Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

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

CLH-O-0000007054-80-01/F

Adopted

26 November 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 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

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 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
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
14.05.2021	Belgium	DAPD Consortium	Company-Manufacturer	1
Comment received				
Please refer to the non-confidential attachment. ECHA note – An attachment was submitted with the comment above. Refer to public attachment DAPD Consortium - Comments on public consultation for CLH proposal - FINAL - 14.5.21 - 96198617_1.pdf				
Dossier Submitter's Response				
Regarding point 7 of the attachment – Comments regarding the use and potential exposure of the substance: According to Regulation (EC) No. 1271/2008, a "substance or a mixture fulfilling the criteria relating to physical hazards, health hazards or environmental hazards, laid down in Parts 2 to 5 of Annex I is hazardous and shall be classified in relation to the respective hazard classes provided for in that Annex" (Article 3 of the regulation). Classification considers the intrinsic hazardous properties of the respective substance, but does not take into account the individual exposure conditions of its uses.				
RAC's response				
RAC agrees with the Dossier Submitter.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
14.05.2021	Belgium	DAPD Consortium	Company-Manufacturer	2

Comment received

Please refer to the non-confidential attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment DAPD Consortium - Comments on public consultation for CLH proposal - FINAL - 14.5.21 - 96198617_1.pdf

Dossier Submitter's Response

Regarding point 4.b of the attachment - relevance of effects in humans:

There is no mechanistic information that raises doubt about the relevance about the reproductive effects for humans.

Regarding point 4.1.f/4.2a, dystocia and maternal toxicity:

Please see Dossier Submitter's Response to comment 3.

Regarding point 4.2.a, studies performed with Sprague-Dawley (SD) rats:

The Sprague-Dawley (SD) rat is a common animal model to be used in toxicology. There is no information on modes of action of the reproductive effects seen in SD rats after treatment with the test substance which would contradict the default assumption of its relevance for humans.

Regarding point 4.2.d to k, supportive information from studies with DPPD - mode of action proposal of prostaglandin inhibition:

The information from studies with BENPAT constituent DPPD were considered by the DS to support a mechanistic explanation. Studies performed with rats show that DPPD acts as prostaglandin F2alpha (PGF2alpha) inhibitor. Even though the process of parturition differs between humans and rats, there is evidence that PGF2alpha is a relevant prostaglandin in humans, e.g. to the uterotonic activity, including contractions of the uterus and initiation of birth. Data from DPPD give supportive information on a mode of action. However, BENPAT is a multi-constituent substance, and it cannot be ruled out that other constituents contribute to the effects seen in the investigated studies.

Regarding point 4.3. consideration of analogues effects with salicylic acid (SA):

With regard to the data mentioned in the attachment of the registrant and the RAC opinion (RAC Opinion proposing harmonised classification and labelling at EU level of Salicylic acid, 2016): Effects seen in a pre-natal developmental toxicity study in rats (oral, diet) include foetal effects (foetal anomalies and growth retardation) after treatment with 165 mg/kg bw/d of SA from GD 8-14 (absence of maternal effects). At 205.9 mg/kg bw/d maternal effects were observed, expressed as temporary body weight loss with toxic symptoms (salivation, piloerection) and the following foetal effects: high foetal mortality (no live foetuses

in 9/15 dams examined), high frequency of complex anomalies (cranioschisis, myeloschisis, pes varus, oligodactyly etc; Tanaka et al., 1973a, reliability 2).
Other studies investigating effects of SA or acetyl salicylic acid (ASA) after oral gavage identified increased malformations in foetuses at 150 mg/kg bw of SA (Tanaka, 1973b), or 250 mg/kg bw/d of ASA (192 mg/kg bw/d as salicylic acid), or 200 mg/kg bw/d of ASA (Nakatsuka and Fujii, 1979; treatment GD 7-17) and 99 mg/kg bw of ASA (Schardein *et al.*, 1969; treatment GD 6-15).

In the pre-natal developmental toxicity study (OECD TG 414) conducted with BENPAT, none of these developmental effects were seen in foetuses after exposure (oral gavage) to 20.0, 70.0, and 200.0 mg/kg bw/d of the test substance from GD 6-15.

