day dose groups but was considered adverse in the 200 mg/kg bw/day dose group due to the magnitude. The number of animals with at least one irregular cycle (cycles < 4 days or > 5 days duration) during the pre-cohabitation period were increased in the 200 mg/kg bw/day dose group. The number of cycles and the cycle duration of the 50, 100 and 200 mg/kg bw/day groups were comparable with the control values.

Lower mean testes, epididymides, and levator ani-bulbocavernosus muscle weights were present in P0 males at 200 mg/kg bw/day compared to controls. In the testes and epididymides, these weight differences, respectively, correlated microscopically with germ cell degeneration/depletion and decreased sperm, respectively.

There were treatment-related reductions in fertility at ≥ 100 mg/kg bw/day. The number of pregnant females was significantly reduced (4 and 0 females pregnant and the male and female fertility indices of 40% and 0%) in the 100 and 200 mg/kg bw/day dose groups. The number of pregnant females were 9, 9, 4 and 0 in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively. The male and female fertility indices were 90%, 90%, 40% and 0% in the 0, 50, 100 and 200 mg/kg bw/day groups, respectively.

**Results**: F1 generation

Clinical signs:There were 9, 9 and 4 litters that appeared normal for most of the observation period in the 0, 50 and 100 mg/kg bw/day dose groups, respectively.

Minor observations, not considered treatment related, included: thin, cold to touch, partially absent appendages, little or no milk in stomach, swollen, twisted, encrustation, ulceration, dark color, pallor. These findings were not considered test material-related because they occurred only in the control group, occurred in the control group in similar incidence, did not occur in a treatment-related manner, was of short duration and/or was considered to be a common finding of young pups in a laboratory situation.

Mortality / viability:There were treatment-related effects on mortality (decreased live birth index and the post-implantation survival index) at 100 mg/kg bw/day.

There were no pregnant animals in the 200 mg/kg bw/day dose group.

The live birth index was significantly reduced (94.3%) in the 100 mg/kg bw/day dose group. The post-implantation survival index was reduced in the 100 mg/kg bw/day dose group (87.3% compared with 95% in the concurrent control group). The number of litters with less than 100% viability was increased in the 100 mg/kg bw/day dose group with only 1 of 4 litters having 100% viability versus 9 of 9 in the concurrent control group.

The viability indices on PND 4, 7 and 13 were comparable with concurrent control values in the 100 mg/kg bw/day dose group, respectively.

All offspring survival index values in the 50 mg/kg bw/day dose group were comparable with the concurrent control values.

Body weight and weight changes:

The mean Pup Weights on PND 1 were reduced in a treatment-related manner in the 100 mg/kg bw/day dose group. The mean litter body weight was reduced in the 100 mg/kg bw/day dose group on PND 1.

On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights in the 50 and 100 mg/kg bw/day dose groups were comparable with the control group values.

Food consumption and compound intake (if feeding study):not examined

Food efficiency:not examined

Water consumption and compound intake (if drinking water study):not examined

Ophthalmological findings:not examined

Haematological findings:not examined

Clinical biochemistry findings:not examined

Urinalysis findings:not examined

Sexual maturation:no effects observed

The mean sex ratio was comparable among groups.

Organ weight findings including organ / body weight ratios:no effects observed

There were no treatment-related organ weight differences in thyroid/parathyroid glands in the F1pups.

Gross pathological findings:no effects observed

Histopathological findings:no effects observed

There were no microscopic findings in the thyroid/parathyroid glands in the F1 pups.

Other effects:Thyroid Hormone:There were no differences from controls for thyroxine (T4) or thyroid stimulating hormone (TSH) on PND 4 in F1 female pups (Groups 1-3). On PND 13, there were no differences from control for T4 levelsin F1 male pups. Many of the individual animal TSH values were below the level of detection in males at 100 mg/kg bw/day and females at 50 and 100 mg/kg bw/day. As there were no thyroid weight changes and/or microscopic findings in the thyroid glands in these animals the significance of these changes was considered unclear and likely not the result of test article administration.

Developmental neurotoxicity (F1)

Behaviour (functional findings):not examined

Developmental immunotoxicity (F1):not examined

Details on results (F1)

Clinical Signs:There were 9, 9 and 4 litters that appeared normal for most of the observation period in the 0, 50 and 100 mg/kg bw/day dose groups, respectively.

Minor observations, not considered treatment related, included: thin, cold to touch, partially absent appendages, little or no milk in stomach, swollen, twisted, encrustation, ulceration, dark color, pallor. These findings were not considered test material-related because they occurred only in the control group, occurred in the control group in similar incidence, did not occur in a treatment-related manner, was of short duration and/or was considered to be a common finding of young pups in a laboratory situation.

Mortality:There were treatment-related reductions in the mortality (decreased live birth index and the post-implantation survival index) at 100 mg/kg bw/day.

There were no pregnant animals in the 200 mg/kg bw/day dose group.

The live birth index was significantly reduced (94.3%) in the 100 mg/kg bw/day dose group. The post-implantation survival index was reduced in the 100 mg/kg bw/day dose group (87.3% compared with 95% in the concurrent control group). The number of litters with less than 100% viability was decreased in the 100 mg/kg bw/day dose group with only 1 of 4 litters having 100% viability versus 9 of 9 in the concurrent control group.

The viability indices on PND 4, 7 and 13 were comparable with concurrent control values in the 100 mg/kg bw/day dose group, respectively.

All offspring survival index values in the 50 mg/kg bw/day dose group were comparable with the concurrent control values.

Body Weight:The mean Pup Weights on PND 1 were reduced in a treatment-related manner in the 100 mg/kg bw/day dose group. The mean litter body weight was reduced in the 100 mg/kg bw/day dose group on PND 1.

On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights in the 50 and 100 mg/kg bw/day dose groups were comparable with the control group values.

