baua:
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Federal Institute for Occupational Safety and Health

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,4-Benzenediamine, N,N'-mixed phenyl and tolyl derivs. (BENPAT)

EC No 273-227-8

CAS RN 68953-84-4

Evaluating Member State(s): Germany

Dated: July 2022

Evaluating Member State Competent Authority

BAuA

Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Year of evaluation in CoRAP: 2013

Before concluding the Substance evaluation a Decision to request further information was issued on 01 October 2015

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance *1,4-Benzenediamine, N,N'-mixed phenyl and tolyl derivs.* ('BENPAT', EC number 273-227-8) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB properties
- Wide dispersive use
- Consumer use
- Aggregated tonnage

During the evaluation, the following additional concerns were identified:

- Mutagenicity (Gene mutations in mammalian cells)
- Repeated dose toxicity
- Carcinogenicity
- Reproductive toxicity

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

BENPAT has been assessed by the PBT Expert Working Group of the Technical Committee of New and Existing Chemicals (TC NES) under the previous EU chemicals legislation. At that point in time, it was decided to delete BENPAT from the list of potential PBT/vPvB substances based on the knowledge at that time.[2]

A dossier for harmonised classification and labelling (CLH) has been prepared and submitted by the evaluating member state competent authority (eMSCA) in March 2021 aiming for the inclusion of BENPAT in Annex VI of Regulation (EC) No 1272/2008 (CLP regulation) for the hazard classes skin sensitisation (Skin Sens. 1, H317) and reproductive toxicity (Repr. 1B, H360FD).[3]

In their opinion on the CLH dossier, adopted in November 2021, ECHA's Committee for Risk Assessment (RAC) has concluded that BENPAT warrants classification as Skin Sens. 1 (H317) and Repr. 1B (H360FD) (RAC, 2021).

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the Substance has led the eMSCA to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	

[2] https://echa.europa.eu/documents/10162/0d9952ad-815e-4b70-a0f8-cd43f1dc1cd8
[3] https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e183955610

No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

The available data is sufficient to conclude that BENPAT acts as a presumed human reproductive toxicant. Therefore, the eMSCA proposed to classify and label the Substance as Repr. 1B (H360FD. May damage fertility. May damage the unborn child).

As the self-classification did not reflect this assessment and Article 36 CLP names reproductive toxicity as a hazard class normally subject to harmonised classification and labelling (CLH), the eMSCA has prepared a CLH dossier to this effect. 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 also be acknowledged throughout the Community, and therefore a CLH process was required.

RAC concluded that BENPAT warrants classification as Repro. 1B for fertility and development (H360FD. May damage fertility. May damage the unborn child) and as Skin Sens. 1 without sub-categorisation (H317. May cause an allergic skin reaction) (RAC, 2021).

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Human Health

RAC has already concluded that classification of BENPAT as Repr. 1B is warranted. Therefore, the Substance fulfils the criteria for identification as SVHC according to Article 57(c) though the eMSCA currently considers the implementation of the suggested (see above) harmonised classification of BENPAT as a sufficient risk management measure with regard to the protection of human health.

Environment

The eMSCA currently considers the available information insufficient to firmly conclude on the PBT/vPvB status of BENPAT. However, available scientific methods are lacking to close this knowledge gap. Therefore, the eMSCA currently considers an SVHC identification of BENPAT due to a clear fulfilment of the PBT and/or vPvB properties of the substance according to Annex XIII according to Article 57(d) or 57(e) as not possible.

An identification of BENPAT as SVHC from the viewpoint of protection of the environment will be considered when the PBT/vPvB properties of BENPAT are clarified.

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable

5.2. Other actions

Not applicable

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date**	**Actor**
CLH dossier (Repr. 1B and Skin Sens. 1)*	03 March 2021 (Final submission)	DE CA

* RAC concluded in 2021 on classification as Repro. 1B for fertility and development (H360FD) and as Skin Sens. 1 without sub-categorisation (H317) (RAC, 2021).

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance *1,4-Benzenediamine, N,N'-mixed phenyl and tolyl derivs.* ('BENPAT', EC number 273-227-8) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB properties
- Wide dispersive use
- Consumer use
- Aggregated tonnage

During the evaluation, the following additional concerns were identified:

- Mutagenicity (Gene mutations in mammalian cells)
- Repeated dose toxicity
- Carcinogenicity
- Reproductive toxicity

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated Initial and additional concerns	**Outcome/conclusion**
Persistence	**Concern unresolved.** Based on the available information the eMSCA does not consider a final conclusion on whether BENPAT fulfils the Annex XIII criterion for persistent (P) or very persistent (vP) as possible.
Bioaccumulation	**Concern confirmed.** Based on the available information constituents of BENPAT meet the criterion for bioaccumulative (B) or even very bioaccumulative (vB) according to Annex XIII.
Toxicity	**Concern confirmed.** Based on the available information BENPAT fulfils the criteria for T according to Annex XIII with regard to effects observed for the whole substance; both for human health (Repr. 1B) and ecotoxicity (Aquatic Chronic 1). Similar effects are expected for the aquatic toxicity of the single constituents.
Overall conclusion on PBT/vPvB status	**Concern unresolved.** While the eMSCA considers a conclusion on the B and T criterion for BENPAT possible, a conclusion on P is not possible at the moment. Based on present knowledge, the eMSCA does not consider currently available validated test methods and information requirements as suitable to clarify the remaining concern for the Substance.
Wide dispersive use	**Concern confirmed.** BENPAT is registered in an aggregated tonnage of >1000 tonnes per year for wide dispersive uses, e.g. as an antioxidant in tyres. Due to the relevance of the service life of articles which consumers may handle, the eMSCA considers the harmonised classification of BENPAT as an important step to indicate its hazardous potential.
Aggregated tonnage	
Consumer use	
Mutagenicity	**Concern refuted.** Based on new information provided by the registrants following a decision, the eMSCA concludes that there is no concern for mutagenicity. No further action required.
Repeated dose toxicity	**Concern confirmed.** BENPAT consistently showed a proliferative activity in the urinary bladder in available repeated dose studies at doses as low as 2.65 mg/kg bw/d (52-week chronic dietary study). However, with an

EVALUATED ENDPOINTS	
Endpoint evaluated Initial and additional concerns	**Outcome/conclusion**
	absence of microscopic findings in the bladder. Registrants should consider self-classifying BENPAT as STOT RE 1.
Carcinogenicity	**Concern refuted.** Based on the available information, BENPAT does not warrant classification for carcinogenicity. During evaluation, the necessity to further clarify this endpoint was made dependent on the outcome of the requested study on *in vivo* gene mutation. As the requested study was negative, no further action is deemed necessary.
Reproductive toxicity	**Concern confirmed.** Available information is sufficient to conclude that BENPAT warrants classification as Repr. 1B, H360FD. CLH process was initiated and completed by the eMSCA.
Additional endpoints evaluated	
Skin Sensitisation	**Concern confirmed.** Available information on the skin sensitising potential of BENPAT warrants classification and labelling of the substance as Skin Sens. 1, H317. The eMSCA has submitted a CLH dossier on this endpoint (BAuA, 2021) and RAC concluded that classification and labelling of BENPAT as Skin Sens. 1, H317, without sub-categorisation, is warranted (RAC, 2021).

7.2. Procedure

BENPAT was suspected to fulfil the PBT properties according to REACH Annex XIII based on the eMSCA's PBT QSAR screening of substances registered in 2010. In January 2013, new information became available. Based on the information from new tests and suspected PBT properties the eMSCA notified BENPAT for substance evaluation (SEV) according to REACH Article 45(5). On 1 July 2013, BENPAT was included in the CoRAP list.[4]

For the environment the evaluation was targeted at the persistency, the bioaccumulation potential and toxicity of BENPAT. Environmental hazard assessment was based on registration dossiers and the information from new tests provided by the registrants in January 2013.

The evaluation of BENPAT with regard to human health was targeted at toxicity. The assessment of exposure was excluded. The data provided in the registration dossiers, original study reports as well as publicly available literature and evaluation reports were considered by the eMSCA for the human health assessment of BENPAT.

The eMSCA prepared a draft decision with further information requirements on BENPAT. Following notification of the draft decision to the registrants and decision making by the Member State Committee (MSC), ECHA took the SEV decision on 1 October 2015.[5]

Following an appeal before ECHA's Board of Appeal (BoA) and the subsequent BoA decision on 8 September 2017,[6] the SEV decision was partly annulled. An action brought against the BoA decision before the General Court resulted in partial annulment of the BoA decision

[4] Inclusion of additional substance for the year 2013 following a REACH Article 45(5) notification: https://echa.europa.eu/documents/10162/17221/additional_corap-substance-inclusion-2013_en.pdf

[5] SEV decision on BENPAT from 1 October 2015: https://echa.europa.eu/documents/10162/12660814-8e9a-22f5-34e8-61c2a426658e

[6] BoA decision on BENPAT (Case A-026-2015) from 8 September 2017: https://echa.europa.eu/documents/10162/140d1f25-70de-b36d-acf8-b850a100f7d9

but was dismissed to the remainder following a judgment of the General Court on 20 September 2019.[7]

The information requirements in the procedurally modified SEV decision were the following:

- Transgenic rodent (TGR) somatic and germ cell mutation assays in rats or mice, oral route (OECD TG 488) or
  In vivo Mammalian Alkaline Comet Assay in rats, oral route (OECD TG 489)
- Full study report and annexes for an unpublished Two-Generation reproductive toxicity study report
- Simulation testing on ultimate degradation in surface water (OECD TG 309) using a representative constituent of BENPAT (R-898).

The last elements of the required information were provided by the registrants on 6 April 2021 and were evaluated by the eMSCA. The eMSCA concluded its assessment based on all available information and submitted its conclusion to ECHA.

