CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. ;

Reaction mass of N-phenyl,N'-o-tolyl-phenylene diamine, N,N'-diphenyl-p-phenylene diamine and N,N'-di-o-tolyl-phenylene diamine

EC Number: **273-227-8**

CAS Number: **68953-84-4**

Index Number: **-**

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Version number: **2.0**

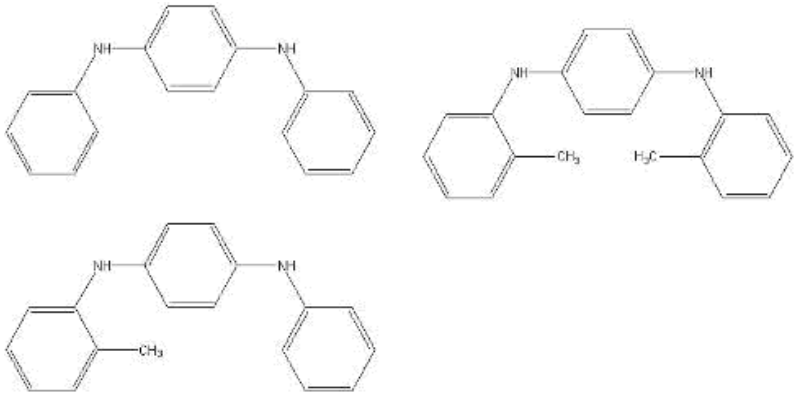
Date: **February 2021**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Reaction mass of N-phenyl,N'-o-tolyl-phenylene diamine, N,N'-diphenyl-p-phenylene diamine and N,N'-di-o-tolyl-phenylene diamine
Other names (usual name, trade name, abbreviation)	BENPAT Wingstay 100, DAPD
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	273-227-8
EC name (if available and appropriate)	1,4-Benzenediamine, N,N'-mixed ph and tolyl derivs.
CAS number (if available)	68953-84-4
Molecular formula	Non available Constituents: C18H16N2 (N,N'-diphenylbenzene-1,4-diamine) C20H20N2 ((2-methylphenyl)benzene-1,4-diamine) C19H18N2 (N,N'-bis, N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine)
Structural formula	
Molecular weight or molecular weight range	260.3 – 288.39 g/mol

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
N,N'-diphenylbenzene-1,4-diamine (CAS: 74-31-7, EC: 200-806-4)	See confidential annex	Skin Sens. 1 – H317 Aquatic Chronic 3 – H412	Skin Sens. 1 – H317 Muta. 2 – H341 Repr. 2 – H361 (fertility) Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410 Aquatic Chronic 3 – H412
N,N'-bis(2-methylphenyl)benzene-1,4-diamine (CAS: 15017-02-4, EC: 239-102-7)	See confidential annex	-	-
N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine (CAS: 27173-16-6)	See confidential annex	-	-

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
2-methyl-N-phenylaniline CAS: 1205-39-6	See confidential annex I	-	-	-
Diphenylamine CAS: 122-39-4; EC: 204-539-4	See confidential annex I	Acute Tox. 3* – H301/H311/H331 STOT RE 2*– H373** Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410	Acute Tox. 3 – H301/H311/H331 Eye Irrit. 2 – H319 Skin Irrit. 2 – H315 Eye Dam. 1 – H318 STOT RE 2 – H373 (several) STOT SE 3 – H335 (Respiratory tract) STOT SE 1- H370 (central nervous system) Aquatic Acute 1 – H400 Aquatic Chronic 1 – H410 Flam. Sol. 2 – H228 Repr. 2 – H361	-
Phenylenediamine oligomers	See confidential annex I	-	-	-
Other low molecular weight diphenylamine derivatives	See confidential annex I	-	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No entry										
Dossier submitters proposal	tbd	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. ; Reaction mass of N-phenyl,N'-o-tolyl-phenylene diamine, N,N'-diphenyl-p-phenylene diamine and N,N'-di-o-tolyl-phenylene diamine	273-227-8	68953-84-4	Skin Sens. 1 Repr. 1B	H317 H360FD	GHS07 GHS08 Dgr	H317 H360FD			
Resulting Annex VI entry if agreed by RAC and COM					Skin Sens. 1 Repr. 1B	H317 H360FD	GHS07 GHS08 Dgr	H317 H360FD			

Table 5: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation	**Harmonised classification proposed**	**Yes**
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity		
Reproductive toxicity	**Harmonised classification proposed**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure		
Aspiration hazard		
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

BENPAT does not have an existing entry in Annex VI of CLP and has not been considered for harmonised classification and labelling previously in the EU. BENPAT has been listed on the Community rolling action plan (CoRAP) in 2013. Concerns for substance evaluation were that BENPAT is suspected to be PBT/vPvB, has consumer and wide-dispersive uses, and the substance is produced/ imported in a high (aggregated) tonnage in the EU (> 1000 t/a). There are still open information requests concerning environmental fate.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Animal studies present in this dossier provide strong evidence that BENPAT causes dystocia (obstructed labour) and high incidences of polycystic kidneys in the offspring. There is no robust data on the Mode of Action (MoA) to conclude that the effects of BENPAT are not relevant to humans or to raise doubt about the human relevance. Therefore, BENPAT acts as a presumed human reproductive toxicant and classification and labelling as Repr.1B (H360FD, May damage fertility. May damage the unborn child) is proposed and the DS disagrees with the current self-classification. According to Article 36 paragraph 1 (d), reproductive toxicity, category 1A, 1B or 2 (Annex I, section 3.7) shall normally be subject to harmonised classification and labelling.

Furthermore, most, but not all of the notifiers to the C&L Inventory self-classified BENPAT as a skin sensitiser, without sub-categorisation, while fewer registrants notified BENPAT as Skin Sens. 1B. In light of its high market volume and consumer/wide-spread uses, the sensitising properties of BENPAT should be acknowledged throughout the Community, and therefore harmonised classification is required.

5 IDENTIFIED USES

According to the ECHA dissemination site (last accessed 19 November 2019), BENPAT is not naturally found in the environment; it is used in synthetic materials such as polymers. Release to the environment of this substance is likely to occur from: outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials), outdoor use in long-life materials with high release rate (e.g. tyres, treated wooden products, treated textile and fabric, brake pads in trucks or cars, sanding of buildings (bridges, facades) or vehicles (ships)) and indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, footwear, leather products, paper and cardboard products, electronic equipment).

6 DATA SOURCES

Data for BENPAT were taken from the publically disseminated REACH Registration Dossier (last accessed 19 November 2019), from study reports on toxicity to reproduction made available by the Registrants in the REACH lead registration dossier, and from the results of a systematic literature screening.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Blue-brown flakes or pastilles with an amine-like odour.	MSDS Data	
Melting/freezing point	93-101°C	(Taylor, 2010)	Experimental data
Boiling point	Decomposition before boiling at 350 °C	(Taylor, 2010)	Experimental data
Relative density	1.2 at 20 °C	(Skrok, 2010)	Experimental data

Property	Value	Reference	Comment (e.g. measured or estimated)
Vapour pressure	Estimated vapour pressures for the 3 main constituents of 1,4-benzenediamine, N,N'-mixed phenyl and tolyl derivatives were in the range of 10^{-7} to 10^{-8} hPa at 25 °C.	(EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation, 2000)	QSAR estimation
Surface tension			The multi-constituent substance 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. does not contain any amphiphilic constituents and as a consequence there is no structural alert for surface activity. In addition, surface activity is not a desired property of the material.
Water solubility	(1) N,N'-diphenyl-p-phenylenediamine; CAS: 74-31-7: 0.13 mg/L; (3) N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine; CAS: 27173-16-6: 0.11 mg/L; (2) N,N'-bis(2-methylphenylbenzene)-1,4-diamine CAS: 15017-02-4: 0.045 mg/L	(Douglas and Kogovsek, 2004)	The water solubility was determined of the 3 main constituents. Experimental data
Partition coefficient n-octanol/water	N,N'-diphenyl-p-phenylenediamine; CAS: 74-31-7: log Kow = 3.3 N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine; CAS: 27173-16-6: log Kow = 3.9 N,N'-bis(2-methylphenylbenzene)-1,4-diamine; CAS: 15017-02-4: log Kow = 4.6	*(Dix, 2000)*	The partition coefficient was determined of the 3 main constituents. Experimental data
Flash point			The study does not need to be conducted because the flashpoint is only relevant to liquids and low melting points solids

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	DAPD is a non-flammable solid.	(Krzysiak-Warzała, 2010)	The sample melted after applying the burner's flame. On the surface the burner's flame was applied to, a small sparking was observed, but when the flame was withdrawn, the sparking stopped.
Explosive properties			There are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature			Testing should not be conducted for substances that melt at <160°C.
Oxidising properties			Test is not needed for organic substances that do not contain oxygen, fluorine or chlorine.
Granulometry	The test substance in its final form (i.e. as supplied to customers) consists of pastilles with a minimum size of approximately 3.9 mm. In a sieving experiment using a 100 micrometer mesh sieve, no particles < 100 micrometer were detected.	(DJCHEM CHEMICALS POLAND SA, 2010)	Experimental data
Stability in organic solvents and identity of relevant degradation products	Recovery data for test chemical in octanol showed all components were > 97 % recovered after 7 days.	(Douglas and Kogovsek, 2004)	Experimental data
Dissociation constant	pKa = 1.47 at 20 °C	(Hambrick, 1994)	Value for N,N'-diphenyl-p-phenylenediamine
Viscosity			Viscosity is relevant only to liquids.

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 7: Summary table of toxicokinetic studies – non-human information

Method	Results	Remarks	Reference
Rat, Sprague-Dawley, male Oral exposure: Gavage Exposure regime: One oral gavage dose administered to each rat. Dose/conc.: ~20 mg DAPD/kg that included ~8 mg R-1679/kg Radioactivity doses Phase 1: ~15 µCi/rat Radioactivity doses Phase 2: ~33 µCi/rat Test material was synthesised with radioactivity (^{14}C) positioned in the centre ring. This radiotracer was added to the test substance to allow quantification of excreted material. Three sampling sites were included in the study - urine, faeces, and bile Samples were analysed for radioactivity using liquid scintillation counting and HPLC with both UV and flow-through scintillation counting.	Identification of metabolites was not conducted. However, HPLC profiles demonstrate (a) in bile, the parent compound R-1679 is a minor fraction (< 5 %) of that administered, and (b) > 90 % of the radiolabel elutes in the chromatographic region associated with metabolites exhibiting higher polarity than the parent compound. 40 % of that radioactivity was located in one peak (B4). Evaluation of results: Bioaccumulation potential cannot be judged based on these study results.	2 (reliable with restrictions) Supporting study Test material: (1) Component: N-phenyl-N'-o-tolyl-p-phenylene-diamine (R-1679), typically 40 % of the registration substance DAPD; CAS No: 27173-16-6 (2) Component: N,N'-di (o-tolyl)-p-phenylenediamine (R-898), typically ~20 % of the registration substance DAPD; CAS No: 15017-02-4	(RTI, 1998a; RTI, 1998b)
Rat, Fischer 344, female (n=6/exposure group) Dermal exposure Solid test material was radiolabelled (^{14}C with 94 % chemical and radiochemical purities), making possible accurate quantitation of absorption kinetics using beta counting assay. Specific activity was 11.7 Ci/mol. The location of the ^{14}C label was the centre ring. Dermal doses: 350 µg ^{14}C R-898 (10 µCi radioactivity) added to 1660 µg of DAPD in solution of acetone; total volume = 50 µL. Dosage for intravenous administration: 35 µg ^{14}C R-898 (1 µCi radioactivity) added to 1660 µg of DAPD in solution of acetone; total volume = 20 µL. These levels of exposure were estimated to lack toxic activity based upon acute toxicity test results.	Comparison of excreted radioactivity following dermal vs. i.v. administration of radioactive R-898 to groups of rats indicated that 60 % of the dermally applied test chemical was absorbed during the 7-day study. The bulk (> 95 %) of absorbed test chemical was excreted via the faeces.	Reliability: 2 (reliable with restriction) GLP-compliant Supporting study Test material: Component: N,N'-di (o-tolyl)-p-phenyl-enediamine (R-898), ~20 % of DAPD (CAS 15017-02-4)	(University of California, 1997)