Table 1: Hematology results – group mean values (adapted from registration dossier).

| Group (Sex) | | RETIC $x10^9/L$ | RDW % | PT Seconds |
|---|---|---|---|---|
| 0 mg/kg/day (males) | Mean | 163.4 | 12.5 | 18 |
| | SD | 9.51 | 0.19 | 0.76 |
| | N | 4 | 4 | 5 |
| 50 mg/kg/day (males) | Mean | 166.3 | 12.7 | 18.7 |
| | SD | 20.02 | 0.49 | 0.54 |
| | N | 5 | 5 | 5 |
| 100 mg/kg/day (males) | Mean | 151.8 | 12.3 | 19.5** |
| | SD | 15.96 | 0.36 | 0.77 |
| | N | 5 | 5 | 5 |
| | | | | |
| 200 mg/kg/day (males) | Mean | 211.7** | 13.4* | 19.6** |
| | SD | 36.29 | 0.80 | 0.40 |
| | N | 5 | 5 | 5 |

*p<0.05 **p<0.01

Table 2: Clinical chemistry results – group mean values (adapted from registration dossier).

| Group (Sex) | | ALKP U/L | BUN mg/dL | CREAT mg/dL | TRIG mg/dL | $Na^+$ mEq/L | $Cl^-$ mEq/L | PHOS mg/dL |
|---|---|---|---|---|---|---|---|---|
| 0 mg/kg/day (males) | Mean | 160 | 12 | 0.3 | 86 | 143 | 103 | 8.5 |
| | SD | 23.5 | 0.0 | 0.05 | 14.4 | 1.4 | 1.1 | 0.41 |
| | N | 5 | 4 | 5 | 5 | 5 | 5 | 5 |
| 50 mg/kg/day (males) | Mean | 166 | 13 | 0.3 | 69 | 143 | 102* | 7.9 |
| | SD | 43.5 | 0.9 | 0.04 | 38.1 | 0.8 | 1.2 | 0.50 |
| | N | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 100 mg/kg/day (males) | Mean | 184 | 14 | 0.3 | 45* | 143 | 102* | 7.8* |
| | SD | 22.8 | 1.3 | 0.0 | 9.1 | 0.5 | 0.9 | 0.11 |
| | N | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 200 mg/kg/day (males) | Mean | 232* | 18** | 0.3* | 43** | 141* | 102* | 8.5 |
| | SD | 61.4 | 1.8 | 0.05 | 18.1 | 0.5 | 0.8 | 0.43 |
| | N | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

*p<0.05 **p<0.01

Table 3: Clinical chemistry results – group mean values (adapted from registration dossier).

| Group (sex) | | ALT | ALKP | CHOL | ALB |
|---|---|---|---|---|---|

| | | U/L | U/L | mg/dL | g/dL |
|---|---|---|---|---|---|
| 0 mg/kg/day (females) | Mean | 183 | 151 | 120 | 3.5 |
| | SD | 39.0 | 19.8 | 30.8 | 0.29 |
| | N | 5 | 5 | 5 | 5 |
| 50 mg/kg/day (females) | Mean | 124** | 210 | 95 | 3.5 |
| | SD | 25.9 | 70.9 | 12.0 | 0.15 |
| | N | 5 | 5 | 5 | 5 |
| 100 mg/kg/day (females) | Mean | 93** | 270* | 85* | 3.2* |
| | SD | 14.0 | 119.1 | 5.4 | 0.21 |
| | N | 4 | 4 | 4 | 4 |

*p<0.05 **p<0.01

Table 4: Functional assessment – group summary of assessments (males week 5) (adapted from registration dossier).

| Dose group | | vehicle | p-cymene | | |
|---|---|---|---|---|---|
| dose (mg/kg/day) | | 1 (0) | 2 (50) | 3 (100) | 4 (200) |
| Forelimb Grip Strength (g) | Mean | 1401.7 | 1109.3 | 1143.7 | 984.4 |
| | SD | 346.3 | 413.1 | 261.7 | 231.9 |
| | N | 5 | 5 | 5 | 5 |
| Hindlimb Grip Strength (g) | Mean | 837.7 | 680.5 | 628.2 | 541.6* |
| | SD | 284.5 | 54.2 | 152.0 | 227.7 |
| | N | 5 | 5 | 5 | 5 |

*p<0.05

Table 5: p-cymene-related organ weight changes in male reproductive organs (% difference relative to controls) in adult P0 male rats at the end of dosing (adapted from registration dossier).

| Group (sex) | 2 (M) | 3 (M) | 4 (M) |
|---|---|---|---|
| Dose (mg/kg/day) | 50 | 100 | 200 |
| Testes[b] | | | |
| Absolute weight (%) | - | - | -14[a] |
| vs. body weight (%) | - | - | -8 |
| vs. brain weight (%) | - | - | -12 |
| Epididymides[b] | | | |
| Absolute weight (%) | - | - | -14[a] |
| vs. body weight (%) | - | - | -8 |

| | | | |
|---|---|---|---|
| vs. brain weight (%) | - | - | -14[a] |
| Levator Ani-Bulbocavernosus Muscle[b] | | | |
| Absolute weight (%) | - | - | -14 |
| vs. body weight (%) | - | - | -9 |
| vs. brain weight (%) | - | - | -15 |
| Seminal Vesicles/ Coagulating glands | | | |
| Absolute weight (%) | -19 | -23 | -22 |
| vs. body weight (%) | -20 | -22 | -14 |
| vs. brain weight (%) | -16 | -23 | -18 |
| Prostate | | | |
| Absolute weight (%) | -26 | 2 | -24[a] |
| vs. body weight (%) | -27[a] | 5 | -16 |
| vs. brain weight (%) | -23 | 3 | -20 |

a Statistically significant difference between mean values for test item-treated and control groups. bValues in table represent those calculated from data combining toxicity and reproductive subsets of animals. - = not test item-related.