Furthermore, a repeated dose toxicity was investigated for 50, 250, 500, and 1000 mg/kg bw/day of the target substance methyl salicylate (Read-across for AS) blended with diet and administered daily for a period of 2 years in (Osborn-Mendel) rats. Effects seen in the study using methyl salicylate (growth retardation and bone lesions at 500, and 1000 mg/kg bw/day) were different from effects seen with doses of 3.3, 20 and 120 mg/kg bw/d of BENPAT in the oral 52-week dietary study (anaemic effects at high dose).

Data show a different profile of target effects and raise doubts whether these studies conducted with ASA or SA are relevant for comparison with BENPAT. As the mode of action for BENPAT is unclear, no arguments are given to support read across considerations. In addition, the classification of AS as Repro Cat. 2 was based on developmental effects only for which animal and epidemiological data were taken into consideration.

<u>Regarding point 5.2, kidney effects in SD rats.</u>

For SD rats it is known that old animals spontaneously develop singular cortical cysts. In the oral 52-week dietary study, none of the investigated animals showed cortical cysts at the 38 week sacrifice, 1/20 male rats (mid dose, 20 mg/kg bw/d) showed cortical cysts, but no female at the 52-week sacrifice. This single case is considered as incidentally.

It is emphasized that in the two-generation reproductive toxicity study, the term "polycystic kidney" was used to "differentiate from those cysts (cortical or medullary) which may arise spontaneously" (Annex III, Two-Generation Reproductive Toxicity Evaluation of Wingstay 100 Administered in the Feed to CD (Sprague-Dawley) Rats). For more information on the characterisation of renal lesions see "Characterisation of polycystic kidneys" in the Annex I of the CLH proposal. Furthermore, the study author cited that "Although the mechanism of cyst formation was not apparent, it did not appear to be associated with a primary hydronephrotic mechanism."
In conclusion, the well characterised renal lesions in BENPAT treated rats shows that the observed type of multicystic lesions differ from those arising spontaneously in aged rats. Furthermore, the (dose-dependent) high incidence in F1 and F2 weanlings and adults support a relevance of these effects. There is no evidence that the mode of action is not relevant to humans.

RAC's response

In general, RAC supports the response from the DS.

<u>Regarding point 4.b and 4.1 of the attachment:</u>

Dystocia and increased gestational length were observed in dams without general toxicity. Dystocia was thus not secondary to maternal toxicity. See also RAC's response to comment 3.

RAC agrees with the DS that there is no mechanistic information that raises doubt about the relevance about the reproductive effects for humans.

Regarding point 4.2.a, studies performed with Sprague-Dawley (SD) rats:
RAC agrees with the DS. Furthermore, the CLP guidance states (VI.4.1 Species and strains): *The most sensitive species for each substance has to be used to determine the potency parameter unless there is clear evidence that the observed effects are not relevant to humans or when there is good evidence for a difference in sensitivity between humans and the test species. This also applies to different strains.*

Adverse effects in kidneys were a pronounced and dose-dependent effect in Sprague-Dawley rat weanlings. Weanlings appeared to be more sensitive to BENPAT induced polycystic kidneys, but from the information available it is unclear whether these effects are solely due to *in utero* exposure or due to exposure during lactation and the diet. In view of that, the information was included as supported evidence for classification for developmental toxicity. The industries stated that this is strain dependent. RAC notes there is no information available to RAC to support this effect is not relevant to humans.

Regarding point 4.2.d to k, supportive information from studies with DPPD - mode of action proposal of prostaglandin inhibition:
RAC agrees with the DS that the studies with DPPD, one of the constituents of BENPAT, do provide some mechanistic explanation, showing that DPPD acts as prostaglandin inhibitor. RAC agrees with the DS that prostaglandin inhibition is relevant for humans. RAC notes that BENPAT consists of other constituents and impurities, with unknown toxicity/modes of action. As a consequence, the effects observed for BENPAT cannot be solely attributed to DPPD.