The information on oral toxicokinetic (and also on oral absorption percentages) stems from two oral toxicokinetic studies in which two components of BENPAT, N-phenyl-N'-(o-tolyl) p-phenylenediamine (CAS No. 27173-16-6) and N,N'-di-(o-tolyl)-p-phenylenediamine (CAS No. 15017-02-4), have been investigated, respectively as radiolabelled admixture to BENPAT. Both oral studies were not performed according to an

acknowledged test guideline. However, the results are useful to assess the extent of oral absorption. Both studies also investigated the extent of biliary excretion and metabolism. Urine, faecal and bile samples were collected over a period of 48 hours following oral dosing to bile duct-cannulated animals. Results showed that 76 to 78 % of the administered radioactivity was excreted within this 48 hour observation period. The main part, about 75 % of the dosed radioactivity was excreted in the faeces. Urinary excretion consisted of (unidentified) metabolites and accounted for maximally 2.5 % of the total amount of radioactivity. Analysis of the biliary fraction showed that 30 to 35 % of the administered radioactivity entered into the gastro-intestinal tract via the biliary route. HPLC analysis of bile demonstrated that > 95 % of the metabolites excreted by this route exhibited greater polarity than the parent compound, suggesting metabolic formation of oxidation and conjugation products of these components.

In both studies, reference is given to a study performed with a further main constituent of BENPAT, DPPD (N,N'-diphenyl-p-phenylenediamine; CAS No.: 74-31-7). However, the study performed with CAS number 74-31-7 is not presented in the CSR or in the IUCLID file. The registrant stated that "the majority (55 %) of orally administered DPPD is excreted in faeces of rats with < 0.1 % excreted in urine" (ECHA dissemination site, last accessed 19 November 2019).

Dermal absorption has been investigated with N,N'-bis(2-methylphenyl) benzene-1,4-diamine (CAS No. 15017-02-4), one of the main constituents of BENPAT. The radiolabelled ([^{14}C]-label) substance had been administered by the dermal and intravenous route to female rats (University of California, 1997). Urinary and faecal excretion was monitored over a period of 168 h after dosing. Recoveries of radioactivity were 70 % and 88 % after intravenous and dermal dosing, respectively. After dermal administration, skin site rinses, tape stripping and skin digestion samples to recover unabsorbed residues were also analysed. Comparison of excreted radioactivity from intravenously and dermally treated animals indicated that 60 % of the dermally applied test chemical was absorbed during the study period (7 days). Most of the absorbed test compound (> 95 %) had been excreted via the faeces.

The study report states that the study has been performed according to EPA FIFRA 40 CFR, part 160 guideline. However, that guideline describes a GLP statement rather than a test guideline and does not address how to conduct *in vivo* dermal absorption studies. Thus, the assignment of Klimisch code 1 (reliable without restriction) is not supported by the DS. Further, important measurements considered essential in OECD TG 427 (*in vivo* dermal absorption study) apparently were not addressed in this study, such as determination of quantities in blood and quantities in the remaining carcass.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Data for oral and dermal toxicokinetics for the registered substance or components thereof are available from animal studies. Human information is not available at present.

Study results show that one main component of BENPAT (CAS No.: 15017-02-4) is dermally absorbable and most amounts of BENPAT components (CAS No. 15017-02-4, 27173-16-6) are excreted in the faeces after oral and dermal administration. Furthermore, bile analysis in oral toxicokinetic studies demonstrated that the major fraction of the administered substances were present as metabolites, however, metabolites had not been identified.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier.

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Skin sensitisation is an immunological process consisting of two phases. At first, during induction, the chemical forms a hapten-protein-complex in the skin of naive individuals. A sequential set of events follows, leading to the production of allergen-specific memory T-cells. In the second phase, the elicitation, exposure of the sensitised individual to the allergen leads to proliferation and activation of these T-cells, secretion of cytokines and mobilisation of other inflammatory cells resulting in a clinical outcome of allergic contact dermatitis (ECHA, 2017).

There is one animal study available addressing the sensitising potential of BENPAT and performed according to OECD TG 406.

Table 8: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results[1]**	**Reference**
According to OECD TG 406 (GPMT) Reliability: 1, reliable without restriction GLP-compliant	Guinea pig, Hartley, male/ female n=20/test group n=10/ control group	BENPAT, test substance, reference 1 Purity: no information	Intradermal induction: (1) 5.0 % test chemical in acetone/propylene glycol), (2) 5.0 % test chemical in acetone/propylene glycol/ FCA emulsion, and (3) FCA emulsion; Topical induction: 100 % test chemical (0.35 g); Epicutaneous challenge: 25 % or 100 % test substance (vehicle: acetone/mineral oil); Reading after 24 and 48h: 24h (25 %): 5/20 positive reactions 48h (25 %): 3/20 positive reactions 24h (100 %): 0/20 positive reactions 48h (100 %): 5/20 positive reactions Re-challenge (day 28): 24h (25 %): 15/20 positive reactions 48h (25 %): 11/20 positive reactions 24h (100 %): 15/20 positive reactions 48h (100 %): 15/20 positive reactions	**Positive** **No sub-categorisation possible due to high intradermal induction dose**	

[1] According to the Guidance on the Application of the CLP Criteria, Version 5.0

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results[1]	Reference
			Positive control (0.1 % DNCB[2]): valid, 6/6 positive reactions		

The sensitising potential of BENPAT was investigated in a GLP-compliant guinea-pig maximisation test (GPMT) performed according to OECD TG 406 (Charles River, 1995). The induction phase included both intradermal and epidermal exposure to solutions of 5 % test substance in Freund's adjuvant or acetone/propylene glycol. Animals received two epicutaneous challenge administrations on day 21 (1st challenge) and day 28 (2nd challenge), respectively. The dermal response following 48h after the first challenge to 25 % or 100 % of chemical resulted in clinical effects (oedema or desquamation) for 15 % or 25 % of test animals, respectively. Forty-eight hours after the second challenge, 55 % of the animals exposed to 25 % of test chemical showed signs of desquamation, oedema or blanching. In the test group exposed to 100 % of test chemical, 75 % of the animals showed signs of a sensitisation reaction after 48 hours. This study indicates that BENPAT acts as a sensitiser. Since the concentration selected for intradermal induction (5 %) is higher than those required for classification as Skin Sens. 1A, this study does not allow for a reliable sub-categorisation.

There are no human data available, addressing the sensitising potential of BENPAT.

Notably, several studies showed that the BENPAT constituent, N, N'-diphenyl-p-phenylene-diamine (DPPD; EC: 200-806-4, CAS: 74-31-7), produced sensitisation reactions in animal models (LLNA BrdU-ELISA, GPMT) and elicits skin sensitisation in humans (human patch test studies, case reports). DPPD has a harmonised classification and labelling in Annex VI to the CLP regulation as Skin Sens. 1, supporting the skin sensitising potential of BENPAT.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

A GLP-compliant GPMT conducted according to OECD TG 406 shows that BENPAT should be classified as a sensitiser. The study does not provide a reliable basis for sub-categorisation.

Data addressing the skin sensitisation potential of BENPAT in humans are not available to the DS.

Furthermore, DPPD, one of the main constituents of BENPAT, already has a harmonised classification as Skin Sens. 1 in Annex VI to the CLP regulation.

10.7.2 Comparison with the CLP criteria

In Table 9, animal studies on skin sensitisation are compared with CLP criteria, as laid down in ECHA's Guidance on the Application of the CLP criteria.

[2] 2,4-Dinitrochlorobenzene, CAS: 97-00-7

Table 9: Comparison of available data (animal studies) for skin sensitisation of BENPAT or DPPD with the CLP criteria

<table>
<tr><th>Reference(s)</th><th>Criteria acc. to CLP regulation, as laid out in (ECHA, 2017)</th><th>Results</th><th>Resulting Classification</th></tr>
<tr><td colspan="4">Animal data</td></tr>
<tr><td>GPMT

(Charles River, 1995)</td><td><u>Skin Sens. 1A:</u>
≥ 30 % responding at ≤ 0.1 % intradermal induction dose or
≥ 60 % responding at > 0.1 % to ≤ 0.1 % intradermal induction dose intradermal induction dose

<u>Skin Sens. 1B:</u>
≥ 30 % to < 60 % responding at > 0.1 % ≤ 1 % intradermal induction dose or
≥ 30 % responding at > 1 % intradermal induction dose</td><td>Following intradermal induction with a test substance concentration of 5 %, 55-75 % of the guinea pigs showed a positive reaction at re-challenge.</td><td>Skin Sens. 1

No sub-categorisation</td></tr>
<tr><td>Annex VI to the CLP regulation, index number 612-132-00-1: N,N'-diphenyl-p-phenylene-diamine
(DPPD; EC 200-806-4, CAS 74-31-7) classified as skin sensitiser, Category 1</td><td>Generic concentration limits (GCL) of components classified as skin sensitiser that trigger classification of the mixture as Skin Sens. 1

<table><tr><th>Component classified as</th><th>Conc. that trigger classification of mixture</th></tr><tr><td>Skin Sens. 1</td><td>≥ 1.0 %</td></tr><tr><td>Skin Sens. 1A</td><td>≥ 0.1 %</td></tr><tr><td>Skin Sens. 1B</td><td>≥ 1.0 %</td></tr></table></td><td>BENPAT component DPPD (≥ 1.0 %), classified as Skin Sens 1
→ BENPAT classified as Skin Sens 1</td><td>Skin Sens. 1

No sub-categorisation</td></tr>
</table>

A GLP-compliant GPMT performed according to OECD TG 406 (Charles River, 1995) reveals that BENPAT is a skin sensitiser (55-75 % of the guinea pigs showed a positive reaction upon re-challenge, following intradermal induction with a test substance concentration of 5 %). Category 1 is appropriate since a lower concentration to show absence of effects at lower doses than 1 % was not tested.

In addition, one of the main BENPAT constituents, DPPD, has a harmonised classification as Skin Sens. 1 in Annex VI to the CLP regulation, without sub-categorisation. In line with Art. 11 (1) and Table 3.4.5 in Annex I of the CLP Regulation, therefore, a substance containing DPPD as a constituent at concentrations ≥ 1.0 % should also be classified as Skin Sens. 1.

In summary, the available data provide conclusive evidence that BENPAT acts as skin sensitiser, but do not allow for sub-categorisation.

10.7.3 Conclusion on classification and labelling for skin sensitisation

The DS proposes to classify BENPAT as a moderate skin sensitiser, i.e. **Skin Sens. 1 (H317 - May cause an allergic skin reaction)**.

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Hazard class not assessed in this dossier.