Table 6:p-cymene-related organ weight changes in liver (% difference relative to controls) in adult P0 male rats at the end of dosing and adult P0 female rats at the end of lactation (adapted from registration dossier).

| Sex | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 50 | 100 | 200 | 50 | 100 | 200 |
| Liver | | | | | | |
| Absolute weight (%) | 8 | 6 | 27[a] | 16 | 26[a] | NT |
| vs. body weight (%) | 6 | 8[a] | 41[a] | 14 | 22[a] | NT |
| vs. brain weight (%) | 13 | 6 | 35[a] | 14 | 22 | NT |

a Statistically significant difference between mean values for test item-treated and control groups. NT = not taken; no organ weights were taken for females at 200 mg/Kg/day as all were euthanized prior to scheduled termination due to non-pregnancy.

Table 7: p-cymene-related findings in the testis and epididymis in adult P0 male rats at the end of dosing (adapted from registration dossier).

| Group (Sex) | 1 (M) | 2 (M) | 3 (M) | 4 (M) |
|---|---|---|---|---|
| Dose (mg/kg/day) | 0 | 50 | 100 | 200 |
| Testis[a] | | | | |
| Degeneration/Depletion, Germ Cell | | | | |
| Minimal | - | - | - | 1 |
| Slight | - | - | - | 5 |

| | | | | |
|---|---|---|---|---|
| Moderate | - | - | - | 1 |
| Total | 0 | 0 | 0 | 7 |
| Depletion, Germ Cell | | | | |
| Minimal | - | - | - | 2 |
| Total | 0 | 0 | 0 | 2 |
| Retention, Spermatid | | | | |
| Minimal | - | - | 7 | 2 |
| Slight | - | - | - | 7 |
| Total | 0 | 0 | 7 | 9 |
| Epididymides[a] | | | | |
| Sperm, Reduced, Luminal | | | | |
| Minimal | - | - | 1 | - |
| Slight | - | - | 1 | 4 |
| Moderate | - | - | - | 5 |
| Marked | - | - | - | 1 |
| Total | 0 | 0 | 2 | 10 |
| Cribriform Change | | | | |
| Minimal | - | - | - | 2 |
| Slight | - | - | 1 | 3 |
| Total | 0 | 0 | 1 | 5 |
| Cell Debris, Luminal | | | | |
| Minimal | - | - | - | 2 |
| Slight | - | - | - | 3 |
| Moderate | - | - | - | 4 |
| Total | 0 | 0 | 0 | 9 |
| Number of Animals Examined | 10 | 10 | 10 | 10 |

aAll changes were bilateral. - = Finding not present.

Table 8: p-cymene-related findings in the liver in adult P0 male rats at the end of dosing and adult P0 female rats at the end of dosing/lactation. (adapted from registration dossier).

| Group (Sex) | 1 (M) | 2 (M) | 3 (M) | 4 (M) | 1 (F[a]) | 2 (F[a]) | 3 (F[a]) | 4 (F[a]) |
|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 0 | 50 | 100 | 200 | 0 | 50 | 100 | 200 |
| Hypertrophy, Hepatocellular | | | | | | | | |
| Minimal | - | - | - | 2 | - | 1 | - | 1 |
| Total | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 1 |
| Number of Animals Examined | 5 | 5 | 5 | 5 | 6 | 6 | 10 | 10 |

- = Finding not present. aFemales include all animals in which liver was examined including those euthanized during gestation period that were not pregnant.

Table 9: Histopathology: p-cymene-related findings in kidneys in adult P0 Male and Female Rats (adapted from registration dossier).

| | Group/Sex | 1 (M) | 2 (M) | 3 (M) | 4 (M) | 1(F) | 2 (F) | 3 (F) | 4 (F) |
|---|---|---|---|---|---|---|---|---|---|
| | Dose (mg/kg/day) | 0 | 50 | 100 | 200 | 0 | 50 | 100 | 200 |
| | No. of animals | 10 | 10 | 10 | 10 | 9 | 9 | 4 | 0 |
| Kidneys | No. examined | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 0 |
| Dilatation, Tubular | Slight | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | Total | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Vacuolation, Tubular Epithelium | Minimal | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Total | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Accumulation, Hyaline Droplets | Minimal | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Slight | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | Total | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 |
| Basophilia,Tubular | Minimal | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| | Total | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |

Table 10: Estrous cycle evaluations – group mean values (adapted from registration dossier).

| Group (sex) | Number of Animals | Regular Cycles | | | | Irregular Cycle[a] | Extended Estrus[b] | Acyclic[c] |
|---|---|---|---|---|---|---|---|---|
| | | 4 Day | 4/5 Day | 5 Day | Total | | | |
| Control (Female) | 10 | 6 | 0 | 0 | 6 | 4 | 0 | 0 |
| | | 60% | 0% | 0% | 60% | 40% | 0% | 0% |
| 50 mg/kg/day (Female) | 10 | 7 | 0 | 0 | 7 | 3 | 0 | 0 |
| | | 70% | 0% | 0% | 70% | 30% | 0% | 0% |
| 100 mg/kg/day (Female) | 10 | 4 | 3 | 0 | 7 | 3 | 0 | 0 |
| | | 40% | 30% | 0% | 70% | 30% | 0% | 0% |
| 200 mg/kg/day (Female) | 10 | 1 | 3 | 0 | 4 | 6 | 0 | 0 |
| | | 10% | 30% | 0% | 40% | 60% | 0% | 0% |

a – At least one cycle of < 4 or > 5 days. b – At least four consecutive days of estrus. c – At least ten days without estrus.

