Ministry of Environ
of Denmark
Environmental
Protection Agency

SUBSTANCE EVALUATION CONCLUSION
as required by REACH Article 48
and
EVALUATION REPORT

for

1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters

EC No 701-339-3 (previously: EC No 271-082-5)

Evaluating Member State(s): Denmark

Dated: 01 January 2022

Evaluating Member State Competent Authority

Danish Environmental Protection Agency (Danish EPA)
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Year of evaluation in CoRAP: 2014

Before concluding the substance evaluation, a Decision to request further information was issued on 19 May 2017. Following the outcome of the requested information, the evaluating Member State concluded the evaluation without any further need to ask for more information from the registrants under Article 46(1) decision.

Further information on the Substance here:

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

DISCLAIMER

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

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, 1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters (List number 701-339-3) (previously 1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters, EC number 271-082-5) was originally selected for substance evaluation in order to clarify concerns related to human health:

- Suspected toxicity for reproduction
- High (aggregated) tonnage
- Exposure/Lack of exposure assessment
- Lack of RCR (risk characterisation ratio)

According to the registration dossier, the Substance has not been classified for reproductive toxicity due to "lack of data" although structurally related substances have a harmonised classification as reproductive toxicant - Repro 1B

During the evaluation, an additional concern was identified:

- Suspected endocrine disrupting properties

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

As of June 2021, no EU legislation applies specifically to the Substance.

In parallel to this substance evaluation, the registrant of the Substance submitted a testing proposal to ECHA: experimental study in rabbits (2nd species) according to OECD TG 414 (Prenatal Developmental Toxicity Study). ECHA sent its decision to the registrants on 13 April 2022 [2].

In addition, ECHA undertook a comprehensive compliance check including of number of endpoints. ECHA sent its decision to the registrants on 13 April 2022 [3].

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State (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	
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level (CCH and TPE are ongoing).	X

[2] Testing proposal: https://echa.europa.eu/da/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e18486039c (latest update 24/04/2020)

[3] Compliance check: https://echa.europa.eu/da/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e1851fbc2b (latest update 16/07/2020)

Based on the available data, the eMSCA has concluded that no further information should be requested. A residual concern for endocrine disruption subsists. However no further request or regulatory action proposed at present.

4. FOLLOW-UP AT EU LEVEL

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

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

The evaluation of the available information on the substance has led the eMSCA to conclude that there is no apparent need for follow-up action at EU level at this point in time (see table below). The reasons are briefly explained for each identified concern below Table 2. For further details and discussions, please see Part B of this Conclusion document.

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/ exposure	x
Actions by the registrants to ensure safety, as reflected in the registration dossiers (e.g. change in supported uses, applied risk management measures, etc.)	

Following the evaluation of the originally available information in the dossier of the Substance, the eMSCA concluded that further information was necessary to clarify the identified concerns of reproductive toxicity and endocrine disruption properties. Thus a Decision to request further information was issued on 19 May 2017.

In the decision issued by ECHA, a modified combined repeated dose toxicity study with reproduction/developmental toxicity screening test (modified OECD TG 422) was requested[4]. The requested study aimed to clarify the concern for fertility, developmental toxicity to male reproductive development and endocrine disrupting mode of action of the Substance.

Based on the data available, including the modified OECD TG 422 study performed in response to the request in the substance evaluation decision of 2017, the eMSCA concludes that there is currently no need for further follow-up action.

However, while the recent modified OECD TG 422 study is accepted and considered sufficient to fulfil the request of the adopted substance evaluation decision, the eMSCA identified several deficiencies related to the study design, examinations and performance. Further, the doses selected did not induce general toxicity in the highest dose group, leading to uncertainty to the evaluation (unpublished study report, 2019).

Below, the reasons for not initiating further follow-up actions are briefly presented for each concern and further discussed in Part B.

[4] ECHA substance evaluation adopted decision, dated 19 May 2017, and available at: https://echa.europa.eu/documents/10162/405c123c-10f6-6570-4b78-e7a6bf720357

The concern for toxicity to reproduction

Adverse effects on fertility were found in a subacute repeated dose toxicity study and a two-generation toxicity study conducted with the Substance. However, this concern was not substantiated in the requested modified OECD TG 422 (unpublished study report, 2019) as the reproductive performance and reproductive organ weights were unaffected in all dose groups (375, 1500 and 6000 ppm).

Adverse effects on male reproductive development were found in a one- and a two-generation study. This concern was not substantiated based on results from the requested modified OECD TG 422 study (unpublished study report, 2019) as male sexual development were unaffected at doses up to 6000 ppm.

Based on the presented results in Part B (section 7.9.7.) and on a weight of evidence evaluation, it is concluded that no further information should be requested as the eMSCA considers that the outcome of the requested study did not substantiate the concern for reproductive toxicity (unpublished study report, 2019). The eMSCA also does not consider the adverse effects observed in the previous studies sufficiently severe to trigger further regulatory action.

ECHA has informed the eMSCA that, a testing proposal to conduct a prenatal developmental toxicity study (OECD TG 414) in a second animal species is currently being assessed by ECHA and expected to be included imminently in a draft decision. Also, a compliance check is ongoing (see section 2).

The concern for endocrine disruption

Anti-androgen mode of action: Due to findings of decreased anogenital distance (AGD), increased nipple retention, delayed sexual maturation and decreases of the weights of several reproductive organs in male offspring exposed to the Substance during development (unpublished study report, 2005), an additional concern regarding endocrine disrupting mode of action was identified. This concern was not substantiated based on results from the requested Modified OECD TG 422 study as the Substance did not seem to have endocrine disrupting properties in relation to adverse effects on male sexual development at doses up to 6000 ppm (367-411 mg/kg/day) (unpublished study report, 2019).

However, it is difficult for the eMSCA to conclusively determine the endocrine disrupting potential related to anti-androgenic activity of the Substance due to deficiencies specifically related to the examination of AGD and nipple retention in this study. Although this concern is not fully clarified, the eMSCA has concluded not to require further information as the requested study was modified to include several sensitive endpoints according to the substance evaluation adopted decision issued by ECHA[4].