10.10 Reproductive toxicity

Table 10: Summary table of animal studies on (statistically significant) adverse effects on sexual function and fertility, and development (Data include changes in organ weight, with an at least 10 % effect level compared to controls.)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group, reference</th><th>Test substance, dose levels</th><th>Results</th></tr>
<tr><td>Two-Generation Reproductive Toxicity Study, according to OECD TG 416

<u>Key study for adverse effects on sexual function and fertility, and development</u>

Reliability: 1, reliable without restriction
GLP-compliant

Rats, CD® Sprague-Dawley, N=30/sex/dose group (F0 & F1 parents)

Exposure F0 and F1-generation: Pre-breeding (10 weeks), mating (two weeks), gestation (three weeks), lactation (three weeks)

Exposure F2-generation: Mating (two weeks), gestation (three weeks), lactation (three weeks)

Necropsy:
F0 males after delivery (histologic evaluation of reproductive & target</td><td>Dietary doses of <u>BENPAT,</u> 1,4-Benzene-diamine, N,N'-mixed Ph and tolyl derivs., <u>CAS: 68953-84-4</u>, test substance reference 1 (in 2 % corn oil) at 120, 400, and 1500 ppm, corresponding to 0, 7.5, 25 and 100 mg/kg bw/d

Purity: No data</td><td>
<table>
<tr><th>F0 adults</th><th>F1 adults</th></tr>
<tr><td colspan="2">Effects on sexual function and fertility[3]</td></tr>
<tr><td colspan="2">100 mg/kg bw/ d</td></tr>
<tr><td>↑ gestational length (5.9 %; 23.5 d vs. control: 22.2 d);
↑ % post-implantation loss (41.6 %; 52.3 % vs. control: 10.7 %);
↓ no. total pups/ litter (12.1 vs. control: 15.7);
↓ no. live pups/ litter (7.6 vs. control: 15.6);
↑ no. of dead pups/ litter (4.1 vs. control: 0.1)</td><td>↑ gestational length (4.5 %; 23.2 d vs. control: 22.2 d);
↑ % post-implantation loss (16.8 %, 23.6 % vs. control: 6.8 %);
↓ no. live pups/ litter (10.8 vs. control: 15.6);
↑ no. of dead pups/ litter (2.5 vs. control: 0.1);

↑ no. with abnormal stage of oestrous cycle (43.3 % (13/30), control: 6.7 % (2/30)); ↑ ♀ in metestrus (31.0 % (9/29), control 10 % (3/30)); ↑ mean cycle length (7.2 d, compared to control: 5.1 d; not statistically significant)</td></tr>
<tr><td colspan="2">25 mg/kg bw/d</td></tr>
<tr><td>↑ gestational length (2.7 %; 22.8 d vs. control: 22.2 d);
↑ % post-implantation loss (15.4 %; 26.1 % vs. control: 10.7 %);
↓ no. total pups/ litter (12.3 vs. control: 15.7);
↓ no. live pups/ litter (11.9 vs. control: 15.6)</td><td>↑ gestational length (4.1 %; 23.1 d vs. control: 22.2 d);

↑ no. with abnormal stage of oestrous cycle (28.6 %, control: 6.7 %);

↑ abnormal cycles (8/30 abnormal cycling and 2/30 not cycling, control 2/30 with abnormal cycle and 0/30 not cycling); ↑ ♀ in metestrus (7/29, compared to control 3/30); cycle length comparable to control</td></tr>
<tr><td colspan="2">7.5 mg/kg bw/d</td></tr>
</table>
</td></tr>
</table>

[3] Values for effects on sexual function and fertility are included in Table 11.

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results	
tissues for high dose and control males). F1/ F2 litters at weaning (three weanlings/sex/litter) F0 females after weaning of F1 litters (histopathology of reproductive & target organs for high dose and control animals) F1 males were necropsied after the delivery period Parental F1 females after weaning of the F2 litters **(RTI, 2001a)**			↑ gestational length (2.7 %; 22.8 d vs. control: 22.2 d); ↑ ♀ in metestrus (8/30; control 3/30); cycle length comparable to control
		General toxicity[4], [5]	
		100 mg/kg bw/ d	
		↓ BW (ca. -7 %) in ♂ for pre-breeding and mating period; ↓ BW (-7 %) in ♀ for SD 7 to SD 63 of pre-breeding period; ↓ BW (-10 %) in ♀ during gestation, ↓ BWG in ♀ during gestation (-18 %) ↓ BW in ♀ for lactation, PND 0, 4, 7 (-11 %); ↑ BWG in ♀ during lactation (PND 0-21) 4 ♀ dead during lactation period (died in process of delivering (1 ♀), euthanised in process of delivering, PND 0 (1 ♀), euthanised moribund, PND 0 (1 ♀), found dead, PND 1 (1 ♀); 3 ♀ died and 1 ♀ euthanised moribund during holding period until scheduled sacrifice, all ♀ had pups that died during delivery; ↑ gestation length (23d -25d for 6 ♀, 2 ♀ died/euthanised in process of delivering); piloerection (6 ♀); vaginal bleeding, GD23/24 (5 ♀); litter with all-dead pups on PND 0 (7 ♀); *Unscheduled necropsy of dams that died during lactation:* Liver: necrosis (7 ♀), inflammation (1 ♀), haemorrhage (1 ♀); kidney: necrosis (5 ♀), inflammation (1 ♀); uterus: haemorrhage (2 ♀), inflammation (3 ♀), retained dead foetuses *in utero* (3 ♀), retained placenta (2 ♀), resorbing implants (1 ♀), uterus was too autolyzed to evaluate (1 ♀); vagina: retained foetus (2 ♀), haemorrhage (1 ♀); adrenal cortex: degeneration (haemorrhage) (2 ♀); ovary: cysts	↓ BW (-7 %) in ♂ for last three weeks of pre-breeding; ↓ BW (-7.0 %) in ♂ for mating period; ↓ BWG (-11 %) in ♀ during gestation; ↓ BW (-9 %) in ♀ for lactation PND 0, 4, 7; ↓ BW (-7 %) in ♂ at scheduled sacrifice One ♀ died (PND 0) while delivering; uterus inflammation, haemorrhage, thrombosis; kidney polycystic, mineralisation, inflammation; liver necrosis; bladder hyperplasia, inflammation; lungs oedema, alveolus; five foetuses retained in left horn of uterus, five foetuses and numerous blood clots in right horn of uterus, and one placenta retained in vagina *Scheduled necropsy*: ↑ relative paired kidney weight (10.1 %) in ♂; ↓ % progressively motile sperm (21.4 %, control: 27.7 %); ↑ polycystic kidneys in ♂ (21/30) & ♀ (18/30); ↑ tubule dilatation in the renal papilla in ♂ (3/30) & ♀ (4/30); ↑ kidney chronic inflammation in ♀ (9/30); ↑ renal tubule regeneration in ♂ (16/30) & ♀ (14/30)

[4] Absolute body weight assumed, not specified by study author

[5] Incidence of polycystic kidneys in F0 and F1 animals is summarised in Table 13.

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results	
		(follicle) (1 ♀); lung: thrombosis (2 ♀), inflammation (2 ♀), congestion (1 ♀) *Scheduled necropsy:* ↑ relative paired kidney weight (10.7 %) in ♂ & (12.0 %) in ♀; ↑ absolute liver weight (14.0 %) in ♀, ↑ relative liver weight (19.8 %) in ♀; ↓ absolute (-23.0 %) and relative (-19.6 %) uterine weight; polycystic kidneys (3/30 ♀), kidney cortical necrosis (5/30 ♀); liver hematopoietic cell proliferation (4/30 ♀), hepatocellular centrilobular necrosis (7/30 ♀); lung inflammation (2/30 ♀), thrombosis (2/30 ♀); adrenal cortex degeneration (2/30 ♀)	
		25 mg/kg bw/d	
		1 ♀ found dead (GD 17), implantation sites not consistent with gestational age; amniotic sacs blood filled; 16 resorbing foetuses *in utero*; ovary mineralisation, oviduct; uterus haemorrhage; mammary gland adenocarcinoma neoplasm (malignant without metastasis) 1 ♀ died in process of delivering (PND 0); vagina: one retained foetus; uterus left horn: three retained foetuses, blood in amniotic sacs; uterus right horn: six retained foetuses; thymus with red foci (haemorrhage); lungs all lobes reddened; liver all lobes pale; kidney cortex pale; adrenals enlarged 1 ♀ found dead (PND 2), prolonged gestation (23 d), delivered six living pups; uterus right horn: two retained foetuses; vagina: one retained foetus; liver necrosis; lungs – congestion; kidney necrosis cortex; uterus haemorrhage, inflammation *Scheduled necropsy:* ↓ absolute (-20.4 %) and relative uterine weight (-20.7 %) kidney cortical necrosis (1/11 ♀); hepatocellular centrilobular	1 ♀ died on PND 3, delivered 10 live pups and 4 dead pups; liver necrosis, all lung lobes congested with irregular patchy consolidation, and one foetus retained in the vagina. *Scheduled necropsy:* ↓ relative paired ovary weight (-17.61%) ↑ polycystic kidneys in ♂ (10/30) & ♀ (1/30); ↑ tubule dilatation in the renal papilla in ♂ (1/30) & ♀ (1/30); ↑ kidney chronic inflammation in ♀ (4/30); ↑ renal tubule regeneration in ♂ (1/30) & ♀ (1/30)

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results	
		necrosis (1/11 ♀)	
		7.5 mg/kg bw/d	
		↓ absolute (-11.5 %) and relative paired ovary weight (-12.0 %); two ♂ with kidneys exhibiting "pits"	↓ % progressively motile sperm (21.7 % vs control: 21.4 %) ↑ polycystic kidneys in ♂ (5/30) & ♀ (2/30); ↑ kidney chronic inflammation in ♀ (2/30); ↑ renal tubule regeneration in ♂ (1/30)
		F1 offspring	**F2 offspring**
		Offspring toxicity (PND 0)	
		100 mg/kg bw/ d	
		F1 pup necropsy findings in dead pups: Ductus (arteriosus) open (patent) and *in utero*; dead pups with closed or open (patent) ductus; cannibalised, autolysed, unable to evaluate	↑ BW (5 %); F2 pup necropsy findings in dead pups: dead pups with closed or open (patent) ductus; autolysed, unable to evaluate; abdominal organs autolyzed, unable to evaluate
		25 mg/kg bw/d	
		↑ BW (7 %); F1 pup necropsy findings in dead pups: Ductus (arteriosus) open (patent) and *in utero*; dead pups with closed or open (patent) ductus; cannibalised, autolysed, unable to evaluate	↑ BW (11 %); F2 pup necropsy findings in dead pups: dead pups with closed or open (patent) ductus; abdominal organs autolyzed, unable to evaluate
		7.5 mg/kg bw/d	
		↑ BW (6 %); F1 pup necropsy findings in dead pups: dead pups with closed or open (patent) ductus; cannibalised, unable to evaluate	↑ BW (9 %); F2 pup necropsy findings in dead pups: dead pups with closed or open (patent) ductus; autolysed, unable to evaluate
		Weanlings toxicity (PND 21)	
		100 mg/kg bw/ d	
		↓ absolute (-11.2 %) thymus weight in ♂, ↑ relative spleen weight (16.6 %) in ♀, ↓ absolute (-10.8%) & relative brain weight (-10.2 %) in ♀; ↑ polycystic kidney in ♂ (10/11) & ♀ (11/11),	↑ polycystic kidney in ♂ (15/16) & ♀ (15/15)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group, reference</th><th>Test substance, dose levels</th><th>Results</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>↑ renal tubule regeneration in ♂ (9/11) & ♀ (10/11)</td><td></td></tr>
<tr><td colspan="2">25 mg/kg bw/ d</td></tr>
<tr><td>↑ BW in ♂ (9.2 %) & (9.1 %) in ♀;
↑ absolute thymus weight (11.6 %) in ♂ & (12.7 %) in ♀;
↑ absolute spleen weight (34.7 %) in ♂ & (23.2 %) in ♀;
↑ relative spleen weight (23.8 %) in ♂ & (13.8 %) in ♀;
↓ relative brain weight in ♀ (-11.2 %);
↑ polycystic kidneys in ♂ (8/20) & ♀ (7/18)</td><td>↑ BW (12.3) in ♂ & (8.6 %) in ♀,
↑ absolute thymus weight (10.9 %) in ♂; ↑ absolute spleen weight (22.7 %) in ♂;
↓ relative brain weight (-13 %) in ♂
↑ polycystic kidney in ♂ (6/19) & ♀ (8/19)</td></tr>
<tr><td colspan="2">7.5 mg/kg bw/ d</td></tr>
<tr><td>↑ absolute spleen weight (20.0 %) in ♂ & (17.3 %) in ♀;
↑ relative spleen weight (13.7 %) in ♂ & (12.7 %) in ♀;
↑ polycystic kidney in ♂ & ♀, chronic inflammation, nephropathy
↑ polycystic kidneys in ♂ (1/25) & ♀ (5/26)</td><td>↑ BW (8.1 %) in ♂;
↑ absolute thymus weight (13.6 %) in ♂;
↑ absolute spleen weight (15.1 %) in ♂;
↑ polycystic kidney in ♂ (3/64) & ♀ (5/64)</td></tr>
</table>
One F0 female at 0 mg/kg bw/d was euthanised on SD 17, probably due to a broken left hind leg; one male at 0 mg/kg bw/d was sacrificed moribund on SD 85; one F1 female at 0 mg/kg bw/d died on SD 97