Table 11: Mating and fertility – group mean values (adapted from registration dossier).

| | Dose Group | Control | 50 mg/kg/day | 100 mg/kg/day | 200 mg/kg/day |
|---|---|---|---|---|---|
| Females paired with males | N | 10 | 10 | 10 | 10 |
| Total number mated | N | 10 | 10 | 10 | 9 |

| | | | | | |
|---|---|---|---|---|---|
| female mating index | % | 100 | 100 | 100 | 90 |
| Total number pregnant | N | 9 | 9 | 4 | 0 |
| female fertility index | % | 90 | 90 | 40 | 0 |
| Males placed with females | N | 10 | 10 | 10 | 10 |
| Total number mated | N | 10 | 10 | 10 | 9 |
| male mating index | % | 100 | 100 | 100 | 90 |
| with females pregnant | N | 9 | 9 | 4 | 0 |
| male fertility index | % | 90 | 90 | 40 | 0 |
| Females with defined | | | | | |
| Day0 of Gestation | N | 10 | 10 | 9 | 8 |
| Number of days to Mating | Mean | 2.5 | 2.4 | 3.2 | 2.6 |
| | SD | 0.97 | 0.97 | 0.97 | 0.92 |
| Day 1 to 4 | N | 10 | 10 | 8 | 8 |
| | % | 100 | 100 | 88.9 | 100 |
| Day 5 to 8 | N | 0 | 0 | 1 | 0 |
| | % | 0 | 0 | 11.1 | 0 |
| Day 9 to 14 | N | 0 | 0 | 0 | 0 |
| | % | 0 | 0 | 0 | 0 |

Table 12: Offspring survival indices - group mean values (adapted from registration dossier).

| Group (sex) | | Post Implantation Survival Index (%) | Live Birth Index (%) | Viability Index (%) Day 4 | Viability Index (%) Day 7[a] | Viability Index (%) Day 13[a] |
|---|---|---|---|---|---|---|
| Statistics Test | | Wi | CA | Ch | + | + |
| Control (Female) | Mean | 95.0 | 100.0 | 100 | 86.7 | 84.5 |
| | SD | 6.55 | 0.00 | 0.00 | 1.24 | 3.27 |
| | N | 9 | 9 | 9 | 9 | 9 |
| | N<100% | 4 | 0 | 0 | 9 | 9 |
| 50 mg/kg/day (Female) | Mean | 97.7 | 97.7 | 100.0 | 84.3 | 84.3 |
| | SD | 4.65 | 4.77 | 0.00 | 4.28 | 4.28 |
| | N | 9 | 9 | 9 | 9 | 9 |
| | N<100% | 2 | 2 | 0 | 9 | 9 |
| 100 mg/kg/day (Female) | Mean | 87.3 | 94.3 | 98.3 | 82.5 | 82.5 |
| | SD | 14.50 | 4.17 | 3.33 | 4.33 | 4.33 |
| | N | 4 | 4 | 4 | 4 | 4 |
| | N<100% | 3 | 3** | 1 | 4 | 4 |

The following data were used for the statistics tests, animal indices for post implantation survival index and animal indices dichotomized to 1 when 100% and 0 otherwise for live birth and viability indices. A + indicates statistics (Fisher's exact tests) were only possible on additional pairwise comparisons. a – Offspring removed for hormone analysis are not excluded from calculation of the viability index.

Table 13: Litter size - group mean values (adapted from registration dossier).

| Group (sex) | | Gestation Length | Corpora Lutea | Implantations | Pre-Implantation Loss (%) | Total @ | Live on Day | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1 | 4 | 7 | 11 | 13 |
| Statistics Test | | Wi | Wi | Wi | Wi | Wi | Wi | Wi | Wi | Wi | Wi |
| Control (Female) | Mean | 21.8 | 16.3 | 16.0 | 1.8 | 15.1 | 15.1 | 15.1 | 13.1 | 12.9 | 12.8 |
| | SD | 0.44 | 2.40 | 2.06 | 3.64 | 1.45 | 1.45 | 1.45 | 1.45 | 1.36 | 1.39 |
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 50 mg/kg/day (Female) | Mean | 21.7 | 14.9 | 14.0 | 5.9 | 13.8 | 13.4 | 13.4 | 11.4 | 11.4 | 11.4 |
| | SD | 0.50 | 3.06 | 3.08 | 6.81 | 2.99 | 2.96 | 2.96 | 2.96 | 2.96 | 2.96 |
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 100 mg/kg/day (Female) | Mean | 21.3 | 17.3 | 16.0 | 7.1 | 14.0 | 13.3 | 13.0 | 11.0 | 11.0 | 11.0 |
| | SD | 0.58 | 1.89 | 1.41 | 1.94 | 2.71 | 2.87 | 2.71 | 2.71 | 2.71 | 2.71 |
| | N | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

@ - Includes offspring that died prior to the designated Day 1 of age.

Table 14: Body weight - group mean values (g) for offspring (adapted from registration dossier).

| Group (Sex) | | Day of Age | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 4 | 7 | 11 | 13 |
| Statistics Test | | Wi | Wi | Wi | Wi | Wi |
| Control (Male) | Mean | 6.8 | 8.9 | 13.1 | 20.4 | 24.5 |
| | SD | 0.62 | 0.89 | 1.31 | 2.08 | 2.70 |
| | N | 9 | 9 | 9 | 9 | 9 |
| 50 mg/kg/day (Male) | Mean | 6.8 | 9.8 | 14.8 | 23.0 | 26.8 |
| | SD | 0.91 | 1.61 | 2.68 | 4.77 | 5.22 |
| | N | 9 | 9 | 9 | 9 | 9 |
| 100 mg/kg/day (Male) | Mean | 6.1 | 8.7 | 13.4 | 21.2 | 25.1 |
| | SD | 0.44 | 0.50 | 0.78 | 1.58 | 1.72 |
| | N | 4 | 4 | 4 | 4 | 4 |
| Control (Female) | Mean | 6.6 | 8.4 | 12.7 | 20.0 | 23.7 |
| | SD | 0.66 | 0.90 | 1.51 | 2.74 | 3.25 |
| | N | 9 | 9 | 9 | 9 | 9 |
| 50 mg/kg/day (Female) | Mean | 6.3 | 9.0 | 14.0 | 22.2 | 26.0 |
| | SD | 0.84 | 1.75 | 2.80 | 4.85 | 5.53 |
| | N | 9 | 9 | 9 | 9 | 9 |
| 100 mg/kg/day (Female) | Mean | 6.0 | 8.6 | 12.8 | 19.9 | 23.7 |
| | SD | 0.30 | 0.53 | 0.81 | 1.04 | 1.16 |
| | N | 4 | 4 | 3 | 3 | 3 |