Regarding point 4.3, consideration of analogue effects with salicylic acid (SA)/ASA:
RAC agrees with the DS that developmental effects observed for salicylic acid or acetyl salicylic acid show a different profile (e.g., malformations such as cranioschisis, craniorachischisis and dose-related growth retardation), so effects different from those observed for BENPAT. In addition, it is noted that the classification for developmental toxicity of SA/ASA was based on evidence from animal studies and a very large epidemiological data base for SA. RAC notes that no such data is available for BENPAT. So, neither the effects observed in animal studies for ASA and BENPAT are comparable, nor are the data available to RAC for SA and BENPAT comparable. In view of the different effects observed, the different animal and human (epidemiological) data available to RAC for ASA and BENPAT, together with the fact that BENPAT is a multiconstituent, RAC agrees with the DS that comparison because of analogue effects is not justified.

Regarding point 5, comments on polycystic kidneys:
RAC concurs with the response from the DS on the polycystic kidneys. RAC considers the polycystic kidneys in weanlings in the two available generation studies as supportive evidence, besides increased post-implantation loss, for BENPAT-induced developmental toxicity. See also RAC's answer to comment 3.

Regarding point 6, comments on the use of studies on DPPD and DPA for the assessment:
Justification for classification of reproductive toxicity is based on available information from reproductive studies for BENPAT. Information on DPPD and DPA is used for an explanation of the mode of action for prolonged parturition (dystocia) or as supportive evidence for polycystic kidneys in this assessment. Furthermore, it has been stressed multiple times by the DS and RAC that BENPAT consists of multiple other constituents and impurities, besides DPPD and DPA, with unknown toxicity/modes of action. RAC does not agree that information on DPPD and DPA are specifically used as justification for classification as suggested in this comment.

Date	Country	Organisation	Type of Organisation	Comment number
12.05.2021	Sweden		MemberState	3

Comment received

Fertility

The Swedish CA agree that the dystocia observed in the 2-generational study as well as in the 1-generation mechanistic study, resulting in a significantly reduced delivery of viable offspring, is an adverse effect that warrants classification, in line with previous conclusions by RAC. In the 2-generational study this effect showed a dose-dependent increase, being significant at 25 and 100 mg/kg/d, but with possible changes already at 7.5 mg/kg/d. SE consider the degree of maternal toxicity as key for a classification in either category 1B or 2 and the proposal would benefit from a clearer description of this. Was the maternal toxicity (e.g. body weight changes, clinical symptoms, necrosis of liver and kidney (including the degree of severity) at 100 mg/kg/d) more pronounced in the dams that did not deliver viable pups? Were there any body-weight changes or clinical symptoms observed at 7.5 and 25 mg/kg/d. If the maternal toxicity can be considered not to be of sufficient magnitude to cause the observed effects Cat 1B is supported. Any data available from repeated dose-studies on the effects of BENPAT on hormonal levels in female rats could provide further mechanistic insights/support as to if the affected cyclicity could play a role in the observed dystocia.

Developmental toxicity

Polycystic kidneys were observed in all generations of the 2-generational study and the 1-generation mechanistic study. The offspring generations (F1 and F2) are clearly more sensitive to this effect as indicated by their significantly higher incidences than the parental generation. However, we do not consider it clear whether this effect should be regarded as a developmental effect or not. If not any structural disturbances of the development of the kidney have occurred, this effect could rather be considered as a systemic toxic effect (relevant for classification). Any additional mechanistic data on the possible development of polycystic kidneys from BENPAT or other similar substances would be helpful.

Dossier Submitter's Response

Fertility

High dose F0 dams that died during delivering/ were euthanized in process of delivery or died during lactation did not show differences in (absolute) body weight (BW) or body weight gain (BWG) during gestation, relative to females that survived until terminal sacrifice at 100 mg/kg bw/d. There were clinical symptoms among dams that died at 100 mg/kg bw/d, including piloerection (gestation day (GD) 23, 24, PND 0) and vaginal bleeding (GD 23, 24, post-natal day (PND) 0). Microscopic observation revealed vaginal haemorrhage; uterus inflammation and haemorrhage; ovary cyst; liver vacuolisation, hematopoietic cell

proliferation, liver haemorrhage, and necrosis; kidney congestion, inflammation, polycystic kidneys, and kidney necrosis; lungs congestion, lungs inflammation, and thrombosis; adrenal cortex degeneration and haemorrhage. Among dams that survived until terminal sacrifice there were females that showed piloerection (GD 23, PND 0-5) and vaginal bleeding (GD 23), but in these dams there were no gross lesions observed during microscopy, except vagina inflammation (1 animal) and polycystic kidneys (2 animals). Body weight (BW), BWG, clinical observation, and histopathological findings of dams for high dose (100 mg/kg bw/d) F0 dams that died/ were euthanized during delivery/lactation and F0 dams that were sacrificed at the end of the study are summarised in Table 1.