A maternal NOAEL of 25 mg/kg bw/d is derived based on lethality of dams during lactation. It was not possible to identify a NOAEL for sexual function and fertility, a LOAEL of 7.5 mg/kg/day is identified, the lowest dose tested, based on prolonged gestation and dystocia. A developmental NOAEL was not established and a developmental LOAEL of 7.5 mg/kg bw/d is derived, based on polycystic kidneys in F1 and F2 generations.
</td></tr>
<tr><td>Prenatal Developmental Toxicity Study, according to OECD TG 414

<u>Key study for adverse effects on development</u>

Reliability: 1, reliable without restriction(defined by the registrant); however the DS assess this study with Reliability 2: reliable with restriction, because evaluation of effects of BENPAT</td><td>Oral gavage of BENPAT, test substance reference 2 (dissolved in corn oil) at doses of 0, 20.0, 70.0 and 200.0 mg/kg bw/d</td><td><u>General toxicity F0 generation</u>
<u>200 mg/kg bw/d</u>
↓ maternal BW at GD 12 (-5.4 %); ↓ maternal weight gain during dosing period, for GD 6-9 (-87.8 % weight gain, compared to control), and 6-15 (-30.5 % weight gain, compared to control) ; no statistical significance for maternal body weight gain, when corrected (minus gravid uterine weight); data for maternal body weight corrected (minus gravid uterine weight) not available

<u>Effect on Development</u>
No treatment-related statistically or biologically significant changes in the incidence of individual or pooled external, visceral (including craniofacial), skeletal or total foetal malformations or variations</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results
during late pregnancy were not possible (no treatment from GD 16) GLP-compliant Rats, CD® (Sprague-Dawley), N=25/dose group Exposure of F0 ♀ on gestation days (GD) 6-15; Scheduled sacrifice on GD 20 Evaluation of dams body, liver, & gravid uterine weights; ovarian corpora lutea; uterine implantation sites (resorptions, dead foetuses, live foetuses) Foetuses were dissected from uterus, counted, weighed, sexed, & examined for external abnormalities; examination for visceral malformations & variations, soft tissue craniofacial malformations and variations, and skeletal malformations and variations **Deviations:** No treatment from GD 16 until GD 20, time window too short to evaluate adverse effects of BENPAT during late pregnancy **(RTI, 1995)**		There were no adverse effects seen during this study, the maternal and developmental NOAEL is 200 mg/kg bw/d, the highest dose tested. A NOEL of 70 mg/kg bw/d was established in maternal animals, due to decreased body weight and body weight gain in high dose animals.
Range-finding study, for Prenatal Developmental Toxicity Study (OECD TG 414) **Supporting study**	Oral gavage of BENPAT, test substance reference 2	**600 mg/kg bw/d** ↓ Mean maternal body weights at GD 9 (-11.6 %), 12 (-16.8 %), and 15 (-11.6 %); significant dose-related downward trends for maternal body weights on GD 18 (-8.0%) and 20 (-6.5 %); no data available for body weight corrected (minus gravid uterine weight);

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results
Rats, CD® (Sprague-Dawley) N=8/ dose group Exposure: GD 6-15 Observation: dams body weight and food consumption (GD 0,6, 9, 12, 15, 18, and 20); clinical signs (once daily through gestation, at least twice daily during dosing period) Necropsy on GD 20: gravid uterus and liver weight, ovarian corpora lutea count, status of uterine implantation sites; total, non-live (early or late resorptions, dead foetuses), live foetuses. All live foetuses were weighed, sexed externally (except tor two litters which were inadvertently not sexed), examined for external malformations (including cleft palate) and variations **(RTI, 1995)** – Appendix V	(dissolved in corn oil) at doses of 0, 20.0, 70.0, 200.0, and 600 mg/kg bw/d	↓ maternal weight gain tor GD 6-9 (-22.8 g vs control: 11.7 g), 6-15 (dosing period; 15.3 g vs control: 51.7 g); ↓ maternal weight gain corrected to weight of the gravid uterus, GD 0-20 (41.8 g vs control: 72.3 g) ↓ maternal feed consumption (g/kg/d) for GD 6-9; -60.0 % (GD 6-15: the lack of statistical significance due to the fewer dams alive at this dose tor this interval); ↑ maternal feed consumption for GD 15-18 (22.6 %) and 15-20 (19.7 %; post-dosing period), consistent with weight gain for this period 4/8 dams died: One dam died on GD 14, evidence of vaginal bleeding on GD 12 and 13; one dam found dead on GD 14, with blood still evident around vaginal area; Two other dams, who died or were sacrificed moribund on GD 12, with resorbing conceptuses; At necropsy: Dead dams exhibited pale organs (e.g. kidneys, lungs, liver, ovaries, spleen) and extremities (ears, eyes, tail, and skin) Evidence of vaginal bleeding in one third dam at GD13 (15 implants and 15 live foetuses at scheduled sacrifice) ↓ Foetal body weights/ litter (-12.6 %; all foetuses, and males and females separately) **200 mg/kg bw/d** ↓ Mean maternal body weights at GD 9 (-7.8 %) and 15 (-8.6 %) ↓ maternal weight gain for GD 6-9 (-7.1 g vs control: 11.7 g), 6-15 (27.1 g vs control: 51.7 g), 0-20 (gestation; (127.8 g vs control: 154.0 g)) ↓ maternal weight gain corrected to weight of the gravid uterus, GD 0-20 (gestation; 48.1 g vs control: 72.3 g) ↓ maternal feed consumption (g/kg/d) for GD 6-15 (58.2 g vs control: 67.3 g) ↓ Foetal body weights/ litter (-6.3 %; all foetuses, and females; value for male foetuses was also reduced but not statistically significantly) **70 mg/kg bw/d** Evidence of vaginal bleeding in one dam on GD 13 (15 implants and 15 live foetuses at scheduled sacrifice) **20 mg/kg bw/d** Evidence of vaginal bleeding in one dam on GD 13 (13 implants and 13 live foetuses at scheduled sacrifice) Maternal gravid uterine weights, and absolute and relative maternal liver weights were equivalent across all groups at scheduled sacrifice

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results
		No statistically significant or biologically important treatment- or dose-related changes in pre- or post-implantation loss/litter, incidence of non-live implants (including resorptions and dead foetuses)/litter, incidence of adversely affected implants (non-live plus externally malformed), of the number at live foetuses/litter, or of sex ratio (% male foetuses)/litter. External examination of all live foetuses indicated no external malformations or variations detected in this study.
One Generation Mechanistic Study, non-guideline study **Supporting study** Reliability: 2, reliable with restriction None GLP-compliant Rats, CD® Sprague-Dawley, N=20/dose group Exposure: Group 1: Negative control, Group 2: Prebreed and mating Group 3: Gestation and lactation Group 4: Prebreed, mating, gestation, and lactation Group 5: Prebreed, mating, gestation, and lactation, plus 600 mg iron gluconate in drinking water (prebreed through lactation) Corresponding to 180-185 mg/kg bw/day of BENPAT during prebreed and mating, 157-167 mg/kg bw/day (gestation), and 347-436 mg/kg bw/day (lactation) Analysis of maternal blood (GD 21 and PND 21), F1 offspring blood	Dietary dose of **BENPAT,** 1,4-Benzene-diamine, N,N'-mixed Ph and tolyl derivs., test substance reference 1 (in corn oil) at 2500 ppm Purity: No data	**F0 generation - effects on sexual function and fertility** **Group 2 (prebreed & mating)** No significant effects One ♀ delivering on GD 21 (no longer exposed) **Group 3 (gestation & lactation)** ↑ gestational length (23.6 d vs control: 22.2 d), ↓ gestational index (no. females with live litters/no. females pregnant; 64.7) ↑ dams delivering litters with all-dead pups, followed by dams euthanasia (5 dams), ↓ live birth index (54.6 vs control: 96.9), ↑ stillbirth index (45.4 vs control: 3.1), ↓ total & average pups/ litter (PND 0; 9.9 vs control: 13.0 and 7.9 vs control: 12.6, respectively) **Group 4 (pre-breeding, mating, gestation, & lactation)** ↑ gestational length (23.8 d vs control: 22.2 d, ↓ gestational index (no. females with live litters/no. females pregnant; 71.4) ↑ dams delivering litters with all-dead pups, followed by dams euthanasia (5 dams), ↓ live birth index (54.0 vs control: 96.9), ↑ stillbirth index (46.0 vs control: 3.1), ↓ average pups/ litter (PND 0; 8.8 vs control: 12.6) **Group 5 (prebreed, mating, gestation, & lactation plus iron)** ↑ gestational length (23.5 d vs control: 22.2 d), ↓ No. implantation sites/ litter (10.64 vs control: 14.5), ↓ total & average pups/ litter (PND 0; 9.5 vs control: 13.0 and 8.2 vs control: 12.6, respectively) **F0 generation - general toxicity** **Group 2 (prebreed & mating)** ↓ ♂ BW gain for study day 0-7 (43.8 g vs. control: 53.7 g); ↓ ♂ feed consumption (g/kg/day) for study day 21-28 (exposed; 5.1 %); ↓ ♀ BW at study days 7 (-6.2 %), 14 (-7.7 %), 21 (-9.0 %), and 28 (-8.1 %) ↓ ♀ BW gain for study days 0-7 (16.0 g vs. control: 27.8 g), 7-14 (11.7 g vs. control: 16.1 g), 14-21 (11.2 g vs. control: 15.9 g), and 0-28 (42.4 g vs. control: 61.2 g) ↓ ♀ feed consumption (g/kg/day) from study day 0-7 (exposed; -12.8 %); ↑ ♀ feed consumption (g/kg/day) from gestation day 0-7 (no longer exposed; 12.1 %) **Group 3 (gestation & lactation)** ↑ ♂ BW gain for study day 0-28 (not exposed; 179.8 g vs. control: 158.1 g); ↓ ♀ BW at gestation day 7 (-8.0 %) and 21 (-12.3 %); ↓ ♀ BW gain from gestation day 0-21 (97.0 g vs control: 140 g);