Thyroid hormone disrupting mode of action: The Substance seems to have some endocrine disrupting abilities related to thyroid disruption as thyroid stimulating hormone (TSH) levels were elevated in parental animals and thyroxine (T4) levels were lowered (unpublished study report, 2019). The concern for thyroid disruption is currently not followed up since the available test designs only cover thyroid endpoints with limited sensitivity. Therefore, the eMSCA does not consider it reasonable to require further testing of the potential thyroid disrupting properties.

In conclusion, it is therefore currently not possible to fully exclude the additional concern that the Substance is an endocrine disruptor due to some indications of disruption of the HPT axis reported in the requested study and due to deficiencies related to the study design, examinations, and performance.

5.2. Other actions

Not applicable.

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

Not applicable.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, 1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters (List number 701-339-3) (previously 1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters, EC number 271-082-5) was originally selected for substance evaluation in order to clarify concerns related to human health:

- Suspected toxicity for reproduction
- High (aggregated) tonnage
- Exposure/Lack of exposure assessment
- Lack of RCR (risk characterisation ratio)

The criterion for selecting the Substance for substance evaluation was a concern for reproductive toxicity. According to the registration dossier, the Substance has not been classified for reproductive toxicity due to "lack of data" although structurally related substances have a harmonised classification as reproductive toxicant - Repro 1B

During the evaluation, an additional concern was identified:

- Suspected endocrine disrupting properties

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Toxicity for reproduction	
Fertility	Concern refuted. Concerns not substantiated in the requested study. No further action.
Developmental toxicity (male)	See section 7.9.7
Endocrine disrupting properties	
Anti-androgenic mode of action	Concern unresolved. No further action. No conclusion can be reached due to uncertainties in the requested study. See section 7.10
Thyroid disrupting mode of action	Concern unresolved. No further action at present as no available guideline studies are considered appropriate by the eMSCA. See section 7.10 Residual concern for endocrine disrupting effects, however no further request or regulatory action proposed at present.

A summary of the evaluation is given above in section 5.1

The Initial concern for toxicity to reproduction

The initial concern triggering the substance evaluation of the Substance was based on the harmonised classification as Repr. 1B for developmental effects and Repr. 2 for effects on fertility of the substance 1,2-Benzenedicarboxylic acid, di-C7-11 branched and linear alkyl esters (C7-11P or DHNUP) (EC no 271-084-6, CAS RN 68515-42-4). The classification was agreed by the technical committee on classification and labelling (TC CL) under the dangerous substance directive (67/548/EC) in 2002. The TC CL concluded that C7-11P induced selective foetal effects (post-implantation loss and high malformation rate and variation rate), depending on the type and extent of branching of the substance, warranting a classification corresponding to Repr. cat 1B for developmental toxicity. The TC CL further concluded that branched C7-11P may cause testicular damage and thus may impair fertility and attributed a classification corresponding to Repr. cat 2 for toxicity to fertility.

The Substance under evaluation contains constituents with the same phthalate chain lengths (i.e. C7-9) as the classified substance C7-11P, and there was a concern that these constituents may cause the adverse effects on reproduction (development and fertility) seen with C7-11P. Thus, this raised a concern that the Substance may have comparable effects.

The concern for adverse effects of the Substance on the developing reproductive system was further strengthened by the fact that the Substance, based on its structure, is expected to be metabolized in the gastrointestinal tract to monoisononyl phthalate and monobenzyl phthalate, both of which are known to be reproductive toxicants.

Monoisononyl phthalate is known as a primary metabolite of diisononyl phthalate (DINP) (CAS RN 68515-48-0 and 28553-12-0) (Clewell *et al*., 2013a). DINP is known to cause adverse effects in the male reproductive system (i.e. reduced sperm count, reduced AGD, and permanent changes in reproductive organs) via interference with foetal testosterone production, although at higher doses (Clewell *et al*., 2013b, Boberg et al., 2011, Gray et al., 2000, ECHA 2013).

The metabolite, monobenzyl phthalate (CAS RN 2528-16-7) itself has been shown to affect male reproductive development when administered to pregnant Wistar rats on gestation day (GD) 15-17, as male foetuses exposed from 250 mg/kg bw/day showed an increase in incidence of undescended testes, reduced AGD and AGD/cube root of bodyweight on GD 21 (Ema *et al*., 2003). Additionally, Butyl benzyl phthalate (BBP), which also shares the monoester metabolite monobenzyl phthalate with the Substance), has been shown to adversely affect male reproductive tract development in numerous studies (Ema and Miyawaki, 2002, Nagao *et al*., 2000, Tyl *et al*., 2004).

Additional concern for Endocrine disruption

Due to findings of decreased AGD, increased nipple retention, delayed sexual maturation and decreases in the weights of several reproductive organs in male offspring exposed to 1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters during development in a non-guideline one-generation study, (unpublished study report, 2005), additional concern regarding endocrine disrupting mode of action was identified.

Thyroid toxicity, e.g. thyroid follicular hyperplasia, has been found for phthalates with backbone lengths of C6-C8 (Bhat *et al.*, 2014, Howarth *et al.,* 2001, Poon *et al.,* 2007, Hinton *et al.,* 1986). Also for di-isodecyl phthalate (DIDP CAS RN 68515-49-1, primarily C10) possible effects on thyroid glands have been described (ECHA, 2013). However, it is unclear whether thyroid toxicity is related to phthalates with specific backbone lengths only.

Thyroid glands were weighed in adult F1 offspring in the two-generation study in accordance with OECD TG 416, (unpublished study report, 2013a), and here a non-significant 10% increase in thyroid weight was seen. Thyroid glands were not evaluated in the non-guideline sub-acute 28-day repeated dose toxicity study (unpublished study

report, 1999), nor in the non-guideline one-generation study (unpublished study report, 2005) on the Substance.