Dystocia was evident in mid (25 mg/kg bw/d) and high (100 mg/kg bw/d) dose F0 animals (OECD TG 416). Clinical observation, live and dead pups on PND 0, and histopathological findings of mid and high dose dams (25 and 100 mg/kg bw/d) with dystocia, which died/ were euthanized during gestation or lactation are summarised in Table 2.

Liver necrosis in 7/9 high dose dams and kidney necrosis in 5/9 high dose dams with dystocia may be discussed as primary cause resulting in dystocia. Due to the fact that some dams with dystocia did not show necrotic lesions in any of these organs and the observation that the majority of necrotic lesions were only minimal to mild it appears as not likely that these necrotic lesions are the cause of dystocia. Moreover, the necrosis could also be secondary to dystocia and haemorrhagic lesions at multiple sites.

F0 dams treated with 25 mg/kg bw/d and died during lactation (litters of all dead pups; viable pups and pups retained in utero) showed gross lesions comparable to dams treated with 100 mg/kg bw/d BENPAT (Table 2). F0 dams (25 mg/kg bw/d) that survived until terminal sacrifice did not show gross lesions, except: ovary cyst, follicle (3 animals); cyst, germinal epithelium (1 animal).

There were no abnormal cycles in F0 dams exposed to 100 mg/kg bw/d BENPAT, including F0 dams that showed signs of dystocia and died during delivery or lactation. Cycle length of high dose F0 dams was comparable to control animals.

Table 1: Body weight (BW), body weight gain (BWG), clinical observation and microscopic evaluation of F0 dams (100 mg/kg bw/d) that died/ were euthanized during delivery/lactation and F0 dams (100 mg/kg bw/d) that were investigated at terminal sacrifice.

Dose (mg/kg bw/d)	**100**	
	F0 ♀, died/euthanized (PND 0 - 8; N=9)[1]	F0 ♀, terminal sacrifice (N=16)
BW GD 0 (g)	308.5±26.9	311.9±26.6
BW GD 7 (g)	338.5±32.9	338.8±27.5
BW GD 14 (g)	366,0±30.7	365.4±32.7
BW GD 21 (g)	440.5±30.9	423.8±66.5
BWG GD 0-7 (%)	9.7	8.6
BWG GD 7-14 (%)	18.6	17.2
BWG GD 14-21 (%)	42.8	35.9
Length gestation (d)	24.1±0.7	23.1±0.5
Average pups in total	12.0 ±4.0	12.7±4.5
Average Live pups, PND 0	0.1	11.6
Average dead pups PND 0,	10.6	1.1
Dams with litter of pups all dead	8/9	1/16
Dams with retained dead foetuses in utero	2/9	-

[1] Necropsy finding, consider death at PND 0-8

Piloerection[2]	7/9	5/16
	Vagina	
Bleeding[2]	5/9	2/16
pup stuck in vagina[2]	1/9	1/16
Inflammation, chronic[3]	-	1/16
Haemorrhage (mild, marked)[3]	1/9	-
	Uterus	
inflammation, acute (mild/moderate marked); chronic-active (moderate) [3]	4/9	-
Haemorrhage (mild/ moderate)[3]	2/9	-
	Ovary	
Cyst, follicle (minimal, mild); mineralisation; oviduct (moderate); cyst, germinal epithelium (minimal) [3]	2/9	2/16
	Liver	
Vacuolisation Cytoplasmic, Hepatocyte, Centrilobular (mild/moderate)[3]	2/9	-
Hematopoietic Cell Proliferation (minimal/mild/moderate)[3]	4/9	-
Inflammation, acute (mild)[3]	1/9	-
Haemorrhage, Centrilobular (mild)[3]	1/9	-
Necrosis, Hepatocyte, Centrilobular (5x minimal or mild/2x moderate)[3]	7/9	-
	Kidney	
Congestion, medulla (moderate)[3]	1/9	-
Inflammation, acute (mild); chronic (mild)[3]	2/9	-
Polycystic kidney (minimal)[3]	1/9	2/16
Necrosis, Cortex (1x minimal, 2x mild, 2x marked)[3]	5/9	-
	Lungs	
Congestion (mild)[3]	1/9	-
Inflammation, acute, artery (marked)[3]	2/9	-
Thrombosis, artery (marked)[3]	2/9	-
Metaplasia, osseous, minimal[3]	-	-
	Adrenal cortex	
Degeneration (moderate/marked)[3]	3/9	-
Haemorrhage (mild)[3]	1/9	-

Table 2: Clinical observation and microscopy evaluation of F0 dams with signs of dystocia at 25 and 100 mg/kg bw/d.