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results
(PND 21) for cellular components and methemoglobinemia F0 females and pups (one pup/sex/litter, for a maximum of ten/sex/group) were necropsied at PND 21. Maternal spleens, livers, kidneys were weighed and retained in fixative; and spleen, liver, kidneys, and heart of pups were weighed and retained fixative. Kidneys of control group & group 5 and offspring kidneys from all groups were examined histopathologically **(RTI, 2000)**		↓ ♀ BW at PND 0 (-12.7 %), 4 (-13.2) and 7 (-12.6 %); ↑ BW gain PND 0-21 (32.6 g vs control: 12.2 g) ↓ ♀ feed consumption (g/kg/day) for gestation day 0-7 (-5 %), and PND 7-14 (-17.5 %) One ♀ found dead (GD 19), 16 retained dead foetuses in utero, blood clots in amniotic sacs, no other findings Five dams "euthanized, entire litter dead" on PND 0/3, prolonged gestation: 24-25d; Findings in uterus: dead foetuses present; vagina: dead foetus present; liver: pale/ white foci present on ventral surface of distal portion of median lobe; kidney: pale, Tail: tip necrotic *Scheduled necropsy:* Pale spleen with white foci on surface in one ♀, kidneys not histopathologically examined **Group 4 (prebreed, mating, gestation, & lactation)** ↓ ♂ BW at study day 7 (-4.7 %), 14 (-5.4 %), and 21 (-5.7 %); ↓ ♂ BW gain for study days 0-28 (137.3 g vs. control: 158.1 g) ↓ ♂ feed consumption (g/kg/day) for study day 21-28 (exposed; 5.6 %) ↓ ♀ BW at study days 7 (-5.5), 14 (-7.3 %), 21 (-9.8 %), and 28 (-8.6 %); ↓ ♀ BW gain for study days 0-7 (16.3 g vs. control: 27.8 g), 7-14 (11.2 g vs. control: 16.1 g), 14-21 (8.3 g vs. control: 15.9 g), and 0-28 (40.1 g vs. control: 61.2 g); ↓ ♀ BW at PND 0 (-12.8 %), 4 (-11.7) and 7 (-11.3 %); ↑ BW gain PND 0-21 (39.3 g vs control: 12.2 g) Five dams "euthanized, entire litter dead" on PND 0/3, prolonged gestation: 24-25 d; Findings in liver: pale; kidney: pale, bilateral, not histopathologically examined; uterus: dead foetuses present *Scheduled necropsy:* ↑ absolute and relative liver weight in ♀, ↑ relative paired kidney weight in ♀; kidneys not histopathologically examined **Group 5 (prebreed, mating, gestation, & lactation plus iron)** ↓ ♂ BW at study day 7 (-4.5 %), 14 (-5.1 %), and 21 (-5.1 %); ↓ ♂ BW gain for study day 0-7 (42.3 g vs. control: 53.7 g) ↓ ♂ feed consumption (g/kg/day) for study day 21-28 (exposed; 4.9 %) ↓ ♀ BW at study days 7 (-5.3 %), 14 (-5.7 %), 21 (-7.2 %), and 28 (-7.2 %), gestation day 21 (-9.4 %); ↓ ♀ BW gain for study days 0-7 (16.2 g vs. control: 27.8 g), 14-21 (11.3 g vs. control: 15.9 g), and 0-28 (43.0 g vs. control: 61.2 g); gestation day 0-21 (112.1 g vs control: 140.0 g); ↓ ♀ BW at PND 0 (-8.8 %), 4 (-8.8) and 7 (-8.8 %); ↑ BW gain PND 0-21 (32.5 g vs control: 12.2 g) One ♀ unscheduled sacrifice post-mating; with foetal/placental remains; kidney: foci; adrenals: congestion; lungs: congestion; spleen: enlarged; thymus: reduced *Scheduled necropsy:* ↑ absolute and relative liver weight in ♀, ↑ relative paired kidney weight in ♀; ↑ polycystic kidneys (15 %) in ♀ **F1 generation - offspring toxicity (PND 0)** **Group 2 (prebreed & mating)** No significant effects

Method, guideline, deviations if any, species, strain, sex, no/group, reference	Test substance, dose levels	Results
		Group 3 (gestation & lactation) ↑ post-implantation (prenatal) loss (57.5 % vs control 12.2%), ↓ live litter size (10 vs control: 15); **Group 4 (prebreed, mating, gestation, & lactation)** ↑ post-implantation (prenatal) loss (55.8 % vs control 12.2%), ↓ live litter size (9 vs control: 15); **Group 5 (prebreed, mating, gestation, & lactation plus iron)** ↑ Average pup BW/litter (PND 0; 14.1 %) **F1 generation – general toxicity weanlings (PND 21)** **Group 2 (prebreed & mating)** No indications of polycystic kidneys **Group 3 (gestation & lactation** ↑ polycystic kidney in ♂ (97 %) & ♀ (96 %), ↑ dilation of collecting tubules in renal papilla (♂: 31/38 vs control: 0/80; ♀: 20/25 vs control: 0/67); ↑ haemoglobin concentration in ♂ (15.2 %); ↑ mean corpuscular haemoglobin concentration in ♂ (1.9 %) & ♀ (2.4 %); ↑ platelets in ♂ (29.5 %) ↑ relative liver weights in ♂ (22.6 %) & ♀ (18.4 %) **Group 4 (prebreed, mating, gestation, & lactation)** ↑ polycystic kidney in ♂ (95 %) & ♀ (91 %), ↑ dilation of collecting tubules in renal papilla (♂: 28/34 vs control: 0/80 ♀: 30/32 vs control: 0/67); ↑ mean corpuscular haemoglobin concentration in ♂(2.1 %) & ♀ (1.8 %); ↑ platelets in ♂ (53.1 %) & ♀ (29.8 %) ↑ relative liver weights in ♂ (26.3 %) & ♀ (19,6 %); ↑ relative heart weights in ♂ (26.1 %) **Group 5 (prebreed, mating, gestation, & lactation plus iron)** ↑ polycystic kidney in ♂ (91 %) & ♀ (100 %), ↑ dilation of collecting tubules in renal papilla (♂: 36/40 vs control: 0/80; ♀: 33/48 vs control: 0/67); ↑ haemoglobin concentration in ♂(15.2 %); ↑ mean corpuscular haemoglobin in ♂ (12.5 %) & ♀ (13.7 %); ↑ mean corpuscular haemoglobin concentration in ♂(2.7 %) & ♀ (2.8 %) ↑ absolute and relative liver weights in ♂ (23.6 % absolute, 25.6 % relative) & ♀ (25.9 % absolute, 25.1 % relative)

*p<0.05
**p<0.0
BW – body weight; BWG – body weight gain; GD – gestation day; PND – postnatal day; SD – study day

Reproductive toxicity of BENPAT was investigated in a GLP-compliant two-generation reproductive toxicity study, performed according to OECD TG 416 (RTI, 2001a). Rats received dietary doses of BENPAT at 0, 120, 400, and 1500 ppm (corresponding to approx. 0, 7.5, 25, and 100 mg/kg bw/d of BENPAT), based on a range-finding study. The DS considers this study as a key study for adverse effects on sexual function and fertility, and on development.

The developmental toxicity of BENPAT was investigated in a GLP-compliant study in line with OECD TG 414 (RTI, 1995). Doses were selected based on the outcome of a range finding study. For the main study, pregnant rats received daily doses of 0, 20, 70, and 200 mg/kg bw/d BENPAT by oral gavage on gestation days (GD) 6-15 (there was no treatment after GD 15 until scheduled caesarean section on GD 20). The DS considers this study as a key study for adverse effects on development.

A one-generation mechanistic, non-guideline, study was conducted to determine the necessary and sufficient exposure of BENPAT to maternal females to produce dystocia, prolonged gestation, and offspring polycystic kidneys, effects previously described in the two-generation reproductive toxicity study (RTI, 2001a). Furthermore, the goal was to determine whether test substance administration results in macrocytic anaemia in maternal animals, and if F0 maternal females exhibit polycystic kidneys after exposure to BENPAT for up to 12 weeks. During this study, also a possible effect of iron supplementation on the above effects was investigated. Rats received a dietary dose of 2500 ppm BENPAT in corn oil, corresponding to approx. 250 mg/kg bw/d. Exposure groups were defined as follows: (1) no exposure (negative control), (2) exposure during pre-breeding (four weeks) and mating (up to two weeks), (3) exposure during gestation (three weeks) and lactation (three weeks), (4) exposure during pre-breeding, mating, gestation, and lactation, and (5) exposure during pre-breeding, mating, gestation, and lactation plus supplementation of 600 ppm iron gluconate in the drinking water (for more details see Annex I). This study provides supportive evidence for adverse effects of BENPAT on sexual function and fertility, and on development.

10.10.1 Adverse effects on sexual function and fertility

(a) Effects of BENPAT on sexual function and fertility

Two-Generation Reproductive Toxicity Study (OECD TG 416)

During the two-generation reproductive toxicity study, effects on gestation and parturition were observed at all dose levels of BENPAT. Gestational length was significantly prolonged at 25 and 100 mg/kg bw/d for F0 dams with F1 litters and at 7.5, 25, and 100 mg/kg bw/d for F1 dams with F2 litters. Both, mid and high dose F0 and F1 females exhibited dystocia (obstructed labour) and animals showed an increased incidence of pallor, piloerection, and vaginal bleeding (mainly F0 dams) during late gestation (days 23-24). Increased delivery length was accompanied by perinatal mortality of F1 and F2 offspring, including litters with all-dead pups. Furthermore, percentages of post-implantation losses per litter were significantly increased for F1 litters, at 25 and 100 mg/kg bw/d, and F2 litters at 100 mg/kg bw/d, and clearly, but not significantly increased for F2 litters at 25 and 7.5 mg/kg bw/d. At the highest dose tested, there was a significant increase in the numbers of dead pups per litter and reduction in live birth index for F0 and F1 dams. The numbers of total pups and live pups per litter were significantly reduced at 25 and 100 mg/kg bw/d for F1 litters and at 100 mg/kg bw/d for F2 litters (statistically significantly reduced for live pups, but not significant for total pups). Additionally, prolonged gestation was associated with increased pup body weights. The effects of BENPAT on reproductive parameters are given in Table 11.

Findings of F1 pups that died during lactation indicate that most deaths occurred mainly on postnatal day (PND) 0, with some deaths on PND 1 to 4. Observations revealed many dead pups with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of the pulmonary alveoli at birth), and no milk in stomach. Many pups however died with closed ductus and air in lungs. At 100 mg/kg bw/d, there was an increased incidence of distended ureter/hydroureter and hydronephrosis, compared to the incidence at low doses. However, due to high number of perinatal deaths at 100 mg/kg bw/d relative to other dose groups, and the low number of pups able to evaluate in all dose groups and controls, data should be considered with care. Clinical observation of F2 pups during lactation revealed treatment related incidence of pups found dead, euthanised moribund, and missing and presumed dead at all dose groups of BENPAT, mainly at PND 0. Prominent findings in all dose groups showed hypothermic pups, no milk in stomach. Examinations

of F2 pups that died during lactation show many pups with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of pulmonary alveoli at birth), many pups, however, died with closed ductus and air in lungs. Kidney lesions, including hydronephrosis were observed at 7.5 and 100 mg/kg bw/d and in controls. However, many pups had autolysed abdominal organs and data should be taken with care due to low animal numbers.

Furthermore, exposure of BENPAT significantly increased the incidence of F1 females (but not F0 females) with abnormal stage of oestrous cycle (25 and 100 mg/kg bw/d) at necropsy. The percentage of females in metestrus was statistically significantly increased at all dose groups, due to non-significant reductions in the percentages of females in dioestrus at all dose levels, and of females in oestrus at 25 and 100 mg/kg bw/d. Cycle length in days exhibited a significant upward trend ($p < 0.05$), but no significant pairwise comparisons. The mean cycle length at highest dose was increased (7.15 days), relative to the control group (5.11 days; and 4.84 days at 7.5 mg/kg bw/d and 5.51 days at 25 mg/kg bw/d).