Thus, further information on the Substance was requested, to elucidate whether this substance has reproductive or endocrine disrupting properties.

7.2. Procedure

Based on an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to suspected CMR (reproductive toxicity); Exposure/Lack of exposure assessment, Lack of risk characterisation ratio, High (aggregated) tonnage, the Substance was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The Competent Authority of Denmark was appointed to carry out the evaluation.

In the course of the evaluation, the eMSCA identified an additional concern regarding endocrine disruption, i.e., disruption of sex- and thyroid hormones.

The evaluation of data under the prioritized endpoints was performed based on the information present on ECHA's dissemination site.

For the three studies originally available in the registration specifically investigating reproductive toxicity, the full confidential study reports were provided for a one-generation study (unpublished study report, 2005), a two-generation study (unpublished study report, 2013a) and a prenatal developmental toxicity study, in a first species (unpublished study report, 2013b).

The open literature was searched for studies on the Substance targeting relevant endpoints, but none were found.

Following the evaluation of the above-mentioned data, the eMSCA concluded that further information was necessary to clarify the concerns. In addition, the eMSCA identified a data gap for a 90-day sub-chronic toxicity study according to the standard information requirements in REACH, Annex IX, 8.6.2. In a decision, issued by ECHA[4], a modified combined repeated dose toxicity study with reproduction/developmental toxicity screening test based on the concerns of reproductive toxicity and endocrine disruption properties was requested. The required 90-day sub-chronic toxicity study was considered fulfilled with the modified OECD TG 422.

The experimental study report of the requested study was submitted by the registrant on 8 January 2020.

Based on the data available including the performed study, the eMSCA concluded that there is currently no need for further follow-up action.

The registrant has submitted a testing proposal for a planned experimental study in rabbits (2nd species) according to OECD TG 414 (Prenatal Developmental Toxicity Study). Furthermore, a compliance check (CCH) of the registration dossier is ongoing See section 2.

7.3. Identity of the substance

The Substance 1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters is a mono constituent substance having the following identity and structural formula.

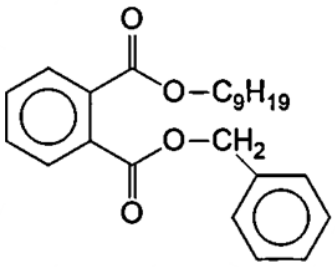
The registrant requested ECHA to change the EC number of the registration. This was accepted by ECHA and the EC number was changed from 271-082-5 to the List number 701-339-3. Additionally, the substance name was changed from "1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters" to "1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters".

Table 4

SUBSTANCE IDENTITY	
Public name:	1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters
EC number:	701-339-3 (previously: 271-082-5)
CAS number:	None (previously 68515-40-2)
Molecular formula:	C24 H30 O4
Molecular weight range:	>= 368.466
Synonyms:	Santicizer 261A

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

Structural formula:



7.4. Physico-chemical properties

The Substance 1,2-Benzenedicarboxylic acid, benzyl isononyl alkyl esters has the following characteristics and physical–chemical properties as given on ECHA's dissemination site[5].

Most of the values listed in Table 5 are based on read across from DEHP, DINP and DIDP proposed by the registrant(s) since the structures and molecular weights of these substances are similar to those for the Substance, such that their properties are expected to be similar. The eMSCA has not verified the read-across proposed by the Registrant.

[5] https://echa.europa.eu/da/substance-information/-/substanceinfo/100.301.215 and https://echa.europa.eu/da/registration-dossier/-/registered-dossier/30693, as of June 2021

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES (ECHA dissemination site)	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid Form: oily Colour: Clear. Pale colour. Odour: Characteristic of aromatic compounds
Freezing point	Based on read across, the freezing point of the Substance is -50°C at 1013hPa. Experimental melting point data is not available for the Substance. A value of -50°C is the average melting point of range of -55°C to -45°C from DEHP, DINP and DIDP
Boiling point	The calculated boiling point of the Substance is 420°C/693K at 1013hPa. Reliable experimental boiling point data is not available for the Substance. A value of 420°C has been calculated using the QSAR MPBPVPWIN The Substance is in the domain of this QSAR. This value is supported by read across from DEHP, DINP and DIDP, which have a boiling point range of 384°C to >400°C. This is supported by company data that reports boiling points of 252 and 390°C at 1.33
Vapour pressure	The calculated vapour pressure of the Substance is 0.000117 Pa at 20°C. Reliable experimental vapour pressure data is not available for the Substance. An indicative value of 0.000117 Pa at 20°C has been calculated using the QSAR MPBPVPWIN. The Substance is in the domain of this QSAR. This value is supported by read across from DEHP, DINP and DIDP, which have a vapour pressure range of 0.000028 to 0.000034 Pa at 20°C. This is supported by company data that reports the vapour pressure to be 0.7 and 13 hPa at 200 and 250°C respectively
Water solubility	The calculated water solubility of the Substance is 0.0098 mg/L at 25°C. Experimental water solubility data is not available for the Substance. A water solubility has been calculated using a QSAR. This value is supported by read across from DEHP, DINP and DIDP, which have a water solubility range of 0.2-1.9 µg/l
Partition coefficient n-octanol/water (Log Kow)	The calculated log n-octanol water partition coefficient for the Substance is 5.75 at 20°C. Reliable experimental partition coefficient data is not available for the Substance. A log Kow of 5.75 has been calculated using a QSAR KOWWIN. The Substance is in the domain of this QSAR. This value is supported by read across from DEHP, DINP and DIDP, which have a log Kow range of 7.5-8.8. This is supported by a Kow value of 1170 from company data
Flash point	Based on read across, the flash point of the Substance is >200°C. Reliable experimental flash point data is not available for the Substance. A value of >200°C has been read across from

	DEHP, DINP and DIDP, which all have flash point >200°C. This is supported by company data that reports a flash point of 224°C
Viscosity	Based on read across, the static viscosity of the Substance is 22 mm^2*s^{-1} at 20°C There is no reliable viscosity data for the Substance. The value of 22 mm^2*s^{-1} will be read-across from DEHP. The Merck Index gives a value of 22 mm^2*s^{-1} for DEHP's static viscosity at 20°C. The Merck Index is a peer reviewed handbook and so can be considered reliable and suitable for use as the key study for this endpoint. This is supported by company data which reports a viscosity of the Substance of 67 mm^2*s^{-1} at 25°C

7.5. Manufacture and uses

7.5.1. Quantities

The aggregated tonnage information as given on the ECHA dissemination site[5] is highlighted in Table 6.