### 3.10.1.9 Study 9: *p*-cymene

Reference: Unnamed 2018

short-term repeated dose toxicity: oral

Non-GLP Dose-Range Finding study

Qualifier:no guideline followed

Principles of method if other than guideline:

This non-GLP dose range-finding study incorporates elements of general regulatory guidelines for toxicity studies but was designed with reference to the following test method and/or guideline: OECD 407: Repeated Dose Oral Toxicity – Rodent 28/14 Day.

GLP compliance:no

Remarks:DRF study conducted in a facility that operates in accordance with GLP principles, however no claim GLP compliance was intended nor is made for this study.

Limit test:no

**Test material**

Reference substance name:p-cymene

EC Number:202-796-7

EC Name:p-cymene

Cas Number:99-87-6

Molecular formula:C10H14

IUPAC Name:1-isopropyl-4-methylbenzene

**Test animals**

Species:rat

Strain:Crl:CD(SD) IGS

The rat is an animal model commonly utilized in toxicity studies. In addition, a historical database is available for comparative evaluation.

Sex:male/female

TEST ANIMALS

- Source: Charles River Laboratories

- Females (if applicable) nulliparous and non-pregnant: Yes

- Age at study initiation: approximately 8 to 9 weeks at initiation of dosing

- Weight at study initiation: Males: Approximately 226 to 300 grams.; Females: Approximately 176 to 225 grams

- Fasting period before study: Not specified

- Housing: Group-housed, in solid bottom cages with cellulose-based contact bedding; Spare animals (1/sex) single housed, in solid bottom cages with cellulose-based contact bedding.

- Diet (e.g. ad libitum): Certified Rodent Diet, No. 2016C (pellets) (Envigo Teklad, Madison, WI) ad libitum.

- Water (e.g. ad libitum): New Jersey-American Water Company, Cherry Hill, NJ; ad libitum, via automated watering system.

- Acclimation period: Approximately 2 weeks

DETAILS OF FOOD AND WATER QUALITY:

Feed Analysis: Analytical certification of batches of feed, provided by the manufacturer, is maintained on file at the Testing Facility. There were no known contaminants in the feed that are expected to interfere with the objectives of this study.

Water Analysis: Water analyses are conducted by New Jersey-American Water Company (Raritan-Millstone Plant) to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis, provided by the supplier, is maintained on file at the Testing Facility. In addition, chemical and microbiological analyses are conducted biannually by a subcontract laboratory on water samples collected from representative rooms in this facility. Results are maintained on file at the Testing Facility. There were no known contaminants that are expected to interfere with the objectives of this study.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20-26°C

- Humidity (%): 30 to 70%

- Air changes (per hr): Not specified

- Photoperiod (hrs dark / hrs light): Twelve hour light/dark cycle provided via automatic timer

IN-LIFE DATES: From: 2018-04-03 To: 2018-04-30

**Administration / exposure**

Route of administration:oral: gavage

Because potential accidental human exposure to the test item might occur via the oral route, the same route via oral (gavage) administration was used in this study.

Vehicle:corn oil

Remarks:NF grade (Spectrum Chemical NC9684514)

- PREPARATION OF DOSING SOLUTIONS: Appropriate amounts of the test item wiere mixed with the vehicle to achieve the desired concentrations.

- VEHICLE

- Justification for use and choice of vehicle (if other than water): Corn oil; not specified

- Concentration in vehicle: 0, 10, 30, and 100 mg/mL

- Amount of vehicle (if gavage): 5 mL/Kg

Analytical verification of doses or concentrations:yes

Details on analytical verification of doses or concentrations:

Dose formulations of the test item, in vehicle, corn oil, were analyzed to confirm that the prepared dose formulations were homogeneous and that the administered

p-cymene concentrations were appropriate under the study conditions.

The analytical method qualified at Envigo (FIA-002-18) involved dilution of p-cymene formulation samples with sample diluent (100% Acetone) followed by gas chromatography with flame ionization detection (GC-FID).

Duration of treatment / exposure:For 14 consecutive days

Frequency of treatment:Once daily

Doses / concentrations: 0, 50, 150 mg/kg bw/day (nominal), 500 mg/kg bw/day (nominal)

No. of animals per sex per dose:3/sex/dose

Control animals:yes, concurrent vehicle

Details on study design:

- Dose selection rationale:

The doses selected for this 14-day repeated dose oral (gavage) range-finding toxicity study in the rat were 0 (vehicle), 50, 150 and 500 mg/kg bw/day. These doses were selected based on the available single dose rat acute oral toxicity data (Jenner et al., 1964) where undiluted p-cymene was shown to be acutely toxic by oral exposure. The LD50 acute oral toxicity was estimated to be 4750 mg/Kg (95% confidence limits of 3720 - 6060 mg/Kg). Clinical signs included depression soon after dosing, coma, bloody lacrimation, diarrhoea, irritable, and scrawny appearance for as long as 2 weeks. In a subsequent study, male Wistar rats were provided p-cymene at the dietary levels of 0.05, 0.5 and 1 % (Imaizumi, et al. 1985). No clinical observation data are available; however body weights were comparable with control values. Serum cholesterol was affected at all levels.