[2] Clinical observation

[3] Microscopic observation

Dose: mg/kg bw/d	25			100								
♀No.	84	150	156	98	220	44	202	108	164	58	14	152
died/ euthanized	GD 17	PND 0	PND 2	PND 0	PND 0	PND 1	PND 0	between PND 1 and 4[4]	PND 4	PND 5	PND 5	PND 8
Length gestation (d)	-	No data[5]	23	24	No data[6]	23	No data[7]	24	24	24	25	25
Live pups, PND 0	-	0	6	0	0	1	0	0	0	0	0	0
Dead pups, PND 0	16 resorbing foetuses in utero	All pups dead (no info on total no.), 9 retained foetuses in utero	0, and 3 retained foetuses in utero	13	6, and 9 retained dead foetuses in utero	7	14, and 3 retained dead foetuses in utero	10	11	17	5	12
Vagina[8]	-	1 retained foetus at necropsy	1 retained foetus at necropsy	bleeding (GD 23, PND 0)	blood in cage and vaginal area (GD 23)	-	bleeding (GD 23, PND 0)	bleeding (GD 23, PND 0)	-	haemorrhage, marked[9]	vaginal bleeding (GD 23)	pups stuck in vagina, bleeding GD 24
Piloerection**Error! Bookmark not defined.**	X (GD 14 - 17)	-	X (PND 0-2)	-	X (GD 23)	X (GD 23, PND 0)	X (GD 23)	X (GD 23, PND 0)	X (PND 0)	-	X (GD 23, PND 0)	X (GD 24, PND 0)
Uterus**Error! Bookmark not defined.**	haemorrhage, mild	-	haemorrhage, mild, inflammation, acute, moderate	-	-	too autolyzed to evaluate	inflammation, acute, mild;	-	haemorrhage, moderate	haemorrhage, mild**Error! Bookmark not defined.**; inflammation, chronic-active, moderate	cervix, too autolyzed to evaluate	inflammation, acute, marked
Ovary**Error! Bookmark not defined.**	mineralisation; oviduct, minimal	-	-	-	cyst, follicle, minimal	-	-	cyst, oviduct, moderate	-	-	-	-
Liver**Error! Bookmark not defined.**	-	vacuolisation cytoplasmic, hepatocyte, centrilobular, mild	necrosis, hepatocyte, centrilobular, mild	necrosis, hepatocyte, centrilobular, mild; vacuolisation cytoplasmic, hepatocyte, centrilobular, moderate	necrosis, hepatocyte, centrilobular, mild; hema cell prolif, minimal	necrosis, hepatocyte, centrilobular, minimal; vacuolisation cytoplasmic, hepatocyte, centrilobular, mild	necrosis, hepatocyte, centrilobular, mild;	-	necrosis, hepatocyte, centrilobular, moderate; hema cell prolif, mild;	necrosis, hepatocyte, centrilobular, moderate	necrosis, hepatocyte, centrilobular, mild; hema cell prolif, moderate; hemorrhage, centrilobular, mild	inflammation, acute, mild; hema cell prolif, minimal
kidney**Error! Bookmark not defined.**	-	-	necrosis, cortex, marked	-	necrosis, cortex, mild; inflammation, chronic, mild	-	necrosis, cortex, mild	-	necrosis, cortex, minimal; congestion, medulla, moderate	necrosis, cortex, marked	inflammation, acute, mild; polycystic kidney, minimal	necrosis, cortex, marked
lungs**Error! Bookmark not defined.**	-	metaplasia, osseous, minimal	congestion, mild	-	-	-	-	-	thrombosis, artery, marked; inflammation, acute, artery,	congestion, mild	thrombosis, artery, marked; inflammation, acute, artery,	-

[4] Not specified in the study report.