Table 6

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

7.5.2. Overview of uses

The Substance is used by consumers (adhesives and sealants and coating products), in articles, by professional workers (widespread uses), in formulation or re-packing and at industrial sites.

An overview is summarised in Table 7 as given on ECHA's dissemination site[5].

Table 7

USES		
	Use(s)	
Formulation	Distribution	**Process category** PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities

		PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) **Environmental release category** ERC 1: Manufacture of the substance ERC 2: Formulation into mixture **Process category** PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) **Environmental release category** ERC 2: Formulation into mixture ERC 3: Formulation into solid matrix
	Formulation and (re)packing of substances and mixtures	**Process category** PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) **Environmental release category** ERC 2: Formulation into mixture ERC 3: Formulation into solid matrix
Uses at industrial sites	Uses in Coatings (industrial)	**Process category** PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises

		PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring **Environmental release category** ERC 4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article) ERC 5: Use at industrial site leading to inclusion into/onto article ERC 6a: Use of intermediate ERC 6b: Use of reactive processing aid at industrial site (no inclusion into or onto article) **Subsequent service life relevant for that use?** no
Uses by professional workers	Uses in Coatings (Professional)	**Process category** PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions PROC 4: Chemical production where opportunity for exposure arises PROC 5: Mixing or blending in batch processes PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring **Environmental release category** ERC 8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor) ERC 8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor) **Subsequent service life relevant for that use?** no
Consumer Uses	Uses in Coatings (Consumer)	**Chemical product category** PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 0: Other: PC10 **Environmental release category** ERC 8c: Widespread use leading to inclusion into/onto article (indoor) ERC 8f: Widespread use leading to inclusion into/onto article (outdoor) ERC 10a: Widespread use of articles with low release (outdoor) ERC 11a: Widespread use of articles with low release (indoor)

		Subsequent service life relevant for that use? no
Article service life	Uses in Coatings (Consumer)	**Environmental release category** ERC 8c: Widespread use leading to inclusion into/onto article (indoor) ERC 8f: Widespread use leading to inclusion into/onto article (outdoor) ERC 10a: Widespread use of articles with low release (outdoor) ERC 11a: Widespread use of articles with low release (indoor) **Article category related to subsequent service life** AC 1: Vehicles AC 3: Electrical batteries and accumulators AC 5: Fabrics, textiles and apparel AC 10: Rubber articles AC 13: Plastic articles AC 0: Other: AC12

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonised classification available.

7.6.2. Self-classification

In the registration(s): Not classified.

There are no notified hazards by manufacturers, importers or downstream users for this substance.

For the previous EC number 271-082-5[6], 116 notifiers do not classify the Substance, whilst 33 classify for Acute Aquatic Toxicity category 1 and 5 notifiers classify the Substance for Aquatic Chronic toxicity category 2.

7.7. Environmental fate properties

No studies were identified for the environmental fate of the Substance. As the required test did not clarify the concerns for endocrine disrupting properties of the Substance, a new concern for the environmental system is thereby not triggered.

7.8. Environmental hazard assessment

Environmental data was not reviewed and thus not included in this document. See section 7.10.1 for considerations of the eMSCA regarding the endocrine disrupting properties of the substance related to the environment.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

No toxicokinetic data on the Substance are available. However, the toxicokinetics of other high molecular weight phthalates, DINP and DIDP, have been studied and are included in the registration. It is suggested by the registrant(s) that these data can be used as read-across information relevant for the evaluation of the Substance. The read across proposed by the Registrant has not been verified by the eMSCA.

[6] https://echa.europa.eu/fr/information-on-chemicals/cl-inventory-database/-/discli/details/11837 as of June 2021

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated by eMSCA.

7.9.3. Sensitisation

Not evaluated by eMSCA

7.9.4. Repeated dose toxicity

7.9.4.1. Subacute repeated dose toxicity (21 days)

Originally, a sub-acute repeated dose toxicity study in rats was provided by the registrant (Table 8). The study was performed in rats and results were used in the performed assessment on adverse effects on fertility. A study report was not provided for this study. Results of endpoints relevant for fertility are presented below and results are also included in the discussion of reproductive toxicity in section 7.9.7.

Table 8

OVERVIEW OF ENDPOINTS RELEVANT FOR REPRODUCTIVE TOXICITY (FERTILITY) IN ORAL REPEATED DOSE TOXICITY STUDY			
Species, strain and number of animals, substance, reliability	**Protocol**	**Results**	**Reference**
Rat (Crl: D(SD)BR) male, n=6 Test substance: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS nr. 68515-40-2) Two different formulations of the substance were tested, one from EU (98.75 % pure) and one from US (>98% pure). Klimisch 2 (reliable with restrictions) 21 days	Non-guideline Sub-acute study. Oral exposure in feed for 21 days. Doses of 0, 0.1, 1 or 2%, corresponding to 0, 60, 600 or 1200 mg/kg /day of the EU and the US version After 21 days of exposure, body weight, and weight of liver, brain, testes and epididymis were investigated as was histopathology of the testes in the high dose group.	Exposure to the 60 mg/kg bw/day dose did not lead to any statistically significant effects. The EU version of the substance caused significantly reduced bw gain and food consumption and significantly increased relative liver weights at the two highest doses (600 and 1200 mg/kg bw/day). The US version caused the same effects only at the highest dose. The substance did not significantly affect absolute weights of brain, epididymides or testes. Relative weights of these organs were not mentioned in the registration dossier. In the EU version of the substance, 4 out of 6 rats from the high dose group showed testicular degradation, while no animals in the control group showed any adverse effects on testes histology. In the US version it was 1/6. Under 'other details on test materials' in the registration dossier it was stated that the two B7-9P versions may contain different relative concentrations of the same substance and/or different components.	Unpublished study report, 1999. Summary only available.