The high dose selected (500 mg/kg bw/day) in the 14-day oral gavage study is approximately one-tenth the acute oral LD50 dose. While the adverse effects at this dose level were unknown, it was anticipated that the high dose level (500 mg/kg bw/day) would not cause mortality but may cause adverse toxicity. The low dose was one log lower (50 mg/kg bw/day) and was anticipated to cause little to no toxicity. The mid-dose was ½ log of the high dose.

- Rationale for animal assignment (if not random): Randomized; Animals are sorted into groups and assigned their permanent identification numbers.

**Examinations**

Observations and examinations performed and frequency:

CAGE SIDE OBSERVATIONS: Yes

- Time schedule: All animals were observed for mortality and general condition at least twice daily (once in the morning and once in the afternoon). During the treatment period, all animals were observed for signs of toxic effects once (by group) at ~2 hours post-dose.

DETAILED CLINICAL OBSERVATIONS: Yes

- Time schedule: Each toxicity study animal was removed from its cage and examined twice pretest (~ weekly) and daily during the treatment period and on Day 15 (prior to necropsy). Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as an evaluation of respiration

BODY WEIGHT: Yes

- Time schedule for examinations: Non-fasted body weights for each study animals were recorded at least once pretest (3 days before start of dosing), on Day 1 (before start of dosing), and on Days 3, 7, 10 and 14. Fasted body weights were obtained on the day of the scheduled necropsy.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes

Food consumption was measured (weighed) for each cage of group housed animals beginning the week prior to treatment initiation and twice weekly (beginning on Day 1) during the treatment phase(study day 1 to4, 4 to 8, 8 to 11 and 11 to 14).

FOOD EFFICIENCY:

- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Not specified

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): Not specified

OPHTHALMOSCOPIC EXAMINATION: No

HAEMATOLOGY: No

CLINICAL CHEMISTRY: No

URINALYSIS: No

NEUROBEHAVIOURAL EXAMINATION: No

IMMUNOLOGY: No

Sacrifice and pathology:

GROSS PATHOLOGY: Yes

A complete macroscopic examination was performed on all study animals, including all unscheduled decedents; and all abnormal observations recorded. The necropsy consisted of an external examination as well as a detailed internal examination.

Necropsy was performed on up to 3 animals/sex/group after animals had been treated for at least 14 days. Animals were fasted overnight prior to necropsy. Euthanasia was carried out by Isoflurane inhalation followed by exsanguination.

Liver, spleen and kidney were taken from all surviving study animals at their scheduled necropsy, weighed, recorded and organ/body weight ratios calculated. Organs were not

weighed for unscheduled decedents during the course of the study. Prior to weighing, all organs were carefully dissected and properly trimmed to remove fat and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection to avoid drying and paired organs were weighed together.

HISTOPATHOLOGY: No

Tissues with abnormalities were retained in 10% neutral buffered formalin except the eyes and testes which were preserved in Modified Davidsons and then transferred to NBF.

Lungs were infused with formalin to ensure fixation.

Statistics:Individual animal data presented in this report may be rounded. Unrounded individual animal data were used to calculate the reported mean and standard deviation values.

Group means and standard deviations for calculated data points (i.e. bodyweight change) were derived from individual animal/cage values.

Food consumption (g/animal/day) was calculated as:grams of food consumed / animal days[a]

[a] Sum of whole days each animal is alive in the cage for each consumption period. If an animal dies the day of death is not counted as an animal day. For example: 4 animals in a cage for 5 days equals 20 animal days; if one animal died on the 3rd day then the animal days equals 17.

Organ weights were presented as absolute and relative to terminal bodyweight using the weight recorded on the day of necropsy.

**Results and discussion**

Clinical signs: Except for the one female in the 500 mg/kg bw/day dose group that exhibited rapid breathing, decreased activity and hunched posture and was sacrificed on Day 13 as moribund, there were no adverse treatment-related clinical signs observed at any dose level in male or female rats.

Mortality:One female in the 500 mg/kg bw/day dose group was sacrificed as moribund on study Day 13. This animal was thin and exhibited rapid breathing, decreased activity and hunched posture. There were no macroscopic findings indicative of a gavage accident. The unscheduled death in this 500 mg/kg bw/day dose group female was considered likely to be test material-related based on its occurrence in the high-dose group and the presence of test material-related organ weight differences and macroscopic abnormalities in animals surviving to terminal sacrifice at this dose level.

Body weight and weight changes: Body weights and body weight changes were reduced in the 150 mg/kg bw/day dose group females. Body weights, bodyweight changes and food consumption (females only) were reduced in male and female rats in the 500 mg/kg bw/day dose group.

Food consumption and compound intake: Food consumption was reduced in female rats in the 500 mg/kg bw/day dose group.

Food efficiency:not specified

Water consumption and compound intake: not specified

Ophthalmological findings:not examined

Haematological findings:not examined

Clinical biochemistry findings:not examined

Urinalysis findings:not examined

Behaviour (functional findings):not examined

Immunological findings:not examined

Organ weight findings including organ / body weight ratios:Fourteen days of oral gavage dosing with p-cymene was associated with organ weight differences in the liver (higher weight at ≥ 150 mg/kg bw/day) and spleen (lower weight at 500 mg/kg bw/day) and macroscopic abnormalities in the testes (small size/soft texture at ≥ 150 mg/kg bw/day).

Gross pathological findings:Necropsy and gross pathology revealed one 150 mg/kg bw/day male with discoloured lungs and bronchi (dark red area on the right caudal lobe [≤ 1mm, 1 (one)] and stomach (black areas on the glandular mucosa [≤ 1mm, 2-5 (few)], one 150 mg/kg bw/day male with discoloured thymus and small epididymides and testes, and two of three males from the 500 mg/kg bw/day group had small, soft testes. A single female from the 500 mg/kg bw/day group had a small thymus, uterus and cervix.