[5] Gestation length not given in the study report. According to the study report, female no. 150 died in the process of delivering on PND 0 (SD 96).

[6] Gestation length not given in the study report. "*Female 220 died while in the process of delivering on postnatal day 0 (study day 95)".*

[7] Gestation length not given in the study report. "*Female 202 was euthanized moribund while in the process of delivering on postnatal day 0 (study day 96)."*

[8] Clinical observation

[9] Microscopic observation at unscheduled necropsy

									marked		marked	
Adrenal cortex**Error! Bookmark not defined.**	-	haemorrhage, mild	-	-	degeneration, moderate	-	degeneration, moderate	-	-	degeneration, marked; haemorrhage, mild	-	-
mammary gland	adenocarcinoma neoplasm (malignant without metastasis)	-	-	-	-	-	-	-	-	-	-	-

In the F1 generation, effects of dystocia were evident (prolonged gestation length relative to controls, litters of dead pups/ pups all dead) in dams treated with 7.5 (1 animal), 25 (1 animal), and 100 mg/kg bw/d (3 animal).
F1 dams that died during/after delivery of dead pups (1 female at 25 mg/kg bw/d, PND 3; 1 female at 100 mg/kg bw/d; PND 0) showed retained dead foetuses in utero at unscheduled necropsy. Microscopic evaluation identified uterus inflammation, haemorrhage, and thrombosis; liver necrosis; lung embolus, lung oedema; and bladder hyperplasia and inflammation. Data are summarised in Table 3. F1 dams (100 mg/kg bw/d) that were investigated at terminal sacrifice (including two dams with litter of pups all dead) did not show gross lesions related to the uterus, liver, lungs, and bladder (as seen in in F1 dams that died). Microscopic findings were only related to the kidney in these animals (kidney mineralisation, polycystic kidneys; regeneration renal tubule). No indication on kidney necrosis has been observed in dams at 25 and 100 mg/kg bw/d showing dystocia or post-implantation loss (2 dams at 25 mg/kg bw/d). Mild respectively marked liver necrosis was seen in only one dam at 25 mg/kg bw/d and at 100 mg/kg bw/d each. In conclusion dystocia or post-implantation loss is not considered as secondary to severe necrosis in liver or kidney. In comparison to F0 dams, necrosis in liver was less often and less prominent in F1 dams, kidney necrosis was absent in F1 dams.

At 25 and 100 mg/kg bw/d, there was an increased number of females with abnormal stage of oestrous cycle (28.6 % (8/30) and 43.3 % (13/30), respectively) relative to control (6.7 % (2/30)). Data on cycling for dams with dystocia are summarised in Table 3.

There were no data on the effects of BENPAT on hormonal levels in female rats from repeated dose-studies available.

Table 3 Clinical observation, microscopy evaluation of F1 dams showing signs of dystocia (7.5, 25, and 100 mg/kg bw/d).

♀No.	472	364	392	528	370	536	326
Dose (mg/kg bw/d)	7.5	25			100		
died/euthanized	End of study	End of study	End of study	PND 3	End of study	End of study	PND 0
Length gestation (d)	ND[10]	-	-	23	24	24	24
Live pups, PND 0	0	implant sites only	implant sites only	10	0	0	1
Dead pups,	3 (3/3 pups			4, and 1 retained	13 (13/13	9 (9/9 pups	5, and 10 retained

[10] Gestational length could not be calculated because sperm were never detected in the vaginal smear.

PND 0	of the litter dead)			dead foetus	pups dead)	all dead)	dead foetuses in utero
Piloerection	X (PND 0)	-	-	-	-	X (PND 0)	X (GD 23)
Uterus					-		Inflammation, chronic, mild; haemorraghe, moderate; thrombosis, vein, moderate
Liver				Necrosis, hepatocyte, centrilobular, moderate	-		Necrosis, hepatocyte, centrilobular, mild
kidney	Mineralisation, corticomedullary junction, minimal	Mineralisation, corticomedullary junction, minimal	Mineralisation, corticomedullary junction, minimal	Mineralisation, corticomedullary junction, minimal	Polycystic, minimal; mineralisation, corticomedullary junction, mild; regeneration renal tubule, minimal	Mineralisation, corticomed jc corticomedullary juntion t, minimal	Polycystic, mild; mineralisation corticomedullary junction, minimal; inflammation, chronic, pelvis, moderate
lungs				Embolus bacterial, artery, mild; congestion, mild			Oedema, alveolus, mild
bladder							Hyperplasia, mild; inflammation, chronic, mild; calculus, mild
Cycle length (d)	4.5	4.0	10	3.0	8.0	17.0	4,0
Abnormal cycle	No (3/30 in total)	No (8/30 in total)	Yes (8/30 in total)	No (8/30 in total)	Yes (13/30 in total)	Yes (13/30 in total)	No (13/30 in total)