The results from this study (unpublished study report, 1999) indicate that 21 days of oral exposure to the Substance through the feed at doses of 600 and 1200 mg/kg induced some adverse effects on the male reproductive system. Absolute weights of testes and epididymis were not significantly affected, and relative weight of these organs were not mentioned in the registration dossier. Testicular degeneration was reported in 67% of high dose males exposed to the EU version of the Substance. As the study is not targeted to detect reproductive effects, there was no exposure during the critical foetal period of male

sexual development. Exposure duration was only 21 days and only 6 animals per dose group were used. Significant adverse effects on the male reproductive system, similar to those seen in the one-generation study (described in section 7.9.7), would not be expected to occur in a repeated dose study unless much higher doses were used.

The results of the sub-acute study are also included in the discussion of reproductive toxicity in section 7.9.7.

In the course of the evaluation, the eMSCA identified a data gap for a 90-day sub-chronic toxicity study according to the standard information requirements in REACH, Annex IX, section 8.6.2. In order to provide further information for the end-points of concern, a modified OECD TG 422 study was requested and thus, performed by the registrant (unpublished study report, 2019). The study protocol is a modified oral combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test. As no sub-chronic toxicity (90-day) study was available for the Substance, the OECD TG 422 was modified to include evaluation of all males and females with regards to haematology, clinical chemistry, and gross and microscopic pathology, including pathology investigations of the additional tissues and organs as normally required in the Sub-chronic toxicity (90-day) study OECD TG 408. The study was performed in rats according to OECD 422 and modified according to ECHA Final Decision dated 19 May 2017[4]

7.9.4.2. Subchronic repeated dose toxicity (98/65 days)

The available information on repeated dose toxicity of the Substance, including the requested **modified OECD TG 422 study**, was thoroughly reviewed by the eMSCA for systemic effects and effects on male and female reproductive performance (Table 9).

Table 9

ORAL SUB-CHRONIC REPEATED DOSE TOXICITY STUDY			
Species, strain and number of animals, substance, reliability	**Protocol**	**Results**	**Reference**
Rat (Crl:CD(SD) rats), n=20/sex/group Test substance: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Klimisch 1 (reliable)	Modified OECD TG 422 study. Oral exposure in feed in feed for 98 days (males) and 65 days (females). Doses of 0, 375, 1500 or 6000 ppm, corresponding to approx. 25, 103, 389 mg/kg /day tested for females during pre-pairing and gestation (66, 250, 975 mg/kg/day during lactation) and for males 17, 67, 280 mg/kg/day throughout the testing period.	No Substance-related mortality or clinical signs of toxicity were observed. Food consumption was not affected and the eMSCA assessed that the tested doses did not cause adverse effects on body weight. A 6000 ppm, kidney weights (both absolute and relative) were increased in males, and in both sexes liver weights (absolute and relative) were increased, and diffuse hepatocyte hypertrophy was seen. Thyroid gland histopathology was also affected at 6000 ppm (in both sexes), seen as an increase in the incidence and severity of follicular cell hypertrophy. These changes corresponded well with observed increases in circulating TSH and decreases in T4. The reproductive/developmental screening test conducted is further presented and discussed in section 7.9.7 (toxicity to reproduction).	Unpublished study report, 2019

Four groups of Crl:CD(SD) rats (20/sex/dose) were administered 0, 375, 1500, or 6000 ppm of Substance in diet, for a duration of 98 days for males (during pre-pairing, pairing, and post pairing phases – until postnatal day, PND 14) or up to 65 days for females (during pre-pairing, pairing, gestation, and lactation phases – until PND 22). The control group was provided access to basal diet ad libitum. For males, the Substance concentrations corresponded to 16-18 mg/kg/day, 61-73 mg/kg/day and 260-299 mg/kg/day throughout the study (pre-and post-pairing phases), in the three dose groups respectively. For the females the average Substance consumption during the pre-pairing and gestation phases, was 24-25 mg/kg/day, 99-106 mg/kg/day and 367-411 mg/kg/day in the three dose groups respectively. During the lactation phase, the average Substance consumption was 66, 250, and 975 mg/kg bw/day.

In this repeated dose toxicity study adults were observed for clinical effects, body weight, food consumption, motor activity, grip strength and foot splay. Complete terminal necropsies with organ weight recordings and microscopic examinations were performed on parental males (PND 14) and females (PND 22). Blood samples for haematology, clinical chemistry, bile acids and thyroid hormone assessments were collected.

The reproductive/developmental screening test that was included in the study is further presented and discussed in section 7.9.7 (toxicity to reproduction).

No Substance-related mortality or clinical signs of toxicity were observed. Food consumption was not affected by oral exposure to the Substance. In males dosed 6000 ppm, body weight gain throughout the dosing period was a bit lower than in the controls (approximately 13%), however, statistical significance was not achieved, except from pre-pairing days 1 to 36, where the body weight gain was statistically significantly reduced by approximately 21%. Terminal body weights in males were 3.8% lower in the highest dose compared to controls, which was not a statistically significant difference. In the females, an overall increase in body weight gain was seen in high dose animals compared to controls. From pre-pairing day 1 to PND 21, this increase was approximately 15%, and the female terminal body weights on PND 22 were 6% higher in high dose females than in controls. This difference was not statistically significant. Hence, in terms of effects on body weight, the eMSCA assessed that the tested doses did not cause adverse effects.

There were no adverse findings in either sex during the functional observational battery and locomotor activity assessments in the parental animals. Also no effects on clinical chemistry parameters were observed.

Haematology results revealed statistically significantly increased haemoglobin distribution width in males administered 6000 ppm, but this change was in the study report considered not to be an adverse treatment-related effect.