However, dietary doses of BENPAT in F0 and F1 female parental animals did not result in treatment-related effects on mating or pregnancy rates or fertility indices. There were no histopathologic findings in F0 or F1 female reproductive organs, no difference between high dose and control F0 and F1 females in paired ovarian follicle counts. There were no effects of treatment on implantation sites/litter or sex ratio of pups at birth (or during lactation).

For male reproductive toxicity, there were no effects on preputial separation in F1 males, no effects on mating or fertility indices, no histopathologic findings in male reproductive organs, and no effects on seminal parameters (epididymal sperm number, motility, morphology) or on calculated testicular daily sperm production.

Table 11: Effects of BENPAT on reproductive parameters (OECD TG 416; (RTI, 2001a))

	F0 animals for F1 litters				**F1 animals for F2 litters**			
Dose (mg/kg bw/d)	0	7.5	25	100	0	7.5	25	100
Abnormal cycles (%)	17.2	6.7	6.7	13.3	6.7	10	**28.6****	**43.3****
Mean cycle length (d)	4.7	4.5	4.7	4.7	5.1	4.8	5.5	7.2
No. of mating pairs	29	30	30	30	30	30	30	30
No. of ♂ sperm positive	26	30	26	27	26	26	24	26
No. of ♀ pregnant	24	27	24	25	22	23	22	24
No. of ♀ with live litters, PND 0	24	26	23	**15****	22	22	20	21
No. of dams with live & dead pups, PND 0	2	4	3	6	3/22	5/23	4/22	11/24
No. of dams with no live litters, PND0	0	1/27[6]	1/24[6]	10/25[7]	0	1/23	2/22[6]	3/24
Gestational length (d)	22.2	22.4	**22.8****	**23.5****	22.2	**22.8****	**23.1****	**23.2****
No. of implantation sites/litter	16.9	15.9	15.6	14.5	16.5	16.4	15.1	15.0
Post-implantation loss /litter (%)	10.7	14.9	**26.1****	**52.3****	6.8	18.5	20.2	**32.6****
No. of total pups/litter, PND 0	15.7	14.9	**12.3****	**12.1****	15.7	14.5	15.2	13.3
No. of live pups/litter, PND 0	15.6	14.1	**11.9***	**7.6****	15.6	13.7	13.4	**10.8****
No. of dead pups/litter on PND 0	0.1	0.3	0.4	**4.1****	0.1	0.7	0.4	**2.5****
Live birth index (%)	99.2	98.0	97.0	**57.5****	99.2	91.9	97.2	**77.8****
Stillbirth index (%)	0.8	2.0	3.0	**42.5****	0.8	7.6	2.8	**22.2****
Sex ratio (% males)	50.4	54.1	55.2	44.1	44.4	47.1	46.2	48.6
Pup body weights (g) on PND 0	6.4	**6.8****	**6.9****	6.6	6.3	**6.9****	**7.0****	**6.6***

* = $p < 0.05$ versus control group value; ** = $p < 0.01$ versus control group value

[6] Implantation sites only

[7] One female with implantation sites only

Mechanistic study with BENPAT (non-guideline)

During the one-generation mechanistic study with BENPAT a significant increase in gestational length associated with dystocia was found in parental females in groups 3 (exposed during gestation and lactation), 4 (exposed from pre-breeding until lactation), and 5 (exposed from pre-breeding until lactation, plus iron), compared to control group and group 2 (only exposed during pre-breeding and mating). There was no difference between group 4 and 5 in the incidence or severity of dystocia. Furthermore, the percentage of post-implantation loss (prenatal) was significantly increased in groups 3 and 4, while in females that received iron (group 5) the number of implantation sites per litter was significantly reduced. On PND 0, live F1 pups per litter were significantly reduced in groups 3, 4, and 5, and numbers of dead pups per litter were significantly increased in groups 3 and 4. The total number of pups per litter were significantly reduced in groups 3 and 5. Additionally, the live birth index was significantly reduced (and stillbirth index significantly increased) in groups 3 and 4, on PND 0. Survival index for the rest of lactation (PND 4, 7, 14, and 21) was high and equivalent among all groups. Body weights on PND 0 of F1 Pups (groups 2, 3, and 4) that were born alive were comparable to control values, except for pups of group 5 (dams received iron supplementation), which showed a significantly increased body weight in comparison to controls. Effect of BENPAT on reproductive parameters are summarised in Table 12.

During the one-generation mechanistic diet study with BENPAT, there were no significant effects on female mating and fertility indices. F0 male mating, fertility, and pregnancy indices were equivalent among all groups.

Table 12: Effects of BENPAT on reproductive parameters from the one-generation mechanistic study (RTI, 2000)

Dose groups and effects	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Pre-breeding and mating, BENPAT (mg/kg bw/d)	0	250	0	250	250[8]
Gestation and lactation, BENPAT (mg/kg bw/d)	0	0	250	250	250[8]
No. of animals started	20	20	20	20	20
Mating index (%)	95.0	95.0	100	95.0	85.0
Fertility index (%)	78.9	73.7	90.0	78.9	70.6
Gestational index (%)	100	92.9	**64.7**[9]	**71.4**[10]	100
Gestational length (d), (No. animals)	22.2 ± 0.1 (13)	22.3 ± 0.1 (13)	**23.6 ± 0.2 (14)**[11]	**23.8 ± 0.2 (13)**[11]	**23.5 ± 0.2 (11)**[11]
No. of live litters, PND 0	15	13	11	10	11
No. of implantation sites/ litter	14.7± 0.8	13.9± 0.6	11.6± 1.2	13.5± 0.8	**10.6 ± 1.4**[12]
Post-implantation loss/litter (%)	12.2 ± 2.8	16.9 ± 6.8	**57.5 ± 10.4**[11]	**55.8± 10.6**[11]	18.0 ± 5.9
No. of live pups/litters, PND 0	12.6 ± 0.6	12.8 ± 0.6	**5.8 ± 1.5**[13]	**6.8± 1.6**[13]	**8.2 ± 1.0**[13]
No. of dead pups, PND 0	0.4 ± 0.2	0.2 ± 0.2	**4.1 ± 1.3**[14]	**5.3± 1.4**[13]	**1.3 ± 0.7**
Live birth index (%)	96.9 ± 1.3	98.9 ± 1.1	**54.6 ± 12.0**[14]	**54.0 ± 12.0**[13]	90.0 ± 5.2
Sex ratio (% males) (PND 0)	57.0 ± 4.3	47.6 ± 3.5	64.9 ± 6.3	44.9 ± 9.4	39.4± 4.6

8 Iron Supplementation

9 P<0.01, Fisher's Exact Test

10 P<0.05, Fisher's Exact Test

11 P< 0.01; Dunnett's TEST

12 P< 0.05; Dunnett's TEST

13 p<0.01; Mann-Whitney U Test

14 p<0.05; Mann-Whitney U Test

(b) General toxicity maternal animals

Two-Generation Reproductive Toxicity Study with BENPAT (OECD TG 416)

During this study, there was an increased number of (mainly F0) females that either died or were euthanised associated with extended parturition and dystocia (at 25 and mainly at 100 mg/kg bw/d). Maternal mortality during lactation included two mid dose F0 dams: one female delivered three live pups but died in the process of delivering, and the second female delivered six living pups but was found dead on PND 2. In both females, retained dead pups were found *in utero* and in the vagina. Eight high dose F0 dams were euthanised in the process of delivering/ died in the process of delivering/ were euthanised moribund, or were found dead during the holding period until scheduled sacrifice. Maternal mortality was highly associated with the delivery of litters with all-dead pups. There were foetuses retained *in utero* (in three dams) and/or *in vagina* (in two dams), and retained placentae were evident in two females. For F1 maternal animals, mortality was relatively low, including one F1 dam, which died at 100 mg/kg bw/d (while delivering). This female delivered one live pup and five dead pups, while foetuses were retained *in utero*, and one placenta in the vagina. One F1 dam at 25 mg/kg bw/d delivered 10 live and four dead pups. This female was found dead on PND 3 with one foetus retained in the vagina. However, there were F0 and F1 females that delivered dead pups per litter or a litter of only dead pups (one female at 100 mg/kg bw/d) and survived until scheduled sacrifice. Unscheduled necropsy of females that died during the lactation and holding period until scheduled sacrifice revealed possible treatment-related effects on the kidneys (necrosis and inflammation); liver (necrosis, inflammation, and haemorrhage); uterus (haemorrhage, inflammation, and retained foetuses, retained placenta) and vagina (retained foetuses and bleeding)**;** lungs (thrombosis, inflammation, and congestion); and adrenal cortex (degeneration and haemorrhage). Deposition of a fibrin-like material in renal glomeruli of the kidney associated with cortical necrosis was present in a few animals. Findings support the premise for the presence of disseminated intravascular coagulation (DIC), which can be activated by endotoxaemia and/or septicaemia (RTI, 2001b).

During the two-generation reproductive toxicity study, at 100 mg/kg bw/d F0 maternal body weight was significantly reduced during the last nine weeks of the ten-week pre-breeding phase (-7 %) and during the three-week gestation period (-10 %). Gestational body weight gain was significantly reduced for high dose females (-18 % compared to controls). Body weight of F0 females was statistically significantly reduced during lactation on PND 0, 4, and 7 at 100 mg/kg bw/d, however, high dose F0 female gained more weight, compared to other dose groups and control group, and lactational weight gain was significantly increased, in comparison to controls. Gestational body weight of F1 females was not significantly different between dose groups and controls, however, high dose F0 dams gained significantly less body weight (-11 %) compared to controls. F0 maternal body weight was significantly reduced during lactation on PND 0, 4, and 7. For F0 and F1 maternal animals of all dose groups, body weight at the end of the lactational period (scheduled sacrifice) was not significantly different from controls (for more information on organ weights and feed consumption, see Annex I).

There was a dose-dependent increase in relative F0 organ weights of the liver (20 %, at 100 mg/kg bw/d) and paired kidneys (9 % at 25 and 12 % at 100 mg/kg bw/d). F1 maternal relative liver and kidney weights were statistically equivalent across all groups. Dose-dependent statistically significant differences in F1 maternal organ weights (compared to controls) were restricted to the brain; absolute female brain weight was significantly reduced at 100 mg/kg bw/d (-6 % vs. controls). Dose-dependent and treatment-related histopathological findings in maternal F0 and F1 animals were limited to the kidneys, indicating a high incidence of polycystic kidneys at scheduled sacrifice. Furthermore, there were statistically significant differences in the weight of reproductive organs (absolute and relative paired ovary, absolute and relative uterine weight) in F0 and F1 maternal animals, however not in a dose-dependent manner (detailed values for F0 and F1 female organ weights are summarised in Annex I).

Mechanistic study with BENPAT (non-guideline)

During the four-week pre-breeding period, body weights of F0 females were significantly reduced in groups 2, 4, and 5 (all exposed), in comparison to controls. Furthermore, F0 female gestational body weights were significantly reduced in group 3 (exposed) on GD 7 and 21, and in group 5 (exposed) on GD 21. Body weight gain during gestation was significantly lower in groups 3 and 5, relative to controls. Examination during

lactation revealed significantly reduced F0 maternal body weights in groups 3, 4, and 5 (all exposed), on PND 0, 4, and 7. However, body weight gain throughout lactation was significantly increased in groups 3, 4 and 5.

Clinical observation of F0 females during gestation included one female each found dead in group 3 (GD 19) and 4 (GD 24). F0 females observed during lactation revealed "dam euthanised, entire litter dead" on PND 0 in groups 3 (two dams) and 4 (three dams), on PND 3 in groups 3 (two dams) and 4 (one dam), and on PND 4 in group 3 (one dam). During gestation and lactation there were findings in groups 2, 3, 4 and 5, including dams with alopecia; pale eyes and tail, pallor; piloerection (including females in process of delivering); and chromodacryorrhea.