7.3. Identity of the substance

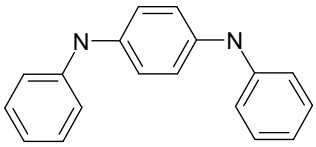
Table 4

SUBSTANCE IDENTITY	
Public name:	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.
EC number:	273-227-8
CAS RN:	68953-84-4
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	N/A
Molecular weight range:	260.3 – 288.39 g/mol
Synonyms:	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs. Accinox 100 Blend of phenyl and tolyl p-phenylenediamines DAPD Mixed di-aryl-p-phenylenediamines Mixed diaryl-p-phenylenediamines Naugard 496 Novazone AS Vulkanox 3100 WINGSTAY 100 WTR Number 4a

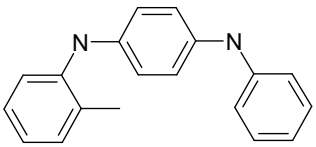
Type of substance ☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

[7] Judgment of the General Court in Case T-755/17 from 20 September 2019: https://curia.europa.eu/juris/liste.jsf?num=T-755/17&language=EN

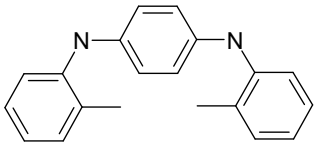
Structural formula:



(1) CAS-No: 74-31-7



(2) CAS-No: 27173-16-6



(3) CAS-No: 15017-02-4

(structures of the three main constituents)

Table 5

INFORMATION ON CONSTITUENTS				
No.	**IUPAC Name**	**Molecular formula**	**CAS RN**	**EC No.**
1	N,N'-diphenylbenzene-1,4-diamine, R-59	$C_{18}H_{16}N_2$	74-31-7	200-806-4
2	N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine, R-1679	$C_{19}H_{18}N_2$	27173-16-6	N/A
3	N,N'-bis(2-methylphenyl)benzene-1,4-diamine , R-898	$C_{20}H_{20}N_2$	15017-02-4	239-102-7

7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	Blue-brown flakes or pastilles with an amine-like odour.
Melting/freezing point	Ca. 97.6 °C at ca. 1 atm, melting range 93-101°C (DSC measurement).
Boiling point	Decomposition before boiling at 350 °C at ca. 1 atm (DSC measurement).
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.
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.

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Water solubility	The water solubility of the 3 main constituents was determined with OECD TG 105 (column elution method): (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
Partition coefficient n-octanol/water (log value)	The partition coefficient of the 3 main constituents was determined (OECD TG 117, HPLC method): (1) N,N'-diphenyl-p-phenylenediamine; CAS: 74-31-7: log K_{ow} = 3.3, 24 °C, pH ca. 7 (2) N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine; CAS: 27173-16-6: log K_{ow} = 3.9, 24 °C, pH ca. 7 (3) N,N'-bis(2-methylphenylbenzene)-1,4-diamine; CAS: 15017-02-4: log K_{ow} = 4.6, 24 °C, pH ca. 7
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 µm mesh sieve, no particles < 100 µm were detected.
Dissociation constant	pK_a = 1.47 at 20 °C (OECD TG 112) (Value for N,N'-diphenyl-p-phenylenediamine)

7.5. Manufacture and uses

7.5.1. Quantities

Table 7

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☒ 1000- 10,000 t	☒ 10,000-50,000 t
☒ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

Currently, there are three active registrants for BENPAT.

7.5.2. Overview of uses

BENPAT is used as an antioxidant and antiozonant in rubber (Engels, H.-W et al. 2011). It is used in the manufacturing of rubber products and tyres.[8]

[8] ECHA dissemination database on BENPAT: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13540/1/1 Last accessed 9 December 2021.

Table 8

USES	Use(s)
Formulation	Formulation of DAPD Anti-oxidant use for manufacturing of general rubber goods (GRG) Anti-oxidant use for manufacturing of tyres and retreading Anti-oxidant use during end of life tyre and GRG waste processing
Uses at industrial sites	Anti-oxidant use during end of life tyre and GRG waste processing
Uses by professional workers	Anti-oxidant use during the service life of GRG – maintenance of GRG articles Anti-oxidant use during the service life of GRG – mounting and dismounting of tyres
Consumer Uses	Anti-oxidant use during the service life of tyres Anti-oxidant use during the service life of GRG
Article service life	Anti-oxidant use during the service life of GRG – maintenance of GRG articles Anti-oxidant use for manufacturing of tyres and retreading Anti-oxidant use during the service life of GRG Anti-oxidant use during the service life of tyres Anti-oxidant use during the service life of GRG – mounting and dismounting of tyres

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

There is no Annex VI entry in CLP available for BENPAT yet. The eMSCA submitted a CLH proposal for the Substance in March 2021 and the RAC opinion was adopted on 26 November 2021: Repr. 1B, Skin Sens. 1.[9]

7.6.2. Self-classification

- In the registration:

Skin Sens. 1B	H317
Repr. 2	H361
Aquatic Acute 1	H400 (M = 10)
Aquatic Chronic 1	H410 (M = 10)

- No additional hazard classes are notified among the aggregated self-classifications in the C&L Inventory.[10]

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

Like other p-phenylenediamines, BENPAT is used as an antioxidant and antiozonant (Engels, H.-W et al. 2011) in tyre manufacturing. Consequently, the main constituents of the Substance are susceptible to oxidation reactions with oxygen, ozone, or reactive radicals.

[9] ECHA overview on CLH process for BENPAT: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/substance/external/100.066.551
[10] ECHA C&L inventory on BENPAT: https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/83611 Last accessed 9 December 2021

Oxidation of p-phenylenediamines has been examined in several studies (Cataldo 2002, Cataldo et al. 2015, Cataldo 2018, Cataldo 2019, Seiwert et al. 2022). A quinonediimine derivative has been observed as a potential oxidation product (Cataldo 2002, Cataldo et al. 2015, Seiwert et al. 2022) which is of particular relevance for diaryl substituted p-phenylenediamines like the constituents of BENPAT (Cataldo et al. 2015).

7.7.1.1.1. Hydrolysis

Two studies are available (Springborn, 1996 and Environment Canada and Health Canada, 2011) which present contradictory results on the hydrolysis of BENPAT.

The final conclusion of the former EU PBT working group (PBT Working Group of the European Chemicals Bureau, 2005) was based on a study on the structural similar substance *N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine* ('6PPD', EC 212-344-0). In this study (Springborn, 1996) 6PPD was found to hydrolyse quickly. It has to be stated, though, that the study showed some deficiencies, e.g. alleged metabolites identified in the study matched substance impurities, no information on precipitates was given and two power cuts disturbed the procedure. Meanwhile, in the light of newer findings (cf. below) the study and its conclusions seem questionable.

A decrease of the initial concentrations of BENPAT's representative constituents was observed in another study on hydrolysis of BENPAT but no degradation products could be observed. Precipitates of the three main constituents were observed on the surface of the glassware. This result indicates that the reported decline of BENPAT in aqueous solution is based on physical precipitation only, and that no hydrolysis occurs (Environment Canada and Health Canada, 2011).

The eMSCA concludes that hydrolysis of BENPAT is unlikely and will not contribute to the fate of BENPAT in environment.

7.7.1.1.2. Phototransformation/photolysis

7.7.1.1.2.1. Phototransformation in air

Model calculations using AOPWIN describe phototransformation to be fast with a DT_{50} of 0.64 h (Environment Canada and Health Canada, 2011). This degradation pathway is of minor importance though, because vapour pressure is low and only negligible amounts of BENPAT will be present in air.

7.7.1.1.2.2. Phototransformation in water

A photodegradation study for one of the structures which is a main constituent of BENPAT showed DT_{50} of 2.5 or 4.7 days at pH 6 or pH 8 (Environment Canada and Health Canada, 2011). As phototransformation may only happen in the upper layers of surface water and having in mind that the Substance will strongly adsorb to suspended organic matter, contribution of photolysis in water will only have small impact on the fate of BENPAT in aquatic environment.

7.7.1.1.2.3. Phototransformation in soil

No data on phototransformation are available. BENPAT shows strong binding affinity to soil (Springborn, 2004). Adsorbed BENPAT is expected not to be available anymore for phototransformation.

7.7.1.1.3. Summary and discussion on abiotic degradation

The Substance and its main constituents are stable to hydrolysis. Phototransformation was observed.

The Substance is an antioxidant and consequently, its main constituents are susceptible to oxidation reactions with oxygen, ozone, or reactive radicals. Respective oxidation reactions may occur during tests conducted under aerobic conditions.

7.7.1.2. Biodegradation

7.7.1.2.1. Estimated data

7.7.1.2.1.1. QSAR results

An extensive overview of QSAR is given by a Canadian Report (Environment Canada and Health Canada, 2011). The applied QSAR models include BIOWIN 3-6, TOPKAT and CATABOL.

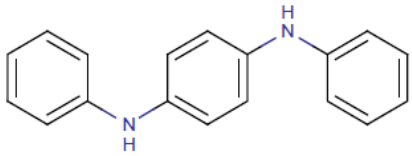
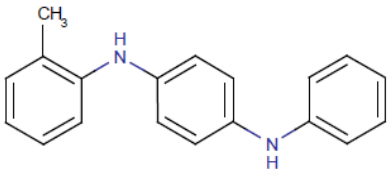
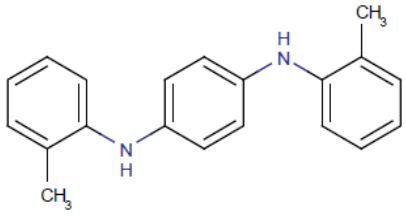
All applied QSAR models predict slow or very slow primary and ultimate biodegradation. However, further documentation would be required to comply with Annex XI of REACH. A possible drawback of biodegradation predictions is that potential abiotic oxidation reactions may not be accounted for.

7.7.1.2.1.2. Discussion of possible biodegradation pathways

The pathways of the main constituents of the Substance (see Table 9) and of the structurally related but less complex diphenylamine were simulated with the University of Minnesota Biocatalysis/Biodegradation Prediction System (UM-PPS). This web application is a rule-based system currently compassing of 250 microbial biotransformation rules based on over 1350 microbial catabolic reactions and about 200 biodegradation pathways. The system compares the organic functional groups of the entered molecules with its set of rules and shows all possible degradation steps. The reaction steps are colour coded according to the likelihood that the respective reaction is catalysed by certain bacteria in water, soil or sediment. An overview of the system can be found in publications by Ellis et al. (Ellis et al., 2008) and Gao et al. (Gao et al., 2010; Gao et al., 2011).