Statistically significantly increased neutrophil counts were observed in females administered 1500 or 6000 ppm. In the study report, this was considered treatment-related, however, in the absence of evidence of inflammation, this finding was not considered to be adverse.

Kidney weights (both absolute and relative) were increased for males given 6000 ppm, which correlated microscopically with an increased incidence and severity of pigment for males given 1500 ppm or 6000 ppm. However, the increased weight and pigment in the kidneys was in the study report considered to be non-adverse, in the absence of any associated tubular degeneration, necrosis or inflammation.

Absolute and relative liver weights were increased for parental animals from the 6000 ppm group. Relative weights were increased by 18% in males and by 29% in females. Diffuse hepatocyte hypertrophy, characterized by the presence of enlarged hepatocytes without consistent zonal pattern was also seen in both sexes. The Substance-related findings in the liver were in the study report considered to be due to adaptive changes.

The eMSCA agrees with the conclusions regarding effects on haematology, kidneys and liver.

For parental animals, dose-related non-significant increases in TSH concentrations and decreases in T4 concentrations were noted in both sexes (ECHA dissemination site)[5].

A statistical analysis performed by the eMSCA showed that the 102% increase in TSH concentration seen in dams dosed 6000 ppm compared to controls was statistically significant (p=0.4%), whereas the 95% increase seen in the males (6000 ppm) was not (p=11.6%). Opposite this, males dosed 6000 ppm also showed a statistically significant 15.8% decrease in T4 compared to control males (p=3.9%), while the 14.5% decrease in T4 seen in females (6000 ppm) was not significantly different from control levels.

It is difficult to determine exactly why TSH levels were only statistically significantly affected in the females whereas T4 levels were only significantly affected in the males, but as the percentage changes in the hormones were rather similar between sexes, it most relates to the high variation in the hormonal analyses. It is also possible that the high exposure (in mg/kg bw/day) the dams experienced during the lactation period, and the longer exposure time in the males (98 vs. 65 days) were contributing factors to the observed differences between the sexes.

There were no effects on thyroid gland weight (absolute or relative) in either males or females. The thyroid gland histopathology was affected in parental males and females dosed 6000 ppm, as an increase in the incidence and severity of follicular cell hypertrophy was seen - characterized by increased height of the follicular epithelium and reduced amounts of colloid leading to decreased follicular size. Thyroid follicular cell hypertrophy was found in 65% of the parental males in the highest dose group (6000 ppm, n=20) and in 55% of the parental females in the same dose group (6000 ppm, n=20).

It was concluded that these histopathology findings were considered non adverse as the microscopic changes (thyroid follicular cell hypertrophy) in the thyroid were considered an adaptive change due to increased thyroid hormone metabolism in the liver, which is commonly associated with liver cell hypertrophy secondary to diffuse hepatocyte hypertrophy.

In relation to the histopathology findings of the thyroid, the eMSCA does not agree with the conclusion from the study report that the changes were non-adverse and adaptive, and finds that when all of the thyroid data is analysed together, it clearly shows adverse effects on the thyroid hormone system after 65-98 days of exposure to a dose of 6000 ppm. The molecular initiating event leading to the observed follicular hypertrophy has not been investigated in this (or any other study), so the assumption that the effects is caused by increased thyroid hormone metabolism, just because the liver weight and histopathology were affected, seems speculative. Indeed, a very large number of chemicals cause adverse liver effects in sub-chronic repeated dose toxicity studies, without causing adverse effects on the thyroid hormone system, so there no is reason to assume causation between these two events. Even if increased liver catabolism of thyroid hormones did turn out to be the Molecular initiating Event (MiE) causing thyroid gland hypertrophy, this would still be viewed as adverse and pose a potential hazard relevant for humans, according to the ECHA/EFSA guidance on endocrine disruptors (2018), unless specifically shown not to be.

It is therefore evaluated by the eMSCA, that since the changes in T4 and TSH levels, as well as the histopathology results were rather similar in males and females, both sexes were probably equally sensitive to the thyroid disrupting effects of the Substance.

7.9.4.3. Summary of repeated dose toxicity

Two repeated dose toxicity studies are available: A sub-acute repeated dose toxicity study (unpublished study report, 1999) and a modified OECD TG 422 study (unpublished study report, 2019).

The information available from the sub-acute repeated dose toxicity study with the Substance (unpublished study report, 1999) indicated that 21 days of oral exposure through the feed at quite high doses (600 and 1200 mg/kg) caused some adverse effects on male testes.

No marked systemic toxicity effects were seen in a modified OECD TG 422 study investigating doses up to 6000 ppm (unpublished study report, 2019). This dose corresponded to exposures around 300 mg/kg bw/day in the males and up to 400 mg/kg bw/day in the females (in all other phases of the study than during lactation), and no adverse effects on body weigh were seen at any dose, including the highest dose level.

This indicates that higher doses should have been included in order to properly assess the full repeated dose toxicity potential of the Substance. As suspected, based on previous phthalate studies, the thyroid gland was a target tissue. Adverse effects were identified whereas reproductive performance in both males and females was unaffected up to the highest tested dose of 6000 ppm.

7.9.5. Mutagenicity

Not evaluated by eMSCA.

7.9.6. Carcinogenicity

Not evaluated by eMSCA.

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

In this section endpoints specific for fertility and developmental toxicity are presented and discussed. Endpoints specific for endocrine disruption are further discussion in section 7.10.

7.9.7.1. Effects on fertility

A two-generation reproductive toxicity study OECD TG 416 (unpublished study report, 2013a) and a modified OECD TG 422 toxicity study (unpublished study report, 2019) were available for assessment of fertility. In addition in a sub-acute repeated dose toxicity study investigated some effects on reproductive organs (unpublished study report, 1999).