Please note a correction to the original CLH dossier, page 27, second paragraph: Gestational body weight of F1 females was reported as not significantly different between dose groups

and controls. However, it is correct that high dose F1 (not as indicated F0) dams gained significantly less body weight (-11 %) compared to controls during the gestation (no data on corrected body weight). F1 (not as indicated F0) maternal body weight was significantly lower (-9%) than the control values during lactation on PND 0, 4, and 7. For F0 and F1 dams 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).

Developmental toxicity

Only treatment of BENPAT during gestation and lactation resulted in a high incidence of polycystic kidneys in all exposure groups (dose-dependent, with 100% in F1 and F2 weanlings at 100 m/kg bw/d; see Table 13 of the CLH report). However, treatment of animals from adulthood on revealed just a low number of (female) rats (3/9 females at 100 mg/kg bw/d) with polycystic kidneys. The DS concludes that the developing kidneys are more sensitive than the adult kidneys, assessing the effect as foetal toxicity. Unfortunately, there are no data on further investigations, e.g. urine analysis, or additional mechanistic data.

RAC's response

The studies show that BENPAT results in dystocia (prolonged parturition or obstructed labour), which in most cases resulted in dead dams and pups. The histopathological effects in liver and kidneys in the highest dose group were minimal to mild and not observed in all dams presenting dystocia. Adverse effects on fertility and sexual function (abnormal cycles, increased gestational length and dystocia) were already observed in absence of general toxicity.

Polycystic kidneys were more pronounced in weanlings as compared to the effects seen in adult rats in the two generation studies but were not observed in repeated dose studies. Polycystic kidneys in weanlings can be the result of exposure to BENPAT *in utero*, a higher sensitivity for the formation of polycystic kidneys in weaning or of exposure to BENPAT via lactation and self-feeding. It is not possible to rule out any of these options. No information is provided on the severity/grade of the observed polycystic kidneys in weanlings. The fact that polycystic kidneys were also observed in F1 adults does indicate the effect is not (fully) reversible. All in all, it is not clear whether polycystic kidneys are the effect of exposure *in utero,* via lactation or via the diet (or a combination thereof) and the effects seem to be non-reversible.

According to the CLP Regulation Annex I paragraph 3.7.1.4, classification for developmental toxicity is primarily intended for effects induced during pregnancy and due to parental exposure. It is unclear from available data whether the increase in polycystic kidneys in weanlings can be considered a developmental effect warranting classification on its own. RAC concludes that the consistent and dose-related post-implantation loss is key for classification for development and polycystic kidneys in weanlings are considered as supportive evidence for developmental effects. Polycystic kidneys in weanlings are considered as permanent and serious but it not clear whether this is due to *in utero* exposure only.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
14.05.2021	Belgium	DAPD Consortium	Company-Manufacturer	4
Comment received				
Please refer to the non-confidential attachment. ECHA note – An attachment was submitted with the comment above. Refer to public attachment DAPD Consortium - Comments on public consultation for CLH proposal - FINAL - 14.5.21 - 96198617_1.pdf				
Dossier Submitter's Response				
As agreed by the registrant, available data on skin sensitisation do not allow for sub-categorisation. Since no further data are available, a harmonised classification of BENPAT as skin sensitiser, category 1 is supported. Data do not allow a conclusion on subcategorisation.				
RAC's response				
RAC agrees with the Dossier Submitter.				

PUBLIC ATTACHMENTS
1. DAPD Consortium - Comments on public consultation for CLH proposal - FINAL - 14.5.21 - 96198617_1.pdf [Please refer to comment No. 1, 2, 4]