It is not possible to predict rate constants with this system. Furthermore, the system predicts biodegradation reactions exclusively. Thus, the susceptibility of the BENPAT constituents towards inorganic oxidation agents is not accounted for in this prediction.

Table 9

Representative Structures used for prediction of biodegradation pathways		
Short name	**Structure**	**SMILES Code**
N,N'-diphenyl-p-phenylenediamine Diphenyl derivative (R-59)		N(C1=CC=CC=C1)C1=CC=C(NC2=CC=CC=C2)C=C1
N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine Phenyl, tolyl derivative (R-1679)		CC1=C(NC2=CC=C(NC3=CC=CC=C3)C=C2)C=CC=C1
N,N'-bis(2-methyl-phenyl)benzene-1,4-diamine Ditolyl derivative (R-898)		CC1=CC=CC=C1NC1=CC=C(NC2=CC=CC=C2C)C=C1

Microbial degradation of aromatic compounds usually proceeds via channelling pathways leading to the formation of few key central intermediates. These intermediates are then subject to degradation by central pathways (Fuchs et al., 2011).

As the components of BENPAT are complex molecules, their degradation pathway is also quite complex. Nevertheless, a comparison of the results shows similarities and patterns.

For the components of BENPAT regarded in this report, the UM-PPS predicts a cleavage of the bond between the amino nitrogen and one of the aryl moieties as a first step (see Figure 1).

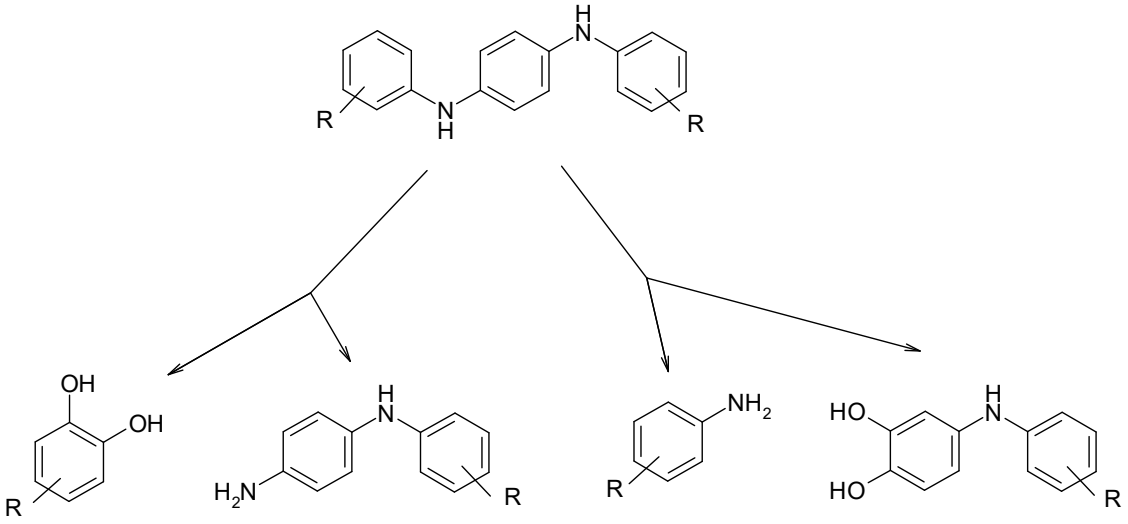


Figure 1: Proposed biodegradation mechanism for the BENPAT constituents: Cleavage of the bond between the amino nitrogen and one of the aryl moieties (R: H, CH_3 or 2 CH_3).

The predicted reaction is supported by information on the structurally similar, but less bulky diphenylamine (EC 204-539-4): The UM-PPS predicts an analogous degradation mechanism based on the same biotransformation rule, which is supported by experimental data (Drzyzga, 2003; Shin and Spain, 2009). The degradation pathways observed for diphenylamine are mainly based on adapted microorganisms. No observed environmental half-life data are available for diphenylamine; however, it is not readily biodegradable and thus, degradation is assumed to be rather slow (European Chemicals Bureau, 2008).

Compared with biodegradation of diphenylamine, biodegradation of the BENPAT components requires another cleavage reaction to yield single aryl units as metabolites. Both diphenylamine and BENPAT are not readily biodegradable, but diphenylamine shows approximately 25 % mineralisation whereas BENPAT yields < 1 % mineralisation, only.

Another predicted pathway is a monooxygenation based on the UM-PPS biotransformation rule 0013. The eMSCA considers that this reaction is predicted erroneously, as the structures do not fulfil the requirements of the biotransformation rule. Rule 0013 refers to certain monosubstituted benzenoids which are monooxygenated by adding a hydroxyl group in para position. However, the amino group is not an accepted substituent for that rule and thus, none of the structures should be regarded. The reaction is predicted for the phenyl, tolyl derivative however, as this structure contains both a monosubstituted benzene ring and another benzene ring substituted with methyl groups. Probably the computer-based structure check generates a mismatch in this situation. Consequently, this reaction path is not discussed further.

7.7.1.2.2. Biodegradation in water and sediments

7.7.1.2.2.1. Screening tests

Several studies are available addressing the ready biodegradability of BENPAT (Ricerca, 1995; Bayer, 1990). In two Manometric Respirometry Tests according to OECD TG 301 F, the test chemical was subjected to aerobic degradation conditions in the presence of activated sludge for 28 days using the oxygen uptake monitoring. No biodegradation of BENPAT was observed (0 and 0.64 %). Another study according to OECD TG 301 C (MITI

(I) Test) on the diphenyl derivative showed 0.2 % degradation and confirms these findings (Environment Canada and Health Canada, 2011). The studies mentioned were submitted in the original registration process. They show that BENPAT is not readily biodegradable. Furthermore a CO_2 Evolution Test according to OECD TG 301 B on the structurally similar substance BENPATAX (EC 273-226-2) showed 1.72 % degradation only (Unpublished report, 1996).

N,N'-phenyl and tolyl-p-phenylene diamine derivatives were tested in a test based on Modified MITI (II) (OECD TG 302C) (Brixham, 2011a). Test conditions deviated from standard test conditions in several aspects. Test duration was 11 days, only. Reference substances were tested but no toxicity control was done. The test substance was applied via silica gel. Bioavailability was enhanced by addition of solubilizer. Inoculum concentration was 3 times higher (300 mg/l) than usual and substance concentration lowered to approx. 2/3 of the usual 30 mg/l. No degradation was observed for the test substance, but the reason remains unclear. Microbial activity may have been too low because degradation of reference substances only reached a low level plateau.

In the second study, a CO_2 Evolution Test (OECD TG 301B) was modified and degradation potential enhanced by several actions (Brixham, 2011b). Test substance was the radiolabelled ditolyl derivative which was applied to silanized test vessels either via silica gel or via solubilizer which was subsequently evaporated. The following changes to standard conditions were done: bioavailability was enhanced by addition of a further solubilizer, test duration prolonged to 56 days and in some cases further test substance was added at the later point in time. Additionally, test substance concentration was lowered. Three treatments were tested (test substance concentration 100, 10 and 1 µg/L) with three replicates each and inoculum concentration was raised tenfold to 300 mg/L. The higher treatment of 100 µg/L was done three times each using a different combination of silica gel and two different solubilizers at test start. Recovery rate was 81-95%. Only one 100 µg/L treatment showed a recovery rate of ≥ 90% in all replicates. Consequently it has to be stated that results for most treatments are doubtful. Test substance was two times added anew in one of the replicates each of the three 100 µg/L treatments. Mineralisation was 9-29 % at day 28 and only slightly raised to 14-33 % at day 56. 41-60 % of applied test substance adsorbed to inoculum and < 5% was extracted from inoculum and vessels.

The radiolabelled ditolyl derivative was again tested in another CO_2 Evolution Test (OECD TG 301B) (Brixham, 2012) under modified and enhanced test conditions. The test substance was applied via silica gel to the test system and bioavailability was enhanced by addition of a solubilizer. Test duration was prolonged to 63 days, temperature increased to 25 °C, test concentration lowered to 10 and 100 µg/l and inoculum concentration raised to 300 mg/l in some replicates. One of the three combinations of test concentration and inoculum concentration showed only a low recovery rate of < 80% which makes this data unreliable. Mineralisation was 12-27% at day 28 and 15-37% at day 63. At test end biomass was extracted following a procedure that is not common in standard testing. The authors state that 15-30% of the applied radioactivity was incorporated by the biomass and another 7-17% was found in the cell walls. However, it remains unclear whether BENPAT is really incorporated in biomass under the abovementioned conditions. Actually, the observed effect might just show the adsorption of the Substance to the biomass. Thus, the informative value of the study is limited both due to the test design as a screening study and due to the various modifications that complicate the comparison with other results.

7.7.1.2.2.2. Simulation tests (water and sediments)

One standard simulation test on biodegradation of BENPAT in surface water (unnamed, 2021) according to OECD TG 309 is available in the registration dossier.

In this test, biodegradation of ^{14}C-R-898, a constituent of BENPAT, in surface water without the addition of suspended sediment was investigated. The test was performed using a flow-

through system with 250 ml surface water with a timeframe of 60 days. The used surface water originates from Lake Biggesee in Olpe, Germany.

The test substance was applied at concentrations of 8 µg/L (low conc.) and 40 µg/L (high conc.) to the surface water and incubated at 12 °C (low conc. for the purpose of kinetic evaluation) and 20 °C (high conc. for the purpose of identification of transformation products). Duplicate samples were incubated for 1, (3), 7, 14, 21, 28, 41, 55, 60 days respectively. As reference substance, ^{14}C-labelled sodium benzoate was used.