Neuropathological findings:not examined

Histopathological findings: non-neoplastic:not examined

Histopathological findings: neoplastic:not examined

Details on results:

One female in the 500 mg/kg bw/day dose group was sacrificed as moribund on study Day 13. This animal was thin and exhibited rapid breathing, decreased activity and hunched posture. Except for this one female in the 500 mg/kg bw/day dose group, there were no adverse treatment-related clinical signs observed at any dose level in male or female rats.

Body weights and body weight changes were reduced in the 150 mg/kg bw/day dose group females. Body weights, body weight changes and food consumption (females only) were reduced in male and female rats in the 500 mg/kg bw/day dose group.

Necropsy and gross pathology revealed one 150 mg/kg bw/day male with discoloured lungs and bronchi and stomach, one 150 mg/kg bw/day male with discoloured thymus and small epididymides and testes, and two of three males from the 500 mg/kg bw/day group had small, soft testes. A single female from the 500 mg/kg bw/day group had a small thymus, uterus and cervix.

Fourteen days of oral gavage dosing with p-cymene was associated with organ weight differences in the liver (higher weight at ≥ 150 mg/kg bw/day) and spleen (lower weight at 500 mg/kg bw/day) and macroscopic abnormalities in the testes (small size/soft texture at ≥ 150 mg/kg bw/day).

Table 1: Daily observations – group total distribution of observations (adapted from registrations dossier).

| Category | Observation | Day | Total incidence/number of animals affected | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Males (n = 3 per Group) | | | | Females (n = 3 per Group) | | | |
| | | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Behaviour | Decreased Activity | 13 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/1 |
| Breathing | Rapid | 13 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/1 |
| Build Conformation | Thin | 13 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/1 |
| Posture | Hunched | 13 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 1/1 |

1 – Group 1 (0 mg/kg/day), 2 – Group 2 (50 mg/kg/day), 3 – Group 3 (150 mg/kg/day), 4 – Group 4 (500 mg/kg/day)

Table 2: Body weight- group mean values (g) (adapted from registration dossier).

| Group | | Day Ral 1 | Day 1 | Day 3 | Day 7 | Day 10 | Day 14 | Change Day 1-3 | Change Day 1-7 | Change Day 1-10 | Change Day 1-14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | | | | | | | | | | | |
| 0 mg/kg/day | Mean | 275 | 299 | 309 | 335 | 344 | 359 | 11 | 36 | 45 | 60 |
| | SD | 15.1 | 15.2 | 18.4 | 20.8 | 17.2 | 18.1 | 3 | 7.4 | 4.4 | 7.2 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 33 | 3 | 3 | 3 |
| 50 mg/Kg/day | Mean | 275 | 303 | 319 | 348 | 358 | 377 | 17 | 45 | 55 | 74 |
| | SD | 20.8 | 23.1 | 23.4 | 27.5 | 27.3 | 28.3 | 2.7 | 4.5 | 5.8 | 7.4 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 150 mg/kg/day | Mean | 266 | 292 | 297 | 329 | 337 | 356 | 6 | 37 | 45 | 64 |
| | SD | 23.2 | 26.8 | 26.0 | 33.1 | 35.2 | 36.7 | 7.0 | 6.8 | 9.8 | 9.9 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 272 | 294 | 291 | 317 | 319 | 333 | -3 | 23 | 25 | 39 |
| | SD | 10.2 | 11.8 | 16.8 | 19.0 | 18.4 | 27.2 | 7.9 | 8.6 | 6.7 | 15.5 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Females | | | | | | | | | | | |
| 0 mg/kg/day | Mean | 197 | 209 | 211 | 221 | 231 | 237 | 2 | 12 | 22 | 28 |
| | SD | 20.3 | 25.9 | 27.0 | 32.0 | 33.2 | 26.1 | 6.8 | 10.5 | 9.1 | 12.0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 50 mg/kg/day | Mean | 215 | 227 | 233 | 248 | 251 | 260 | 6 | 21 | 24 | 32 |
| | SD | 16.8 | 18.8 | 23.6 | 31.8 | 28.7 | 31.3 | 4.9 | 13.0 | 10.9 | 14.2 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 150 mg/kg/day | Mean | 191 | 202 | 199 | 209 | 220 | 219 | -2 | 8 | 18 | 18 |
| | SD | 16.1 | 18.8 | 18.0 | 21.2 | 27.7 | 25.9 | 2.9 | 6.0 | 10.1 | 9.9 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 196 | 208 | 205 | 208 | 198 | 205 | -3 | 0 | -10 | -2 |
| | SD | 7.3 | 8.6 | 13.0 | 22.1 | 20.3 | 28.3 | 8.6 | 15.0 | 14.9 | 16.5 |
| | N | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 |

Table 3: Body weight change – group mean values (g) (adapted from registrtaion dossier).

| Group | | Day Ral 1-1 | Day 1-3 | Day 3-7 | Day 7-10 | Day 10-14 |
|---|---|---|---|---|---|---|
| Males | | | | | | |
| 0 mg/kg/day | Mean | 23 | 11 | 25 | 9 | 15 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | SD | 1.5 | 3.3 | 5.3 | 3.6 | 2.9 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 50 mg/kg/day | Mean | 28 | 17 | 29 | 10 | 19 |
| | SD | 5.0 | 2.7 | 4.6 | 3.5 | 1.6 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 150 mg/kg/day | Mean | 26 | 6 | 31 | 9 | 19 |
| | SD | 3.8 | 7.0 | 8.8 | 3.4 | 5.3 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 23 | -3 | 26 | 2 | 14 |
| | SD | 1.8 | 7.9 | 3.3 | 4.9 | 8.8 |
| | N | 3 | 3 | 3 | 3 | 3 |
| Females | | | | | | |
| 0 mg/kg/day | Mean | 12 | 2 | 10 | 10 | 6 |
| | SD | 5.7 | 6.8 | 5.2 | 3.8 | 3.0 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 50 mg/kg/day mg/kg/day | Mean | 12 | 6 | 15 | 3 | 8 |
| | SD | 3.7 | 4.9 | 8.3 | 7.1 | 3.7 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 150 mg/kg/day | Mean | 11 | -2 | 10 | 10 | -1 |
| | SD | 3.0 | 2.9 | 4.0 | 6.7 | 3.5 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 12 | -3 | 3 | -11 | 2 |
| | SD | 1.6 | 8.6 | 10.0 | 5.2 | 2.7 |
| | N | 3 | 3 | 3 | 3 | 2 |