The **sub-acute repeated dose toxicity study** with the Substance (study report, 1999) indicates that oral exposure through the feed at doses of 600 and 1200 mg/kg, caused some adverse effects on male rats, e.g. testicular degeneration was reported in 67 % of the high dose males exposed to an EU marketed version of the Substance. Significantly increased relative liver weights were seen at both doses. Absolute weights of brain, epididymides or testes were not significantly affected, but the relative weights of these organs were not mentioned in the robust study summary in the registration dossier(s). The results are also described in section 7.9.4.

7.9.7.1.1. Two-generation reproductive toxicity study (OECD TG 416)

A two-generation reproductive toxicity study performed according to the OECD TG 416 (unpublished study report, 2013a) of the Substance has been provided by the registrant. Table 10 records the results regarding reproductive toxicity. The results are further discussed below and in section 7.9.7.3.

Table 10

TOXICITY TO REPRODUCTION: TWO-GENERATION REPRODUCTIVE STUDY			
Species, strain and number of animals, substance, reliability	**Protocol**	**Results**	**Reference**
Rat, Sprague-Dawley, n=28 Test substance: 1,2-Benzenedi-carboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2)	Two-generation reproductive toxicity study (OECD TG 416) The animals were exposed to 0, 750, 2500 or 5000 ppm in diet for two generations. For	At the F0 male necropsy, relative liver and kidney weights were increased at 2500 and 5000 ppm, in the absence of any effects on body weights or other organ weights. F0 males showed increased dose-related percentages of abnormal sperm at 750 ppm and above. For the F0 female necropsy, there were no effects on terminal body weight but significantly increased relative right kidney weights at 2500 ppm and significantly increased absolute and relative liver weights at 2500 and 5000 ppm.	Unpublished study report, 2013a

Key study Klimisch 1, reliable without restriction. Study report used for the evaluation.	the females these concentrations corresponded to 50,167 and 333 mg/kg bw /day. The investigated endpoints included gonadal function, the oestrous cycle, mating behaviour, conception, gestation, parturition, lactation in the F0 and F1 generations and pre- and postnatal growth and development of the offspring in the F2 generation (to weaning).	No effects on mating, fertility, fecundity, pup sex ratio/litter, at any dose were seen. In F0 males, a statistically significant dose-related increase in the percentage of abnormal sperm was seen in all treated groups. The other sperm parameters in the F0 males were not affected by the exposure. AGDs on PND 0 for both F1 male and female pups were equivalent across all groups. Pup body weights were significantly reduced in the highest dose group (5000ppm) in the pre-weaning period, but not after weaning. At the scheduled necropsy for F1 pups on PND 21, male absolute brain, spleen, and thymus weights were significantly reduced at 5000 ppm and relative liver weights were significantly increased at 2500 ppm but not at 5000 ppm. Reproductive organs, testicular descent, AGD and malformations were not evaluated on PND 21. Age at vaginal patency and PPS were unaffected in F1 offspring. There was no evidence of affected fertility or litter sizes during the F1 breeding to produce F2 offspring, but precoital interval was significantly longer at 5000 ppm. At the F1 male necropsy, relative liver weights were significantly increased at 750 ppm and at 5000 ppm. Reproductive organ weights were not significantly affected. As seen in the F0 males, a statistically significant dose-related increase in the percentage of abnormal sperm was also seen in F1 adult males in all treated groups. All F2 litter data, including total litter size on PND 0, AGD, live litter size, live birth ratio, survival ratio, pup body weights during lactation were equivalent across all groups. On PND 21 relative liver weights were significantly increased at 5000 ppm in F2 males and at 2500 and 5000 ppm in F2 females.	

In this OECD TG 416 study, the animals were exposed to 0, 750, 2500 or 5000 ppm in diet for two generations. For the pregnant females these concentrations corresponded to 50, 167 and 333 mg/kg bw/day, thus, a lower dosing than in the previously performed one-generation reproductive and developmental toxicity study was chosen (unpublished study report, 2005) (see section 7.9.7.2.). The investigated endpoints relevant for toxicity to reproduction included gonadal function, the oestrous cycle, mating behaviour, conception, gestation, parturition, lactation in the F0 and F1 generations and pre- and postnatal growth and development of the offspring in the F2 generation (to weaning).

A detailed evaluation of reproductive toxicity data from the two-generation study did not indicate effects on fecundity, except for a dose-related increase in the percentage of abnormal sperm. At the F1 male necropsy around PND 105, reproductive organ weights were not significantly affected but a dose-related increase in the percentage of abnormal sperm was seen. This effect was seen in F1 adult males at 250, 750, 2500, and 5000 ppm, and similarly in F0 males, as these males also showed a dose-related increase in percentages of abnormal sperm from 750 ppm and above.

The registrant concluded that there was no scientific consistent evidence of reproductive toxicity for either males or females at any dose. This was due to the sperm abnormalities observed in exposed F0 and in F1 males are regularly seen in the control males, and the

percentage of abnormal sperm values in this study was within the historical control values for this rat strain and supplier.

Since the statistically significant increase in abnormal sperm was dose-dependent and seen in both F0 and F1 males, the eMSCA considers that the effect was not likely to be a chance finding, but rather a fertility effect caused by exposure to the Substance.

7.9.7.1.2. Modified OECD TG 422 study

In addition to the fertility studies, the requested modified OECD TG 422 study (unpublished study report, 2019) investigated some fertility parameters as it was performed as a modified oral combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test (Table 11).