Mineralisation of the reference substance sodium benzoate in the surface water used in for the OECD TG 309 tests amounted 51.3-55.9% applied radioactivity (AR) at 12 °C after 63 days. Thus, the study is regarded valid as the surface water contains an active microbial community.

Mean total recoveries in the three individual experiments of the OECD TG 309 study, namely low concentration/12 °C, high concentration/12 °C and high concentration/20°C, were as follows: 91.7% AR, 98.9% AR and 92.2% AR. In the low concentration/12°C experiment, recoveries at day 28, 35, 41, 55 and 59 are below the quality criteria of 90% applied radioactivity. The continuous decrease of radioactivity from day 28 onwards indicates a constant loss of either parent compound or transformation products as mineralisation was low (< 3% AR) and no other volatile residues were formed in this test. Consequently, only data points of day 0 to 21 are available for possible kinetic modelling, which leads to the necessity of extrapolation and thus increase the uncertainty of the obtained half-lives. In the high concentration/12 °C experiment for the purpose of kinetic evaluation, recoveries at date 28 and 35 were below 90%. After exclusion of these both, 9 data points would remain for possible kinetic evaluation based on the high concentration 12 °C experiment allowing the derivation of a kinetic half-life without extrapolation. In the high concentration 20 °C experiment, recoveries at day 28, 42 and 59 are below the quality criteria of 90%. As also described for the low concentration/ 12°C experiment, the radioactivity continuously decreased from day 28 onwards until the end of incubation, indicating a loss from either parent compound or transformation products as volatile resides were low in this test (mineralisation < 3% AR). In summary, results of the low concentration 12 °C and the high concentration 20°C experiment should be treated with caution due to the uncertainties regarding the radioactive losses with time.

Mineralisation was low in the following setups: low concentration/12°C, high concentration/12°C and high concentration/20°C. At the end of the study, $^{14}CO_2$ was < 3% AR. No other volatile residues were detected in the test system.

Extractable residues decrease with time for all three individual experiments. Most of the radioactivity were found to be extractable from the surface water by ethyl acetate in the beginning of the experiment (day 21). From day 28 onwards, surface water was additionally extracted using dichloromethane subsequently after the ethyl acetate extraction. At the end 26.9% (low concentration/12°C), 70.8% (high concentration/ 12°C) and 33.9% AR (high concentration/20°C) were found to be extractable. Radioactivity remaining in the surface water after extraction with dichloromethane and ethyl acetate amounted 45.9% (low concentration/12°C), 18.0% (high concentration 12°C) and 33.8% (high concentration/20°C) at the end of the study according to the ECHA dissemination site.

Non-extractable residues defined by the combustion of the filter material used for the filtration of surface water before the extraction step were 5.4%, 2.4% and 1.7% AR for the low concentration/12°C, high concentration/12°C and high concentration/20°C experiment, respectively.

Extractable residues (sum of ethyl acetate and dichloromethane extracts) provided after the extraction of the surface water were analysed with regard to the parent compound ^{14}C-

R-898 and possibly formed transformation products. However, the remaining ^{14}C in the surface water (up to 45% AR) after extraction was not further analysed.

Amounts of parent compound in the extractable residues quickly decrease after application of ^{14}C-R-898 to the surface water (day 0) by an immediate oxidation of the parent compound to N,N'-bis(1-methylphenyl)-1,4-cyclohex-(2,5)diene-diimine (referred to as 'diimine species' in the following sections) in the presence of oxygen. Formation of such quinonediimine derivatives has been observed for several p-phenylenediamines (see section on abiotic degradation). After the initial formation of the diimine species in the low concentration/12°C, high concentration/12°C and high concentration/20°C experiment, concentrations of this primary degradation product decrease with time. Beside the diimine species, further degradation products were observed in course of the incubation. Some of them represent degradation products formed by subsequent degradation of the diimine species, indicating that the primary oxidation is followed by further degradation steps.

Immediately after application of ^{14}C-R-898 to the surface water, a major percentage of the parent substance is oxidised to the diimine species. This is observed in the samples from day zero. However, it cannot be fully excluded that this oxidation starts partly during sample work-up – as an antioxidant, the Substance is challenging for aerobic studies. Furthermore, the fate of the remaining parent substance is not consistent across the three sub-studies (low concentration/12°C, high concentration/12°C and high concentration/20°C).[11]

The registrant conducted a kinetic assessment based on added concentrations of parent and diimine species. However, for PBT assessment, assessment of half-life data for single species would be relevant. The eMSCA conducted a separate kinetic assessment based on the observed parent concentration. Due to the conflicting sub-study results, the derived parent half-life data were not consistent with each other and do not allow conclusions on persistence of the Substance.

In addition to these conflicting results, uncertainty arises from several factors:

- problems with recovery in two sub-studies
- high levels of unidentified ^{14}C in the surface water after extraction
- background signals
- open questions on the interpretation of the initial fast oxidation.

In summary, the specific properties of the Substance are highly challenging for aerobic tests in water. Although the study was conducted according to guidelines and the state of the art, it is not possible to derive a reliable half-life in water with enough certainty from the results.

7.7.1.2.2.3. Summary and discussion on biodegradation in water and sediment

The Substance is not readily biodegradable in screening tests. The enhanced ready biodegradability tests show low biodegradation of BENPAT. These findings are confirmed by the low mineralisation observed in a simulation study on biodegradation in surface water. However, a fast initial oxidation of a major percentage of the Substance is observed in the same study. This reaction is assumed to be abiotic. The respective sub-studies yield conflicting results on the fate of the remaining parent. Interpretation of these data is further complicated by technical issues and open questions on the role of the initial oxidation.

Although the study was conducted according to guidelines and state of the art, it is not possible to derive a reliable half-life in water with enough certainty from the results. As an antioxidant, the substance is highly challenging for aerobic tests in water.

[11] As the respective data are confidential, no further details are given.

7.7.1.2.3. Biodegradation in soil

There is no information on biodegradation in soil. A field dissipation study according to US-EPA 164-1 (Springborn, 2004) on R-898 (ditolyl derivative) is available but it is not relevant for biodegradation assessment (PBT Working Group of the European Chemicals Bureau, 2005). Instead, it provides evidence of adsorption of BENPAT to soil.

7.7.1.2.4. Summary and discussion on biodegradation

Please see section "Summary and discussion on degradation" below.

7.7.1.3. Summary and discussion on degradation

The Substance and its main constituents are stable to hydrolysis. Phototransformation was observed.

The Substance is an antioxidant and consequently, its main constituents are susceptible to oxidation reactions with oxygen, ozone, or reactive radicals. Respective oxidation reactions may occur during tests conducted under aerobic conditions.

The Substance is not readily biodegradable in screening tests. The enhanced ready biodegradability tests show low biodegradation of BENPAT.

These findings are confirmed by the low mineralisation observed in a simulation study on biodegradation in surface water. However, a fast initial oxidation of a major percentage of the Substance is observed in the same study. This reaction is not predicted as a potential biodegradation pathway, but assumed to be abiotic. The respective sub-studies yield conflicting results on the fate of the remaining parent. Interpretation of these data is further complicated by technical issues and open questions on the role of the initial oxidation.

Although the study was conducted according to guidelines and state of the art, it is not possible to derive a reliable degradation half-life in water with enough certainty from the results. As an antioxidant, the Substance is highly challenging for aerobic tests in water.

Given the uncertainties and the lacking conclusion of the surface water study, an extrapolation to sediment or soil appears to be inadequate.

A field dissipation study shows high adsorption to soil, but does not allow to derive degradation half-life data either.

In summary, a reliable conclusion on environmental degradation cannot be drawn. The Substance properties are highly challenging for degradation testing. Consequently, significant technical problems and / or inconclusive study results are expected for other degradation tests as OECD TG 307 or OECD TG 308 as well. Therefore, no further degradation studies are recommended at this point in time. However, technical and scientific progress may allow for an adequate assessment in the future.

7.7.2. Environmental distribution

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

7.7.3.1.1. Measured data

There is one experimental study available which investigated the bioconcentration potential of BENPAT. Bioaccumulation was studied in carp *Cyprinus carpio*. The test was conducted in accordance with MITI Method for Testing the Degree of Accumulation of Chemical Substances in Fish Bodies (analogous to the OECD TG 305C method).

The two test concentrations in this study (Kurume, 1998) were 0.05 and 0.005 mg/L. The concentrations were based on a preliminary test result for a 48h-hour LC_{50}. Ten fish were

exposed over 8 weeks in a flow-through system. The mean lipid content of these fish was 3.6%. BCF values were lipid normalised to a lipid content of 5%. The concentration of BENPAT in water was measured twice a week throughout the study. Five fish were used in the control group. Two fish were analysed during the weeks 1, 2, 4, 6 and 8 using high-performance liquid chromatography. Control fish were analysed at the initiation and termination of exposure. Additional fish were investigated concerning the depuration after the termination of exposure.

The test concentrations ranged from 60 to 100% of the nominal values. The bioconcentration factors (BCFs) were calculated from individual data for fish at each time point and by using time-weighted averages for water concentrations. Although the study shows some deficiencies the eMSCA nevertheless considers it as useful and suitable for assessment. A re-assessment of this study is available (Unpublished report, 1998) in which this exact report data had been statistically re-evaluated with the authors coming to the conclusion that the data once re-evaluated can be used for assessment. It supports plausibility of the study results (Kurume, 1998)

As BENPAT is a complex reaction product there is a range of different BCF values for different components of the product. From the HPLC analysis five peaks were identified: Peaks 1 and 2 were identified as minor components (1.8% and 2.8 % percentage of content) and Peaks 3 to 5 were identified as the major components (19.2%, 42.5% and 21.1% percentage of content). Furthermore, Peak 3 was identified as N,N-bis-phenyl-1,4-benzenediamine (R-59), Peak 4 was identified as N,N-phenyl-tolyl-1,4-benzenediamine (R-1679), and Peak 5 was identified as N,N-bis-tolyl-1,4-benzenediamine (R-898) of BENPAT. The different BCF for the different components of BENPAT are summarized in Table 10 and Table 11. In accordance with the degree of methylation the BCF increases. There is an upward trend from Peak 1 to Peak 5 as can be expected with an increase in the degree of methylation of the aryl rings.