Table 4: Organ weights – group mean absolute values (g) (adapted from registration dossier).

| Group | | Terminal Body Weight | Kidneys | Liver | Spleen |
|---|---|---|---|---|---|
| Males | | | | | |
| 0 mg/kg/day | Mean | 337.5 | 2.387 | 8.557 | 0.691 |
| | SD | 10.1 | 0.281 | 0.470 | 0.132 |
| | N | 3 | 3 | 3 | 3 |
| 50 mg/kg/day | Mean | 356.1 | 2.429 | 9.670 | 0.671 |
| | SD | 28.0 | 0.215 | 0.275 | 0.055 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 150 mg/kg/day | Mean | 325.8 | 2.583 | 11.071 | 0.665 |
| | SD | 36.4 | 0.139 | 0.917 | 0.126 |
| | N | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 298.3 | 2.557 | 12.885 | 0.503 |
| | SD | 21.6 | 0.284 | 1.138 | 0.022 |

| | | | | | |
|---|---|---|---|---|---|
| | N | 3 | 3 | 3 | 3 |
| Females | | | | | |
| 0 mg/kg/day | Mean | 222.7 | 1.512 | 6.357 | 0.460 |
| | SD | 37.0 | 0.241 | 1.609 | 0.074 |
| | N | 3 | 3 | 3 | 3 |
| 50 mg/kg/day | Mean | 246.2 | 1.678 | 7.648 | 0.546 |
| | SD | 30.5 | 0.185 | 0.687 | 0.065 |
| | N | 3 | 3 | 3 | 3 |
| 150 mg/kg/day | Mean | 203.5 | 1.720 | 8.198 | 0.467 |
| | SD | 21.6 | 0.310 | 1.157 | 0.060 |
| | N | 3 | 3 | 3 | 3 |
| 500 mg/kg/day | Mean | 191.0 | 1.685 | 7.835 | 0.359 |
| | SD | 28.8 | 0.329 | 1.495 | 0.032 |
| | N | 2 | 2 | 2 | 2 |

Table 5: organ weights – group mean values realative (%) to body weight (g) (adapted from registration dossier).

| Group | | Terminal Body Weight | Kidneys | Liver | Spleen |
|---|---|---|---|---|---|
| Males | | | | | |
| 0 mg/kg/day | Mean | 337.5 | 0.706 | 2.54 | 0.205 |
| | SD | 10.1 | 0.063 | 0.17 | 0.038 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 50 mg/kg/day | Mean | 356.1 | 0.683 | 2.72 | 0.188 |
| | SD | 28.0 | 0.054 | 0.15 | 0.001 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 150 mg/kg/day | Mean | 325.8 | 0.799 | 3.41 | 0.204 |
| | SD | 36.4 | 0.088 | 0.22 | 0.024 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 500 mg/kg/day | Mean | 298.3 | 0.859 | 4.32 | 0.169 |
| | SD | 21.6 | 0.109 | 0.27 | 0.013 |
| | N | 3 | 3 | 3 | 3 |
| Females | | | | | |
| 0 mg/kg/day | Mean | 222.7 | 0.679 | 2.83 | 0.207 |
| | SD | 37.0 | 0.014 | 0.24 | 0.014 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 50 mg/kg/day | Mean | 246.2 | 0.683 | 3.12 | 0.222 |

| | | | | | |
|---|---|---|---|---|---|
| | SD | 30.5 | 0.015 | 0.13 | 0.005 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 150 mg/kg/day | Mean | 203.5 | 0.841 | 4.02 | 0.230 |
| | SD | 21.6 | 0.078 | 0.20 | 0.024 |
| | N | 3 | 3 | 3 | 3 |
| | | | | | |
| 500 mg/kg/day | Mean | 191.0 | 0.879 | 4.09 | 0.189 |
| | SD | 28.8 | 0.040 | 0.17 | 0.012 |
| | N | 2 | 2 | 2 | 2 |

Table 6: Macropathology – group distribution of findings – terminal necropsy (adapted from registration dossier).

| Number of animals affected | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| Group | 0 mg/kg/day | 50 mg/kg/day | 150 mg/kg/day | 500 mg/kg/day | 0 mg/kg/day | 50 mg/kg/day | 150 mg/kg/day | 500 mg/kg/day |
| No. of animals | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 |
| Tissue / Organ and Findings | | | | | | | | |
| Number of animals within normal limits | 3 | 3 | 1 | 1 | 3 | 3 | 3 | 1 |
| Epididymides (small) | 0 | 0 | 1 | 0 | - | - | - | - |
| Lungs and Bronchi (Dark area(s)) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Stomach (Dark area(s)) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Testes | | | | | | | | |
| - Small | 0 | 0 | 1 | 2 | - | - | - | - |
| - Soft | 0 | 0 | 0 | 2 | - | - | - | - |
| Thymus | | | | | | | | |
| - Dark area(s) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| - Small | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Uterus and Cervix (Small) | - | - | - | - | 0 | 0 | 0 | 1 |

### 3.11 Specific target organ toxicity – single exposure

Not evaluated.

### 3.12 Specific target organ toxicity – repeated exposure

Not evaluated.

### 3.13 Aspiration hazard

Not evaluated.

## 4 ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.