F0 maternal absolute and relative liver weights were significantly increased in groups 4 and 5. Furthermore, relative (but not absolute) paired kidney weights were significantly increased in both groups. Examination of F0 females of groups 1 (controls) and 5 (iron supplementation) did not provide gross evidence of polycystic kidneys, but there were findings of polycystic kidneys in group 5 (2/20 (15 %) of F0 females), but no findings in group 1 (control) after microscopy. The haematological profile indicated no demonstrable macrocytic anaemia on GD 21 in F0 dams in any treatment group. However, at PND 21, there was evidence of macrocytic anaemia (increased MCV, measure of red blood cell size; evidence of release of larger, immature erythrocytes into the peripheral circulation) in F0 maternal animals in groups 4 (exposure during mating, gestation, and lactation) and 5 (exposure during mating, gestation, and lactation plus iron), without any differences between group 4 and 5. In conclusion, iron supplementation did not affect PND 21 maternal anaemia or dystocia.

(c) Relevance for humans

There were no data available to the DS, including e.g. epidemiological studies or case reports, addressing an effect of BENPAT on sexual function, fertility or development in humans. Valid animal data discussed in the sections above are considered as relevant in humans.

Furthermore, several studies are available from animal models which suggest that BENPAT constituent DPPD (a) causes similar effects as BENPAT (dystocia, prolonged parturition followed by maternal mortality) and (b) acts as a prostaglandin inhibitor (Fujimoto *et al.*, 1984; Marois, 1998). Studies are summarised in Annex I.

Prostaglandins are involved in several physiological processes, including ovulation, luteolysis, pregnancy, birth, inflammation, gastric secretion, and blood flow in humans (Bakker *et al.*, 2017; Bennegard *et al.*, 1991; Hahlin *et al.*, 1988; Wiltbank and Ottobre, 2003). There is a large body of evidence that human birth originates from processes leading to elevated levels of prostaglandins (Bakker *et al.*, 2017; Mitchell *et al.*, 1978; Reece *et al.*, 1996; Romero *et al.*, 1994). They play critical roles in cervical ripening, functional progesterone withdrawal, contraction modulation of the human myometrium, and stimulation of proteins responsible for uterine activation for labour (Astle *et al.*, 2005; Madsen *et al.*, 2004; Parkington *et al.*, 1999; Xu *et al.*, 2015).

Studies with the BENPAT constituent DPPD as described above, give supportive information for a possible mode of action. Furthermore, there are no robust data on the MoA to conclude that the effects of BENPAT are not relevant to humans or to raise doubt about the human relevance. These findings are therefore considered relevant to humans.

10.10.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Reliable animal data give strong evidence that BENPAT causes adverse effects on fertility. Studies show that BENPAT causes dystocia (obstructed labour), including a significantly prolonged pregnancy in F0 dams ((RTI, 2000; RTI, 2001a), ≥ 25 mg/kg bw/d), and F1 dams ((RTI, 2001a), ≥ 7.5 mg/kg bw/d), associated with an increased incidence of pallor, piloerection, and vaginal bleeding, in high dose females. The mechanistic study performed with BENPAT (approx. 250 mg/kg bw/d) revealed that treatment during gestation (groups 3, 4, and 5) produces dystocia and prolonged gestation and further indicated that dystocia was not caused by iron deficiency. The increased delivery length was accompanied with a statistically significant increase in the post-implantation loss in F1 (≥ 25 mg/kg bw/d) and F2 litters (100 mg/kg bw/d), pups that retained in vagina, vaginal bleeding, and uterus haemorrhage. Furthermore, there was a BENPAT treatment-related increased offspring mortality, represented by an increased stillbirth index of F1 and F2 pups at 7.5, 25 (not significant),

and 100 mg/kg bw/d (statistically significant). Death of F1 and F2 pups occurred mainly on PND 0, with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of pulmonary alveoli at birth). The available data strongly support that pup mortality occurred perinatal, due to prolonged parturition/ dystocia. However, there were also pups that died with closed ductus and air in lungs.

Dystocia resulted in severe consequences for (mainly F0) maternal animals. There was maternal mortality during the lactation and holding periods (until scheduled sacrifice) for F0 dams at ≥ 25 mg/kg bw/d. However, maternal mortality was relatively low in F1 dams (at 25 and 100 mg/kg bw/d). Dams that died or were euthanised moribund during the process of delivery, lactation, and holding periods revealed treatment-related effects on the uterus and vagina**,** lungs, liver, and kidneys, characterised by retained foetuses and bleeding, vascular congestion or haemorrhage, degeneration or necrosis and inflammatory lesions including vascular thrombosis. Kidney and lung alterations are most likely changes associated with dystocia and maternal infection consequent to resorbing foetuses *in utero*.

Furthermore, BENPAT exposure starting possibly *in utero* until the end of lactation affected oestrous cycling of F1 females (25 and 100 mg/kg bw/d), including a significant increase in the percentage of F1 females in metestrus at necropsy. However, there were no obvious effects on mating or fertility of F1 females. Oestrous cycling of F2 females was not evaluated.

F0 mothers exposed prior to and during mating, gestation, and lactation showed macrocytic anaemia at PND 21. During a repeated dose 28-day oral toxicity study (similar to OECD TG 407) macrocytic anaemia was the primary change in both genders of rats after dietary exposure to BENPAT (120 mg/kg bw/d). Furthermore, macrocytic anaemia was identified in male and female rats during a one-year dietary toxicity study (none-guideline) with BENPAT (120 mg /kg bw/d; repeated dose toxicity studies are summarised in the Annex I). Therefore, maternal anaemia might not be a consequence of dystocia. As shown in the mechanistic study, maternal anaemia (PND 21) is not induced by iron deficiency.

Statistically significant maternal effects were observed at 100 mg/kg bw/d and included decreased body weights during pre-breeding and gestation (less than 10 % reduction compared to controls), and a reduced gestational body weight gain in F0 and F1 animals (18 % and 11 % decrease compared to controls, respectively). Body weight of F0 and F1 dams was reduced during lactation, but only for PND 0, 4, and 7, and in contrast to the other groups, lactational weight gain was increased for high dose F0 animals. Investigations of maternal organ weights revealed a significant increase of F0 liver weight (absolute and relative) at 100 mg/kg bw/d (20 % increase compared to relative control weight). Absolute and relative paired kidney weights were significantly increased in F0 dams at 25 (9 % increase, compared to relative control weight) and 100 mg/kg bw/d (12 %, increase, compared to relative control weight). Polycystic kidneys were observed with a low incidence in F0 maternal animals (only kidneys with gross lesions were histologically examined) and with a much higher incidence in F1 maternal animals.

Any effect of a substance that has the potential to interfere with parturition should be considered an adverse effect on sexual function and fertility. A large body of evidence from animal studies indicates that BENPAT causes adverse effects on fertility, namely dystocia, post-implantation losses, and increased stillbirth (for RAC opinions considering dystocia as an adverse effect on fertility, please see (RAC, 2012; RAC, 2015)). Moreover, the DS concludes that data on body and organ weights do not represent severe maternal toxicity and do not explain the adverse effects on fertility. Therefore, effects on fertility are not considered to be a secondary non-specific consequence of maternal toxicity. Furthermore, there is no robust data on the MoA to conclude that the effects of BENPAT are not relevant for humans or to raise doubt about their human relevance.

10.10.2 Adverse effects on development

From the two-generation reproductive toxicity study (OECD TG 416) and from the one-generation mechanistic study conducted with BENPAT, there are meaningful data on a treatment-related and dose-dependent incidence of polycystic kidneys (for characterisation see Annex I) in the F1 and F2 generation in both sexes. Polycystic kidneys were found in some high-dosed F0 dams as well (for F0 adults, only kidneys with gross lesions were examined histologically in any group). From macroscopic examination, there was no evidence of polycystic kidneys in pups, which died during parturition or lactation. However, microscopic examination of pups kidneys was not conducted. Polycystic kidneys were observed in F1 weanlings (PND 21), F1 adults, and

F2 weanlings at all dose groups (Table 13). Controls had neither macroscopic nor microscopic indications of polycystic kidneys.

Table 13: Incidence of polycystic kidneys in F0 and F1 animals from OECD TG 416 with BENPAT (RTI, 2000)

	Polycystic kidneys in male animals				**Polycystic kidneys female animals**			
[mg/kg bw/d]	0	7.5	25	100	0	7.5	25	100
F0 adults[15]	0 %	0 %	0 %	0 % 0/1	0 %	0 %	0 % 0/2	33 % 3/9
F1 weanlings[16]	0 % 0/23	4 % 1/25	40 % 8/20	91 % 10/11	0 % 0/22	19 % 5/26	39 % 7/18	100 % 11/11
F1 adults[17]	0 % 0/30	17 % 5/30	33 % 10/30	70 % 21/30	0 % 0/30	7 % 2/30	3 % 1/30	60 % 18/30
F2 weanlings[16]	0 % 0/60	5 % 3/64	32 % 6/19	94 % 15/16	0 % 0/60	8 % 5/64	42 % 8/19	100 % 15/15

The one-generation mechanistic study with BENPAT was performed to determine whether F0 parental females exhibit polycystic kidneys after exposure to dietary doses of BENPAT for up to 12 weeks. Therefore, all kidneys from females of group 5 (treated during pre-breeding, mating, gestation, and lactation with iron supplementation) were examined and compared to controls. There were no macroscopic indications of polycystic kidneys. Histopathological examination revealed evidence of polycystic kidneys in F0 dams of group 5 (15 % of dams), but no incidence of polycystic kidney in controls. Other treated groups of F0 females (treatment during pre-breeding, mating; or pre-breeding, mating, gestation, and lactation without iron supplementation) or parental F0 males were not examined. There was no gross evidence of polycystic kidneys in pups that died during lactation, but histopathology was not performed. Investigations of F1 weanlings on PD 21 revealed polycystic kidneys in group 3 (exposure during gestation and lactation), group 4 (pre-breeding, mating, gestation, and lactation), and group 5 (pre-breeding, mating, gestation, and lactation plus iron). Microscopic findings at scheduled sacrifice revealed polycystic kidneys with a high incidence reaching 96 % (24/25) in F1 females and 97 % (37/38) in F1 males in group 3; 91 % (29/32) in F1 females and 95 % (38/40) in F1 males in group 4; and 100 % (48/48) in F1 females and 91 % (31/34) in F1 males in group 5. Results of group 4 and 5 show, that iron supplementation did not affect the incidence of polycystic kidneys. There were no indications of polycystic kidneys in group 2 (exposure during pre-breeding and mating) or controls.

Literature data show that diphenylamine (DPA) induced polycystic kidneys (cystic dilation of the renal tubules, mainly collecting tubules and distal convoluted tubules) in different animal species after subchronic exposure (at least 6 months) (Evan and Gardner, 1976; Gardner *et al.*, 1976; Rohrbach *et al.*, 1993; Thomas *et al.*, 1967). Furthermore, in rats treated for two years cystic changes were accompanied with chronic nephritis (cystic tubule changes at 40.6 and 35.7 mg/kg/d in females and males, respectively, chronic interstitial nephritis at 203.0 and 178.5 mg/kg/d in females and males, respectively (Thomas *et al.*, 1967). Crocker and colleagues found out that diphenylamine derivatives chemically induced polycystic kidneys in newborn rats after treatment of dams from gestation day 14 until term (Crocker *et al.*, 1972). Subsequent investigations identified N,N,N'-triphenyl-p-phenylenediamine, a reaction product of diphenylamine, to induce polycystic kidneys in newborn rats (Clegg *et al.*, 1981). Diphenylamine (CAS: 122-39-4; EC: 204-539-4) and *other low molecular weight diphenylamine derivatives* are listed as impurities of BENPAT (for concentration ranges see Annex I). However, there is no further information available to the DS on the characterisation of *other low molecular weight diphenylamine derivatives*.