Table 10

Mean lipid normalised BCFs for low level (0.005 mg/L)					
Peak	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**
1	139	293	192	253	206
2	321	439	504	590	418
3 (R-59)[12]	1283	1972	2785	3194	1483
4 (R-1679)[12]	2657	4597	3924	4146	3514
5 (R-898)[12]	3826	9257	11132	9965	10056
HMW[13]	5979	4458	6778	4611	2625

Table 11

Mean lipid normalised BCFs for high level (0.05 mg/L)					
Peak	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**
1	171	199	360	192	222
2	422	483	781	454	596
3 (R-59)[12]	1590	1814	1535	1485	1783
4 (R-1679)[12]	3681	2389	3611	3056	2757
5 (R-898)[12]	5194	7264	12132	8333	9639
HMW[13]	1603	1625	2153	1611	1868

[12] Peaks 3-5 are the major components of BENPAT.
[13] HMW: High Molecular Weight (residuals remaining in organism at end of depuration phase)

A depuration test was performed on BENPAT to clarify the clearance process of concentrated test substance from the test fish for exposure concentrations of 0.05 and 0.005 mg/L (Kurume, 1998). The depuration half-lives obtained are summarized in Table 12.

Table 12

Half-lives (days) in the depuration phase		
Peak	**Low level (0.005 mg/L)**	**High level (0.05 mg/L)**
1	0.4	1.0
2	2.3	0.7
3 (R-59)[12]	0.7	1.2
4 (R-1679)[12]	3.6	3.0
5 (R-898)[12]	44.4	5.7
HMW[13]	8.8	193.0

Depuration results for components indicated half-lives were below six days for all components with the exception to one estimate of 44-days for Peak 5. This inconsistent value appears to be an outlier since it is much higher than the value of 5.7 days obtained for the other concentration. Also, the value is inconsistent with the trend. Substantial variation occurred at each time point of the bioaccumulation test as a maximum of 2 fish were analysed. In order to calculate BCFs according to OECD concepts, a statistical assessment was conducted (Unpublished report, 1998). The kinetic relationship between bioaccumulation & depuration rates in fish, estimations of BCFs from pharmacokinetic modelling, and a Monte Carlo analysis to estimate most likely values for BCFs and residual half-lives were taken into account.

Table 13

Bioaccumulation data and BCF estimates for test chemical peaks 3-5						
	Steady state levels in fish [mg/kg]		**Average water levels [µg/L]**		**BCF**	
Peak	**Level 1**	**Level 2**	**Level 1**	**Level 2**	**Level 1**	**Level 2**
3 (R-59)	52.9	6.5	38	4.2	1392 (1933 lipid norm.)	1548 (2150 lipid norm.)
4 (R-1679)	85	8.6	37	3.7	2297 (3190 lipid norm.)	2324 (3228 lipid norm.)
5 (R-898)	222.1	21.3	32	3.3	6941 (9640 lipid norm.)	6455 (8965 lipid norm.)
level 1 = media target level 50µg/L, level 2 = media target level 5 µg/L						

Table 14

Monte Carlo estimates for BCF and half-life for peak 5						
	BCF level 1		**BCF level 2**		**Half-life (days)**	
	mean	**min:max**	**mean**	**min:max**	**mean**	**min:max**
Peak 5 (R-898)	6820 (9472 lipid norm.)	5500-8020 (7639-11139 lipid norm.)	6410 (8903 lipid norm.)	5140-8290 (7139-11514 lipid norm.)	4.7	4.0-5.5

Another study on the diphenyl derivative confirms the results mentioned above for Peak 3. BCF were 1420 and 2150 for the two concentrations 0.1 and 0.01 mg/L tested on *Cyprinus carpio* (CHRIPc, 2008).

One further study investigated the biomagnification potential of BENPAT in rainbow trout (*Oncorhynchus mykiss*) (Brixham , 2011c). The fish-feeding study is based on the proposal for updating the guideline 305, as the test was conducted before the adoption of the new revised OECD TG 305. In principle the study was conducted in accordance with the new revised OECD TG 305 guideline (OECD, 2012). The fish were exposed to the Substance via the diet over 14 days in a flow-through system and then were fed uncontaminated food for 14 days during the depuration phase. The fish were fed dosed or clean food at a rate of 2% of wet body weight per day.

The nominal concentration in the dosed food was 1000 µg/g. One of the major constituents with a proportion of approximately 20% and with the highest degree of methylation (two methyl groups; N,N-bis-tolyl-1,4-benzenediamine, R-898, ditolyl derivative) and the highest partitioning coefficient was radiolabelled. The choice of this constituent was justified assuming worst case bioaccumulation. For spiking the fish food radiolabelled ^{14}C-R-898 was diluted with non-radiolabelled BENPAT in a ratio of 1:10. This should give a total nominal concentration of 1000 µg/g. The mean concentrations in the treated food were 991 µg/g and 972 µg/g at the start and end of the exposure phase. In BENPAT the proportion of R-898 is approximately 20%. Due to adding of radiolabelled R-898 to BENPAT, the actual total nominal concentration in the 1000 µg/g dosed food was 280 µg/g (28%).

Five fish were sampled from each of the test vessels on days 7 and 14 of the exposure phase, and days 1, 3, 7, 10 and 14 of the depuration phase. The gut was removed from each fish and analysed separately. Fish carcass or gut samples were combusted and the $^{14}CO_2$ was analysed. The growth corrected kinetic biomagnification factor (BMF) is 0.065 and the lipid normalized and growth corrected kinetic BMF is 0.174. The lipid content changed considerably (>25%) during the test from 7.2-11% w/w. Though this is not a criterion for the validity of the study it does however imply uncertainties. An estimated BCF of 2107 was also determined. The estimation was conducted in accordance with Sijm et al. (1995a, 1995b).

7.7.3.2. Terrestrial bioaccumulation

A substance is considered as potentially bioaccumulating in airbreathing organisms if its log K_{OW} > 2 and its log Koa > 5. For all three constituents, experimental log K_{OW} values are available and > 2. The octanol-air partition coefficient was estimated by QSAR; predicted log K_{OA} values > 5 for all constituents (see table 15). Hence, the screening criterion for terrestrial bioaccumulation is met for all three main constituents.

A further in-depth assessment would be needed to conclude on the potential terrestrial bioaccumulation. The available studies on toxicokinetics in mammals can be used as supporting evidence. However, a further refinement is not considered necessary in the context of this substance evaluation as there is still no guidance for the assessment and a conclusion on the bioaccumulation criterion is considered possible based on the aquatic data.

Table 15

Estimated log K_{OA}		
constituent	**Based on exp log K_{OW}**	**Based on pred log K_{OW}[14]**
R-59	11.38	12.12
R-1679	11.93	12.62
R-898	12.59	13.12

7.7.3.3. Summary and discussion of bioaccumulation

The main constituents of the substance fulfil the screening criteria for terrestrial bioaccumulation. However, a further refinement is not considered necessary in the context of this substance evaluation as there is still no guidance for the assessment and a conclusion on the bioaccumulation criterion is considered possible based on the aquatic data.

The registration dossier contains information on two studies on the bioaccumulation potential of BENPAT in fish. One study on bioconcentration of BENPAT in carp at two different exposure concentrations is available (Kurume, 1998). Based on a statistical assessment of these data, the following lipid normalized BCF values were determined for the high and low test concentration, respectively:

1933 and 2150 for constituent R-59,

3190 and 3228 for constituent R-1679,

9640 and 8965 for constituent R-898.

In summary, BCF values for R-59 are about 2000 with one of two observed BCF values > 2000, both BCF values for R-1679 are > 2000 and both BCF values for R-898 are > 5000.

A biomagnification factor (BMF) of 0.174 was observed in a second study on the biomagnification potential of R-898. At present no clear trigger value exists for BMF to conclude on the basis of a numerical criterion whether a substance is bioaccumulative or not. A BMF larger than 1 indicates biomagnifications from the diet into the fish. However, even a transfer of the substance i.e. a BMF < 1 may be a matter of concern. Experiences with revision or development of test guidelines show that even substances known to be bioaccumulative may show only BMF < 1 in laboratory test systems (Inoue et al., 2012).

BCF values may be estimated using biomagnification data. In this study a BCF was estimated to be 2107 for R-898. This estimated BCF is lower than the measured BCF values. However, this approach is highly uncertain (Crookes and Brooks, 2011). Therefore, these calculations can only be regarded as estimates.

Considering all available information, BCF values > 2000 were observed in fish for all three constituents and therefore, all three constituents are considered to fulfil the B criterion.

The constituent R-898 has a BMF of 0.174 and a BCF in fish of > 5000. Therefore, R-898 is considered to fulfil the vB criterion as well.

[14] Estimated by KOWWIN as implemented in KOAWIN v1.11; U.S. Environmental Protection Agency, 2015. Predicted values are larger than experimental values and therefore also fulfill the log KOW > 2 criterion.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

Short-term toxicity tests were conducted either as range finding tests for longer-toxicity tests to fish (Springborn, 1997; Springborn, 1998a) or as preliminary tests for bioaccumulation (Kurume, 1998). Consequently, concentrations were not analytically confirmed for all tests conducted and therefore the results display a certain level of uncertainty.

The first test was part of a bioaccumulation test (Kurume, 1998). The test was conducted in accordance with the Japanese Industrial Standard. The orange-red killifish (*Oryzias latipes*) was used for this test. Five concentrations (3.75 mg/L to 60 mg/L) were tested in a semi-static system. Ten fish per test vessel were exposed for 48h. The LC_{50} was estimated to be 17.2 mg/L for this acute study according to the Doudoroff method of calculation.

The first range finding test (Springborn, 1997) was conducted with trout (*Oncorhynchus mykiss*). Data were generated to assess acute toxicity over a 7-day period. No mortality was seen at the three lowest nominal levels (0.1, 0.23, 0.51 mg/L) whereas 100% mortality was observed after three day of exposure at the highest concentration of 2.5 mg/L nominal (0.81 mg/L measured). Intermediate nominal levels (not analysed) induced 0 - 80% mortality along with varying degrees of lethargy and skin discoloration.

Both available tests (Springborn, 1998a; Springborn, 1997) were performed according to the OECD standard guidelines (OECD TG 204) under GLP conditions. The lowest toxicity values were detected in rainbow trout, displayed by an EC_{50} of 0.26 mg/L and a NOEC of 0.14 mg/L after 14 days of exposure. According to ECHA Guidance R.7b p. 25 the OECD TG 204 study cannot be regarded as suitable long-term fish test but is in effect a prolonged acute study with fish mortality as the major endpoint examined.

In the first study (Springborn, 1997) rainbow trout (*Oncorhynchus mykiss*) were exposed to five different concentrations 0.094, 0.19, 0.38, 0.75, 1.5 mg/L (nominal) and 0.062, 0.093, 0.14, 0.35, 0.66 mg/L (measured) over fourteen days under flow-through conditions. Young trout exhibited effects in a dose-related manner. Adverse effects were seen only in 2 highest exposure levels out of 5 test concentrations. The non-lethal effects were lethargy, darkened pigmentation of skin, and loss of equilibrium. Both top levels were also lethal with 100% mortality occurring by day 5 in high concentration media whereas 85% mortality occurred in next lower dose (0.35 mg/L) by day 14. The LC_{50} was 0.26 mg/L. The NOEC was determined to be 0.14 mg/L.

The second study (Springborn, 1998a) was conducted with the common carp (*Cyprinus carpio*). Fish were exposed under flow through conditions to five different concentrations of BENPAT. Concentrations were 0.10, 0.23, 0.51, 1.1 and 2.5 mg/L (nominal) and 0.053, 0.12, 0.19, 0.28 and 0.67 mg/L (measured). For fish exposed to the highest concentration (0.67 mg/L), lethargy, loss of equilibrium and darkened pigmentation was observed on day 2. The first instance of mortality was observed on day 3. 50% of fish in this group were dead at day 8. All fish in this group were dead by day 14. There were no adverse findings in lower exposure groups or controls during the 14-day study. The LC_{50} was 0.43 mg/L, the NOEC 0.28 mg/L.

7.8.1.1.2. Long-term toxicity to fish

No data available.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The acute toxicity test on water fleas *Daphnia magna* (Springborn, 1998b) was conducted according to OECD TG 202 and GLP. The concentrations used in this test were 1.3, 2.2, 3.6, 6.0 and 10 mg/L (nominal). Concentrations were analytically checked at 0 and 48 h. The measured concentrations were 0.20, 0.36, 0.68, 1.1, and 1.8 mg/L. Daphnia were exposed for 48h in a flow-through system. Immobilisation of daphnids was recorded. The 48-h EC_{50} for this chemical was < 1.8 mg/l (measured), 10 mg/L (nominal). The NOEC was 0.36 mg/L and 2.2 mg/L respectively.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

There is one test available which evaluates the long-term toxicity effects towards aquatic invertebrates according to OECD TG 211 and GLP (Chemex, 2010a). *Daphnia magna* were exposed to BENPAT for 21 days in order to evaluate the effects of the Substance on the reproductive output of the organisms. Daphnia were exposed to 0.02, 0.04, 0.08, 0.16 and 0.32 mg /L (nominal) in a semi-static test system. Concentrations were determined analytically. The measured concentrations were 0.007, 0.016, 0.036, 0.075, 0.178 mg/L (mean measured values). At the end of the test, the live offspring per parent daphnia (alive) was evaluated. A 21-day EC_{10} based on reproduction of 0.0045 mg/L (estimated by extrapolation) was calculated and the lowest observed effect concentration (LOEC - reproduction) after 21 days was determined at 0.007 mg/L. In the same test the NOEC for parent daphnia survival (21 days) was 0.016 mg/L, the EC_{10} (survival, 21 days) was 0.027 mg/L.

The test was conducted using the whole substance, but the three constituents are structurally similar and differ only by the degree of methylation. It is expected that they share the same narcotic mode of action and that they exert a very similar degree of toxicity that is only influenced by the increase in lipophilicity with increasing methylation. Based on this assumption, the relevant constituent R-898 is expected to show even higher toxicity than the other constituents. ECOSAR[15] results for daphnid toxicity support this assumption (Table 16). The software offers the choice to enter experimental log K_{OW} values manually or to use log K_{OW} values that are automatically predicted by the software. In both cases, predicted toxicity is highest for R-898 as the substances are assigned to the "Neutral Organics" Class and the respective toxicity is directly predicted based on log K_{OW}. [16]

Table 16

Estimated toxicity towards daphnids				
constituent	Daphnid 48-hr [mg/L]		Daphnid ChV [mg/L]	
	Based on exp log K_{OW}	Based on pred log K_{OW}[17]	Based on exp log K_{OW}	Based on pred log K_{OW}[18]
R-59	9.310	2.160	1.268	0.356
R-1679	2.999	0.772	0.477	0.146
R-898	0.791	0.275	0.150	0.060

[15] ECOSAR v1.11; U.S. Environmental Protection Agency, 2012.

[16] ECOSAR v1.11; U.S. Environmental Protection Agency, 2012.

[17] Estimated by KOWWIN as implemented in ECOSAR v1.11; U.S. Environmental Protection Agency, 2012.

[18] Estimated by KOWWIN as implemented in ECOSAR v1.11; U.S. Environmental Protection Agency, 2012.

7.8.1.3. Algae and aquatic plants

There is one test available for algae (Springborn, 1998c). BENPAT was tested on *Selenastrum capricornutum* (new name: *Pseudokirchnerella subcapitata*) according to OECD TG 201 (algae growth inhibition test). Nominal test concentrations were 0.016, 0.031, 0.063, 0.13, 0.25 and 0.50 mg/L. Mean measured concentrations were 0.0075, 0.013, 0.014, 0.028, 0.05 and 0.079 mg/L. Algae were exposed for 72 h and growth inhibition was established either on the basis of biomass or growth rate. The 72-h EC_{50} based on growth rate was estimated to be > 0.079 mg/L, the highest mean concentration. The NOEC was determined to be 0.013 mg/L.

7.8.1.4. Sediment organisms

Toxicity towards sediment organisms was evaluated in a GLP compliant OECD TG 218 (Sediment-Water Chironomid Toxicity used spiked sediment) study (Chemex, 2010b). The organisms (*Chironomus riparius*) were exposed to a control, solvent control, 25, 64, 160, 400 and 1000 mg/kg dry sediment (nominal). The test chemical was added once at the beginning of the study.

These nominal concentrations corresponded to 0, 0, 6.8, 30.2, 84.4, 255.5 and 615.3 mg/kg dry sediment, respectively (days 0 and 28 - average, measured). Recovery of the measured levels of BENPAT in sediment ranged from 27-63% whereas there was no measured levels of the chemical in overlying water at day 28 in any of the test concentrations. The average numbers of emerging midges at the respective concentrations were: 16.2, 14.7, 13.5, 14, 15.7, 14, 14. These results indicated that no effects could be observed in any of the concentrations. In addition, there was no impact of the test substance on developmental rates of the organisms. Therefore the NOEC (28 days) both for emergence and development rate is equal or higher than 1000 mg BENPAT/kg dry sediment (nominal) or 615.25 mg BENPAT/kg dry sediment (measured).

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro-organisms

In a first range finding study, the acute toxicity towards earthworms (*Eisenia fetida*) exposed to chemically-treated artificial soil at levels of 0.1, 1.0, 10, 100 and 1000 mg/kg dry soil for 14 days was evaluated (Mambo-Tox, 2010a). This study was performed according to OECD TG 207, but not according to GLP. Adult worms were monitored for lethality and body weight changes during the exposure period. No effects were observed on either one of these endpoints. The NOEC (14d) was determined at 1000 mg /kg dry soil (nominal values) for both endpoints (growth and mortality).

Subsequent to the acute toxicity study, a 56-day chronic toxicity study according to OECD TG 222 was performed to assess the impact of BENPAT levels on the earthworm Eisenia fetida (Mambo-Tox, 2010b; Mambo-Tox 2010a). This study was compliant to the GLP guideline. Compound levels added to soil were 125, 250, 500, and 1000 mg /kg dry soil (nominal values). No chemical analysis was performed in this study due to the high degree of adsorption of the Substance to soil. Earthworm mortality and body weight changes were monitored after 28-day exposure period. Egg cocoons which were observed at day 28 were returned to the treated soil to allow hatching. Numbers of juveniles appearing by day 56 were recorded as unhatched cocoons. There were no statistically significant changes resulting from BENPAT exposures with regard to worm mortality, body weight changes, appearance of juveniles or unhatched cocoons during the 56-day study. Therefore, the NOEC was determined at equal to or higher than 1000 mg /kg dry soil (endpoints: mortality, growth, reproduction).

7.8.3. Microbiological activity in sewage treatment systems

Not part of the assessment.

7.8.4. PNEC derivation and other hazard conclusions

Not part of the assessment.

7.8.5. Conclusions for classification and labelling

Not part of the assessment.

7.9. Human health hazard assessment

7.9.1. Toxicokinetics

Not part of the assessment.

7.9.2. Acute toxicity and Corrosion/Irritation

Not part of the assessment.

7.9.3. Sensitisation

A GLP-compliant guinea pig maximisation test (GPMT) conducted according to OECD TG 406 shows that BENPAT acts as a skin sensitiser. Upon re-challenge, 55 - 75 % of the animals showed a positive reaction following intradermal induction with a test substance concentration of 5 %. The study does not provide a reliable basis for sub-categorisation, since concentrations ≤ 1 % selected for intradermal induction were not tested.

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

Notably, several studies showed that the BENPAT constituent N,N'-diphenyl-p-phenylenediamine (EC no. 200-806-4, CAS no. 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). This constituent has a harmonised classification and labelling in Annex VI to the CLP Regulation as Skin Sens. 1.