During the range-finding study for the prenatal developmental toxicity study, pregnant rats (8/dose group) were administered by gavage once daily, on GD 6 through 15, using doses of 600, 200, 70, and 20 mg/kg bw/d of the test substance. Four out of eight high dose dams died (two dams at GD 12 and two dams at GD 14). Among dams, there was evidence of vaginal bleeding (on GD 12, 13) in two dams. The other two pregnant

15 F0 parental animals, only kidneys with gross lesions were examined histologically in any group

16 kidneys of three F1 and F2 weanlings per sex per litter were examined histologically in any group

17 F1 parental animals, all kidneys were examined histologically in any group

females died or were sacrificed moribund on GD 12 with resorbing conceptuses. Dams exhibited pale organs and extremities, going along with internal bleeding. Furthermore, vaginal bleeding was evident in one dam at each 600 mg/kg bw/d, 70 mg/kg bw/d, and 20 mg/kg bw/d. Maternal body weights and weight changes were significantly reduced at 600 and 200 mg/kg bw/d, with an increased weight gain in the post-exposure period. Maternal food consumption was significantly reduced at 600 and 200 mg/kg bw/d (RTI, 1995). Observation of foetuses revealed significantly reduced body weight at 600 mg/kg/day and at 200 mg/kg/day.

For the main prenatal developmental toxicity study (OECD TG 414 (RTI, 1995)), rats were exposed to BENPAT by oral gavage at doses of 20.0, 70.0 and 200.0 mg/kg from GD 6-15. During the study, no female rat died, aborted, or delivered early. Pregnancy rates were high and equivalent across all groups (92.0 - 96.0 %). There were no statistically significantly differences in the number of pregnant females. All pregnant animals had one or more live foetuses at sacrifice (GD 20), without statistically significant differences in the number of pups/dam among dose groups. At 200 mg/kg bw/day, maternal body weight was significantly reduced on GD 12, and maternal weight gain was significantly reduced for GD 6-9, and 6-15 (dosing period). There were no specific treatment-related clinical signs, although dams at 200 mg/kg bw/d exhibited a significant decrease in weight gain from GD 6 to 15. Maternal feed consumption was significantly reduced at 200 mg/kg bw/d for GD 6-9, 9-12, and 6-15 (treatment period) and was significantly increased for GD 18-20 (in the post-treatment period). There were no treatment-related effects on any gestational parameters, including pre- or post-implantation loss, number of live foetuses per litter, foetal sex ratio (% males per litter) or foetal body weight per litter. Foetal body weight per litter exhibited a significant dose-related downward trend at 200 mg/kg bw/d (for sexes pooled and separately). There were no treatment-related statistically or biologically significant changes in the incidence of individual or pooled external, visceral (including craniofacial), skeletal or total foetal malformations or variations in this study.

There was no evidence of malformation in embryonic kidneys analysed in the prenatal developmental toxicity study with dams exposed from GD 6 to GD 15. However, in rats the metanephros, forming the permanent and functional adult kidneys, start to develop between embryonic day E12 and E13. Therefore, exposure time might be insufficient to investigate adverse effects of BENPAT on embryonic kidney development as well as adverse effect on fertility, identified in the two-generation developmental toxicity study and one-generation mechanistic study, namely dystocia.

Table 14: Effects of BENPAT on developmental toxicity (OECD TG 414; (RTI, 1995))

Dose (mg/kg bw/d)	**0**	**7.5**	**25**	**100**
No. of dams	23	23	23	24
No. of implantation sites per litter	15.9 ± 0.6	16.0 ± 0.5	16.4 ± 0.4	16.5 ± 0.4
% pre-implantation loss	14.1 ± 3.7	8.9 ± 2.7	11.2 ± 2.2	7.8 ± 1.7
% resorptions/ litter	2.7 ± 0.9	2.4 ± 0.9	2.2 ± 0.9	3.4 ± 1.0
No. of litters with resorptions (non-live implants)	7	7	6	10
% litters with resorptions	30.4	30.4	26.1	41.7
% late foetal deaths/ litter	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% adversely affected implants/ litter	8.8 ± 1.7	9.9 ± 2.2	6.3 ± 1.4	7.3 ± 1.4
% litters with adversely affected implants	69.6	65.2	60.9	66.7
No. of live foetuses/ litter	15.4 ± 0.6	15.6 ± 0.5	16.0 ± 0.4	15.9 ± 0.5

10.10.3 Short summary and overall relevance of the provided information on adverse effects on development

A large body of evidence resulting from reliable animal studies indicates that BENPAT causes, with a high incidence, polycystic kidneys in F1 and F2 offspring. While polycystic kidneys were detected at low rates in F0 maternal animals, there was no evidence of polycystic kidneys in male or female adult rats exposed to BENPAT, from a 28-day oral toxicity study (similar to OECD TG 407; ca. 7.5, 30, and 120 mg/kg bw) or from a one-year dietary toxicity study (none-guideline; ca. 3.3, 20 and 120 mg/kg-day; studies are summarised in the Annex I).

The mechanistic study with BENPAT shows that exposure of F0 dams during gestation and lactation generated polycystic kidneys in the F1 weanlings. Exposure during the pre-breeding/mating periods did not increase the effects produced from gestation/lactation exposures only. Furthermore, polycystic kidneys are not related to iron deficiency. Because there were no groups only exposed during gestation or only during lactation, it is not possible to further define how the timing of exposure affects this endpoint (possible exposure of pups at the end of lactation caused by self-feeding might also be involved). Furthermore, from the two-generation developmental toxicity study (OECD TG 416) and the one-generation mechanistic study performed with BENPAT, comprising treatment of dams during the whole gestation, there is no information on microscopic investigation of newborn kidneys. In the developmental prenatal developmental toxicity study (OECD TG 414) dams were exposed from GD 6 to GD 15. However, the chosen time window limits to investigate adverse effects of BENPAT on embryonic kidney development. Altogether, the above-mentioned studies do not allow concluding that the high incidence of polycystic kidneys in F1 and F2 weanlings are caused through BENPAT-exposure in utero or are a consequence of exposure of pups during lactation and/or self-feeding.

However, there is evidence from literature data that DPA and a DPA derivative cause polycystic kidneys in newborn pups after exposure of dams during late gestation (GD 14 until term). DPA and *other low molecular weight diphenylamine derivatives* (without further specification) are listed as BENPAT impurities. Therefore, it cannot be excluded that BENPAT induces polycystic kidneys in embryos during prenatal development. Most likely, the developing embryonic kidney is more sensitive to BENPAT, compared to the adult kidney, supported by the low incidence of polycystic kidneys in F0 females and the high incidence in F1 and F2 offspring.

Polycystic kidneys are accompanied by structural abnormalities and impaired kidney function and the manifestation of developmental toxicity includes functional deficiency of the kidneys.

In summary, BENPAT causes adverse effects on development of the offspring, namely polycystic kidneys. There is no robust data on the MoA to conclude that the effects of BENPAT are not relevant to humans or to raise doubt about the human relevance.

10.10.4 Comparison with the CLP criteria

There are no human data available. Therefore, classification of BENPAT as a reproductive toxicant in Category 1A is not warranted.

The proposed classification of BENPAT is based on data from animal studies. A chemical is classified as presumed human reproductive toxicant (Category 1B), if *"data provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects"* (Guidance on the Application of CLP Criteria, 2017, 3.7.2.2).

Doubt about the relevance in humans strengthens classification in Category 2. "Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification" (Guidance on the Application of CLP Criteria, 2017, 3.7.2.2).

Toxic effects in the mother may be caused through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally mediated mechanisms. Maternal toxicity shall be considered in the context of offspring development throughout gestation and during the early postnatal stages (Guidance on the Application of CLP Criteria, 2017, Annex I: 3.7.2.4).

A large body of evidence resulting from animal studies indicates that BENPAT causes adverse effects on sexual function. BENPAT prolongs parturition time, induces dystocia, and impairs oestrous cycling. Obstructed labours result in an increased number of pups born dead. Furthermore, an elevated number of maternal rats died/were euthanised during the process of prolonged delivery or during lactation. Dams showed retained dead foetuses *in uterus/in vagina*, vaginal bleeding, and alterations in the lungs, livers, and kidneys. Kidney and lung alterations (vascular congestion or haemorrhage, degeneration or necrosis and inflammatory lesions including vascular thrombosis) are most likely changes associated with dystocia and maternal infection subsequent to resorbing foetuses *in utero*. Altogether, it is warranted to classify BENPAT as a presumed human reproductive toxicant on fertility. There are no data available on the mode of action of BENPAT. However, BENPAT constituent DPPD was found to act as a prostaglandin inhibitor. Nevertheless, data on DPPD give just supportive information as no mechanistic data on BENPAT are available that demonstrate prostaglandin involvement.

Furthermore, BENPAT causes adverse effects on development, namely a high incidence of polycystic kidneys in F1 and F2 offspring. According to the CLP regulation *"any effect which interferes with normal development of the conceptus either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation"*, in the widest sense, should be considered as an adverse effect on development of the offspring. *"However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy or as a result of parental exposure"* (CLP regulation, Annex I, section 3.7.1.4). Available data does not show that polycystic kidneys in F1 and F2 weanlings were induced after treatment of dams only during gestation. However, data of DPA and its derivative (induction of polycystic kidneys in newborn rats after treatment of dams from gestation day 14 until term) indicate BENPAT-induced developmental toxicity. Therefore, it is warranted to classify BENPAT as a presumed human reproductive toxicant on development.

There is no information that the mode of action on sexual function and fertility, and development is not relevant in humans or is of doubtful relevance for humans. Therefore, the effects of BENPAT observed in the animal studies are potentially relevant also for humans.

In conclusion, it is warranted to classify BENPAT as a presumed human reproductive toxicant on fertility and development, Category 1B.

ED_{10} values were calculated (using BMDS 2.7.0.4 software) resulting in a value of 23.8 mg/kg bw/d for post-implantation loss of F0 dams with F1 litters and 4.3 mg/kg bw/d for polycystic kidneys in F1 females weanlings (PND 21). No SCL is proposed.

10.10.5 Adverse effects on or via lactation

A one-generation mechanistic study was performed with BENPAT, herein 20 CD (SD) rats per sex and groups received dietary doses of BENPAT (2500 ppm in corn oil, corresponding to approx. 250 mg/kg bw/d). Exposure groups were defined as follows: (1) no exposure (negative control), exposure during (2) pre-breeding (four weeks) and mating (up to two weeks), (3) gestation (three weeks) and lactation (three weeks), (4) pre-breeding, mating, gestation, and lactation, and (5) pre-breeding, mating, gestation, and lactation plus supplementation of 600 ppm of iron gluconate in the drinking water. The study design does not give information if exposure of F0 dams only during gestation or only during lactation is sufficient to induce polycystic kidneys in F1 weanlings. Therefore, it is unclear if polycystic kidneys in the F1 generation are due to the transfer of test chemical in the milk during lactation.

10.10.6 Conclusion on classification and labelling for reproductive toxicity

Based on the available data, classification of BENPAT as a "presumed human reproductive toxicant" in Category 1B, H360FD is warranted. It is recommended to place BENPAT into the medium potency group, with the (lower) ED_{10} value of 4.3 mg/kg bw/d.

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Hazard class not assessed in this dossier.

13 ADDITIONAL LABELLING

Hazard class not assessed in this dossier.

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15 ANNEXES

Annex I

Conf. Annex