Available information on the skin sensitising potential of BENPAT (and on its constituent N,N'-diphenyl-p-phenylenediamine) warrants classification and labelling of the Substance as Skin Sens. 1, H317. The eMSCA submitted a CLH dossier on this endpoint (BAuA, 2021). RAC concluded that classification and labelling of BENPAT as Skin Sens. 1, H317, without sub-categorisation, is warranted (RAC, 2021).

7.9.4. Repeated dose toxicity

The main effects observed in the oral repeated-dose studies on BENPAT (oral 3 week gavage study (AHF, 1994b), a 4-week dietary study (AHF, 1994a), and a 52-week chronic dietary study (AHF, 1996)) were haematologic findings indicative of a macrocytic anaemia, signs of extramedullary erythropoiesis in the spleen and proliferative activity in the urinary bladder. An dose-dependent and significant increase of the proliferative activity in the urinary bladder was present in all treated dose groups after 38 and 52 weeks of exposure to BENPAT, with the exception of females after 52 weeks (increase not significant), and during the recovery period (64 weeks). However, proliferative activity in the urinary bladder was not accompanied by microscopic findings in this tissue.

The eMSCA does not necessarily consider proliferative activity in the urinary bladder a consequence of haematological changes, since proliferation occurs at lower dose levels compared to doses resulting in haematological changes. In general, neither urinary bladder

proliferation nor a similar phenomenon has been observed or identified as crucial in the pathogenesis of haemolytic anaemia.

Altogether, proliferative activity in the urinary bladder has been consistently observed in different repeated-dose studies performed with BENPAT, however, with an absence of microscopic findings. The relevance of these effects could not finally be evaluated due to a lack of data after longer exposure to BENPAT (longer than 52 weeks) (see also section 7.9.6).

Due to the fact that severe effects in the bladder after exposure to BENPAT for longer than 52 weeks cannot be excluded, the eMSCA considers that the registrants should self-classify BENPAT as STOT RE 1, based on the lowest dose tested (2.65 mg/kg bw/d) resulting in a significantly increased proliferative activity in the urinary bladder of male rats.

7.9.5. Mutagenicity

During the evaluation, the eMSCA identified a concern for gene mutation in somatic cells *in vivo* based on a positive result in the bacterial gene mutation assay. Therefore, based on the ECHA decision dated 1 October 2015 the Registrant(s) were required to carry out a Transgenic Rodent Somatic and Germ Cell Mutation Assay (TGR) or an *in vivo* Mammalian Alkaline Comet Assay. The registrants provided the results to a Comet Assay (OECD TG 489) to the eMSCA in 2018. Induction of genotoxic effects was observed solely in the presence of clear evidence of cytotoxicity (induction of irritation and inflammation), therefore the study was assessed as not positive and the concern for mutagenicity is considered by the eMSCA as clarified.

7.9.6. Carcinogenicity

During the evaluation, the eMSCA identified an additional concern for carcinogenicity based on a 52-week dietary study (proliferating effect of BENPAT on the urinary bladder and the liver (AHF, 1996)). Clarification of the concern of carcinogenicity was made dependent on the outcome of the requested *in vivo* gene mutation study. As this study was assessed as negative (see section 7.9.5), it can be ruled out that the Substance acts as a genotoxic carcinogen. The Substance might act via a non-genotoxic (threshold) mode of action. However, the request for a carcinogenicity study to further clarify the concern might be disproportionate from a regulatory perspective, as a potential classification for carcinogenicity would not add more stringent risk management measures with regard to consumer protection compared to the classification as Repr. 1B for which a CLH dossier has already been submitted by the eMSCA and agreed to by RAC.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

In a two-generation reproduction toxicity study conducted according to OECD TG 416 (RTI, 2001), BENPAT exposure resulted in a dose-dependent increase in gestational length and in dystocia (prolonged parturition or obstructed labour), which in most cases resulted in dead dams and pups. Effects such as prolonged gestation and dystocia were also observed in a mechanistic non-guideline one-generation study (RTI, 2000) performed with one dose of BENPAT.

In the F1 generation of the OECD TG 416 study, an increase in abnormal cycles was noted. Furthermore, a dose-dependent increase of post-implantation loss was observed in BENPAT treated F0 and F1 dams of the two-generation study. Post-implantation loss was also noted in the one-generation mechanistic study after gestational exposure to BENPAT.

Adverse effects on female fertility (abnormal cycles, gestational length, dystocia, and pup mortality) and development (post-implantation loss) are considered not secondary to maternal toxicity. Furthermore, effects are considered relevant to humans.

Available information on the potential of BENPAT for reproductive toxicity warrants classification and labelling of the Substance as Repr. 1B, H360FD. The eMSCA submitted a CLH dossier on this endpoint (BAuA, 2021).

RAC concluded that BENPAT warrants classification as Repro. 1B for fertility and development (H360FD. May damage fertility. May damage the unborn child) (RAC, 2021).

7.9.8. Hazard assessment of physico-chemical properties

Not part of the assessment.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Only consumers were addressed in this assessment.

Table 17

Overview of relevant dose-descriptors			
Endpoint	**Study used**	**Dose-descriptor**	**Remarks**
Repeated dose toxicity: Chronic toxicity	Oral 52-week dietary study	LOAEL: 2.65 mg/kg bw/d	In contrast to the registrants, a LOAEL of 2.65 mg/kg bw/d is derived based on a significant increase in urothelial cell proliferation. This is in line with an assessment performed by Environment Canada (Environment Canada and Health Canada 2011).
Repeated dose toxicity Short-term toxicity	Oral 4-week dietary study	LOEL: 7.5 mg/kg bw/d	In contrast to the registrants, a LOEL of 7.5 mg/kg bw/d is derived based on a significant increase in urothelial cell proliferation. This is in line with an assessment performed by Environment Canada (Environment Canada and Health Canada 2011).
Reproductive toxicity fertility	Oral Two-Generation Reproductive toxicity study in rats	Systemic LOAEL: 7.5 mg /kg bw/d; Female reproductive toxicity: LOAEL: 7.5 mg/kg bw/d Male reproductive toxicity: NOAEL: 100 mg/kg bw/d	Based on polycystic kidneys and prolonged gestation, NOAELs for systemic toxicity and female reproductive toxicity could not be derived, as effects were evident even at the lowest dose tested.
Reproductive toxicity Developmental toxicity	Oral developmental toxicity study in rats	Systemic and developmental toxicity: NOAEL: 70 mg/kg bw/d	Based on this study, a systemic NOAEL of 70 mg/kg bw/d and foetal NOAEL of 200 mg/kg bw/d was established. However, dose-descriptors for developmental toxicity should be considered from the oral two-generation reproductive toxicity study in rats.
Reproductive toxicity Developmental toxicity	Oral 2 Generation Reproductive toxicity study in rats	Systemic LOAEL: 7.5 mg/kg bw; Developmental toxicity: NOAEL: 7.5 mg/kg bw/d	Based on polycystic kidneys, a NOAEL for systemic toxicity could not be derived, as effects were evident even at the lowest dose tested. A NOAEL of 7.5 mg/kg bw/d for post-implantation loss is considered as developmental NOAEL (RAC 2021).

The LOAEL of 2.65 mg/kg bw/d derived from a 52-week chronic feeding study in rats is taken as a starting point for DNEL derivation for long-term systemic effects after oral, dermal or inhalation exposure to BENPAT. The leading health effects are urothelial cell proliferation and hyperplasia.

Conversion of LOAEL into NOAEL: division by a factor of 3.

This results in a NOAEL of 0.9 mg/kg/d as starting point for DNEL derivation.

7.9.9.1. Calculation of oral DNEL (long-term, systemic)

As oral absorption by rat and human are assumed to be equal, no further correction of the NOAEL value is required.

Assessment Factors applied:

- interspecies differences: 10 (4 (for rat vs. human) and 2.5 (for differences not related to calorimetric differences))
- intraspecies differences for consumers/man exposed via environment (MvE): 10
- differences in duration of exposure: 1
- dose-response and endpoint specific/severity issues: 1
- quality of the database: 1

Overall Assessment Factor: 100

Endpoint-specific DNEL (general population, long-term, systemic, oral route):

0.9 / 100 = 0.009 mg/kg bw/d

7.9.9.2. Calculation of dermal DNEL (long-term, systemic)

The eMSCA derives an oral absorption percentage of 61 % and a dermal absorption percentage of 69 %.

The NOAEL is corrected for difference in absorption between the oral and the dermal route in rats. As no data are available concerning probable differences in dermal absorption between humans and rats, 69 % dermal absorption in humans is taken as the worst case.

Corrected dermal human NOAEL = oral rat NOAEL x (absorption oral rat/absorption dermal rat) = 0.9 mg/kg bw/d x (61/69) = 0.8 mg/kg bw/d

Assessment Factors applied:

- interspecies differences: 10 (4 (for rat vs. human) and 2.5 (for differences not related to calorimetric differences))
- intraspecies differences for Consumers/man exposed via environment (MvE): 10
- differences in duration of exposure: 1
- dose-response and endpoint specific/severity issues: 1
- quality of the database: 1

Overall Assessment Factor: 100

Endpoint-specific DNEL (general population, long-term, systemic, dermal route):

0.84/100 = 0.008 mg/kg bw/d

7.9.9.3. Calculation of inhalation DNEL (long-term, systemic)

Starting point: NOAEL = 0.9 mg/kg/d

The NOAEL is corrected according to the default physiological parameters under the allometric scaling principle (table R8.2 and section R.8.4.2 of the respective ECHA guidance document (ECHA, 2010)).

Corrected NOAEL for the general population via inhalation = (NOAEL oral rat/1.15 m^3/kg bw) = 0.9/1.15 mg/m^3 = 0.78 mg/m^3

Assessment Factors applied:

- allometric scaling factor: 2.5 (for differences not related to caloric differences)
- assessment factor for oral to inhalation extrapolation: 1.64 (taking into consideration value from the oral absorption study: AF= 100 / 61)
- intraspecies differences for Consumers/man exposed via environment (MvE): 10
- differences in duration of exposure: 1
- dose-response and endpoint specific/severity issues: 1
- quality of the database: 1

Overall Assessment Factor: 2.5 x 1.64 x 10 = 41

Endpoint specific DNEL (general population, long-term, systemic, dermal route): 0.02 mg/m^3

DNEL derivation for local effects after long-term exposure:

The eMSCA concurs with the calculation performed by the registrants.

The general population long-term DNEL for local effects by the dermal route is 0.014 mg/cm^2, corresponding to 0.023 mg/kg bw.

Table 18

CRITICAL DNELS/DMELS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL/ DMEL**	**Justification/ Remarks**
Repeated dose toxicity	urothelial cell proliferation and hyperplasia	52 week oral study	0.9 mg/kg/d	0.009 mg/kg bw/d	Oral, systemic
Repeated dose toxicity	urothelial cell proliferation and hyperplasia	52 week oral study	0.8 mg/kg bw/d	0.008 mg/kg bw/d	Dermal, systemic
Repeated dose toxicity	urothelial cell proliferation and hyperplasia	52 week oral study	0.78 mg/m^3	0.02 mg/m^3	Inhalation, systemic
Repeated dose toxicity	urothelial cell proliferation and hyperplasia	52 week oral study		0.023 mg/kg bw	Long-term, dermal, local

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The eMSCA submitted a CLH dossier pertaining to the conclusions on classification and labelling for human health (BAuA, 2021). RAC concluded that BENPAT warrants classification as Repro. 1B for fertility and development (H360FD. May damage fertility.

May damage the unborn child) and Skin Sens. 1 (H317 – May cause an allergic skin reaction) (RAC, 2021).

7.10. Assessment of endocrine disrupting (ED) properties

Not part of the Substance Evaluation.

7.11. PBT and VPVB assessment

7.11.1. Persistence assessment

The Substance is an antioxidant and consequently, its main constituents are susceptible to oxidation reactions with oxygen, ozone, or reactive radicals. Respective oxidation reactions may occur during tests conducted under aerobic conditions. BENPAT is stable to hydrolysis. The available studies show no biodegradation of BENPAT in standard test systems for ready biodegradability. Furthermore, the analysis of possible biodegradation pathways supports that BENPAT biodegrades slowly. The available tests on enhanced ready biodegradability show a low biodegradation.

These findings are confirmed by the low mineralisation observed in a simulation study on biodegradation in surface water. However, a fast initial oxidation of a major percentage of the Substance is observed in the same study. This reaction is assumed to be abiotic. Although the study was conducted according to guidelines and state of the art, it is not possible to derive a reliable degradation half-life in water with enough certainty from the results. Due to its properties as an antioxidant, investigating the Substance in aerobic tests in water is highly challenging.

Given the uncertainties and the lacking conclusion of the surface water study, an extrapolation to sediment or soil appears to be inadequate. A field dissipation study shows high adsorption to soil, but does not allow to derive degradation half-life data either.

In summary, a reliable conclusion on persistence cannot be drawn.

7.11.2. Bioaccumulation assessment

The screening criterion for terrestrial bioaccumulation is met for all three main constituents of the Substance. A further in-depth assessment would be needed to conclude on the potential terrestrial bioaccumulation. In this context, the available studies on toxicokinetics in mammals can be used as supporting evidence. However, a further refinement is not considered necessary in the context of this substance evaluation as there is still no guidance for the assessment and a conclusion on the bioaccumulation criterion is considered possible based on the aquatic data.

Data on aqueous bioaccumulation are available. One study investigated the bioconcentration potential of BENPAT in fish. Both test concentrations exceeded the water solubility of the substance which impairs its informative value. However, another study enhanced the informative value by statistical re-evaluation of the data. This, accompanied by the fact that data shows a clear trend of rising bioconcentration with higher level of methylation of each respective constituent, results in a plausible and reliable picture. As the Substance is a complex reaction product, there is a range of BCF values for its different constituents. The lowest BCF among the main constituents is close to a BCF of 2000. However, the major component shows BCF values well above 2000. For the third major constituent the BCF values are even above 5000.

A second study investigated the biomagnification potential of BENPAT via a fish feeding study. This study showed that the BMF does not exceed 1. However, at present there is no numerical criterion to decide on bioaccumulative behavior according to REACH Annex XIII based on a BMF. Whether a BMF above one or even below one is of concern is still under discussion and substances known to be bioaccumulative may show only BMF < 1 in laboratory test systems. The estimated BCF using the biomagnification data can only be

regarded as an estimation. Therefore, the results of the second study do not contradict or exonerate the findings from the first study. According to the REACH legislation (Annex XIII), a substance is considered to fulfill the "bioaccumulative" criterion (B) when the bioconcentration factor in aquatic species is higher than 2000 and to fulfil the "very bioaccumulative" criterion vB if the bioconcentration factor is higher than 5000. It can be concluded that the three main constituents of BENPAT are bioaccumulative according to Annex XIII (B) and that the constituent R-898 is very bioaccumulative (vB).

7.11.3. Toxicity assessment

The toxicity of BENPAT was characterised in short term and long term toxicity tests on algae, daphnia and fish. Additionally toxicity was tested with benthic as well as terrestrial organisms. According to the REACH legislation (Annex XIII), a substance is considered to fulfil the toxicity criterion (T) when: the long-term no-observed effect concentration (NOEC) for marine or freshwater organisms is less than 0.01 mg/L, therefore only the aquatic toxicity data are considered.

The chronic test on daphnia reproduction shows that BENPAT is highly toxic. The value is below the relevant trigger value NOEC < 0.01 mg/L. The test was conducted on the whole substance, but the three constituents are structurally similar and differ only by the degree of methylation. It is expected that they share the same narcotic mode of action and that they exert a very similar degree of toxicity that is only influenced by the increase in lipophilicity with increasing methylation. The narcotic mode of action leads to increasing toxicity with increasing lipophilicity. Based on the expected narcotic mode of action, the constituent R-898 is expected to show even slightly higher toxicity than the other constituents. QSAR results for daphnid toxicity support this assumption.

Therefore, based on a test on the whole substance, the high structural similarity between the constituents and their predicted common mode of action, both BENPAT as a whole substance and its single constituents (including R-898) are considered to fulfil the T criterion with regard to aquatic toxicity.

Additionally, BENPAT fulfils the criteria for classification as Repr. 1B (cf. Section 7.9.7) which would also fulfil the T criterion according to Annex XIII with regard to toxicity for human health.

7.11.4. Summary and overall conclusions on PBT and vPvB Properties

BENPAT is a multi-constituent substance. All three constituents are considered to fulfil the P/vP screening criterion based on ready biodegradability tests. However, the constituents are susceptible to oxidation and results from a simulation study do not yield unambiguous results on degradation. In summary, a definitive conclusion on persistence is not possible.

All three constituents show BCF values > 2000 in fish and are therefore considered to fulfil the B criterion. Its constituent R-898 has a BMF of 0.174 and a fish BCF of > 5000. The constituent R-898 is therefore considered to fulfil the vB criterion as well.

A NOEC < 0.01 mg/L was observed in a study on long-term toxicity of BENPAT to daphnids. Based on the high structural similarity between the constituents and their predicted common mode of action, both BENPAT as a whole substance and its single constituents (including R-898) are considered to fulfil the T criterion.

7.12. Exposure assessment

Not part of the evaluation.

7.13. Risk characterisation

Not part of the evaluation.

7.14. References

AHF (1994a): Four week dietary study of Wingstay 100 in Fischer 344 rats. American Health Foundation. Unpublished study report.

AHF(1994b): Preliminary oral 3-week range finding study for administration of Wingstay 100 and R-59 in male & female Fischer 344 rats.American Health Foundation. Unpublished study report.

AHF (1996): 52-Week chronic feeding toxicity study of Wingstay 100 in rats with 12-week recovery. American Health Foundation. Unpublished study report.

BAuA (2021): 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, date: February 2021. Federal Institute for Occupational Safety and Health.

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7.15. Abbreviations

6PPD	N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine
AR	applied radioactivity
B	bioaccumulative (pertaining to Annex XIII REACH)
BCF	bioconcentration factor
BENPAT	1,4-Benzenediamine, N,N'-mixed phenyl and tolyl derivs.
BENPATAX	1,4-Benzenediamine, N,N'-mixed Ph and tolyl and xylyl derivs.
BMF	biomagnification factor
BMF_K	growth corrected kinetic biomagnification factor
bw	body weight
CAS RN	CAS registry number
CLH	Harmonised classification and labelling
CLP	Classification, labelling, and packaging of substances
DMEL	Derived minimum effect level
DNEL	Derived no-effect level
DT_{50}	degradation half-life
EC	effect concentration
eMSCA	evaluating Member State competent authority
GLP	Good Laboratory Practice
HMW	High Molecular Weight (residuals remaining in organism at end of depuration phase)
HPLC	high performance liquid chromatography
K_{OA}	octanol-air partition coefficient
K_{OW}	octanol-water partition coefficient
LC_{50}	Lethal concentration to 50% of test animals
LOEC	lowest observed effect concentration
MSC	Member State Committee
NOAEC	No observed adverse effect concentration
NOEC	no observed effect concentration
P	Persistent (pertaining to Annex XIII REACH)
PBT	persistent, bioaccumulative and toxic
QSAR	quantitative structure-activity relationship
RAC	Committee for Risk Assessment
R-59	N,N'-diphenylbenzene-1,4-diamine
R-1679	N-(2-methylphenyl)-N'-phenylbenzene-1,4-diamine
R-898	N,N'-bis(2-methylphenyl)benzene-1,4-diamine
SEV	Substance Evaluation
SVHC	Substance of very high concern
STOT RE	Specific Target Organ Toxicity – Repeated exposure
T	Toxic (pertaining to Annex XIII REACH)
TG	Testing guideline
TGR	Transgenic rodent
UM-PPS	University of Minnesota Biocatalysis/Biodegradation Prediction System
vB	very bioaccumulative (pertaining to Annex XIII REACH)
vP	very persistent (pertaining to Annex XIII REACH)
vPvB	very persistent and very bioaccumulative (pertaining to Annex XIII REACH)