Table 11

ORAL SUB-CHRONIC REPEATED DOSE TOXICITY STUDY			
Species, strain and number of animals, substance, reliability	**Protocol**	**Results**	**Reference**
Rat (Crl:CD(SD) rats), n=20/sex/group Test material: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Klimisch 1 (reliable)	Modified OECD TG 422 study. Oral exposure in feed in feed for 98 days (males) and 65 days (females). Doses of 0, 375, 1500 or 6000 ppm, corresponding to approx. 25, 103, 389 mg/kg /day tested for females during pre-pairing and gestation (66, 250, 975 mg/kg/day during lactation) and for males 17, 67, 280 mg/kg/day throughout the testing period.	P0: No Substance-related mortality or clinical signs of toxicity were observed. Food consumption was not affected and the eMSCA assessed that the tested doses did not cause adverse effects on body weight. A 6000 ppm, livers, kidney and thyroids were affected by the exposure (for further details, see description of repeated dose toxicity, section 7.9.4). Reproductive organ weight and reproductive performance/fertility (including semen quality) was unaffected at all three doses.	Unpublished study report, 2019

In the modified OECD TG 422 study, repeated dose administration of the Substance was evaluated for systemic effects and effects on male and female reproductive performance. In addition to the endpoints and observations presented in the repeated dose toxicity part of the study (see section 7.9.4.), oestrous cycling, mating, fertility and pregnancy indices were also investigated. Reproductive organs and thyroid gland weights were recorded and microscopic examinations performed at terminal necropsies, and parental sperm quality was assessed.

Male and female fertility and reproductive performance were not adversely affected following oral dietary administration of the Substance. No statistically significant effects on reproductive organ weights or histopathological changes in these were observed. The number of mated pregnant females, the number of females delivering, and the duration of gestation were unaffected. The number of implantation sites and the percentage of post-implantation loss were also not affected. No Substance-related findings were noted for sperm count, sperm motility, or sperm morphology, and no Substance-related effect were

seen on the number of pups delivered, the number of live born, or the number of still births.

7.9.7.2. Developmental toxicity

Originally three developmental toxicity studies were available for assessment of developmental toxicity. A prenatal developmental toxicity study performed according to OECD TG 414 (unpublished study report, 2013b) (Table 12), a one-generation study (unpublished study report, 2005) (Table 12), which was based on a one-generation extension study developed by the US EPA Endocrine Disruptors Screening Program and a two-generation study (unpublished study report, 2013a). The two-generation study is presented in Table 10.

Because of unresolved concern for adverse effects on reproductive development and endocrine disruption based on the results of these studies, an additional study was requested in the Final Decision of 19 May 2017 issued by ECHA[4]. The results from this modified OECD TG 422 (unpublished study report, 2019) are presented in section 7.9.4, 7.9.7.1 - 7.9.7.2 and discussed together in the summary of reproductive toxicity, section 7.9.7.3.

Table 12

OVERVIEW OF THE DEVELOPMENTAL TOXICITY STUDIES			
Species, strain and number of animals, substance, reliability	**Protocol**	**Results**	**Reference**
Rat, Sprague-Dawley, n=22 Test substance: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Klimisch 1, reliable without restriction. Study report used for the evaluation.	Prenatal developmental toxicity study (OECD TG 414) with termination on GD 20. Pregnant Sprague Dawley Crl:CD rat dams were fed 0, 250, 750 or 3750 ppm of the Substance from GD6-20. This corresponded to 16.7, 50 or 250 mg/kg bw/day.	There were no treatment- or dose-related maternal findings at any dose or any time during gestation or at scheduled necropsy, including no effects on maternal body weights, weight gains, clinical observations, or pregnancy indices. The only maternal findings were the statistically significantly increased absolute and relative liver weights at term in the highest dose group, with no evidence of hepatocellular hypertrophy or hyperplasia. There were no treatment-related developmental toxicity findings at any dose level, including no effects on pre- or post-implantation loss, foetal numbers, sex ratio, body weights, or foetal external, visceral, or skeletal malformations or variations.	Unpublished study report, 2013b
Rat, Sprague-Dawley, n=25 Test substance: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Key study Klimisch 1, reliable without restriction.	Non guideline one-generation extension study protocol developed by the EPA Endocrine Disruptors Screening Program Female Sprague Dawley rats were exposed to the Substance at concentrations in the diet of 0, 750, 3750 or 7500 ppm from	Gestational body weight change GD6-20 and gestational feed consumption was unaffected by exposure. Maternal body weight was significantly reduced (by 6 %) on PND 7 in the highest dose group, but otherwise was not affected in any dose group from PND 1-21. At necropsy on PND 21 maternal body and reproductive system organ weights were unaffected. Paired kidney and liver weights were increased in the two highest dose groups. There were no treatment related effects in post-implantation loss, live born or	Unpublished study report, 2005

Study report used for the evaluation.	GD 6 to weaning on PND 21. These concentrations corresponded to 50, 250 and 500 mg/kg bw /day. AGD was measured on PND 1 and on PND 12 males were examined for the presence of nipples/areolae. Observations of preputial separation (PPS) in weaned males began at PND 35 and continued until acquisition. At necropsy on PND 21 and PND 75 male AGDs were measured, and males were checked for malformations. Reproductive organs, pituitary, liver, adrenals and kidneys were excised and weighed in all dose groups, and histopathology was performed on organs from selected males from controls and the highest dose group.	stillbirth indices, or on survival indices throughout lactation. On PND 21 body weight reductions were around 4% in the two lower dose groups and continued like this throughout the study. In the highest dose group reductions were around 11% at PND 21 and between 5-6 % during the rest of the study. Feed consumption from PND 21-75 was reduced significantly (by 3-5%) in all dose groups. Clinical observations did not exhibit any apparent dose-related differences across groups. On PND 21 relative liver weight showed a significant increase in the highest dose group. Incidence of kidney hydronephrosis was significantly increased in the two highest dose groups. On PND 75 absolute liver weight were significantly reduced by between 4-7 % in all three dose group, whereas relative liver weights were not significantly affected. Sexual development appeared affected by exposure to the higher doses of the substance as can be seen in Table 14 (annex c). Male (and female) AGDs were reduced on PND 1 and 21, the incidence of epispadias and undescended testes was increased and several reproductive organ weights were reduced on PND 21. Acquisition of puberty in males (PPS) was significantly delayed and on PND 75 absolute weights of prostate, testes and LABC were all affected. Relative weights of the pituitary, adrenals and testis were increased in the highest dose group.	
Rat, Sprague-Dawley, n=28 Test material: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Key study Klimisch 1, reliable without restriction.	Two-generation reproductive toxicity study (OECD TG 416)	See table 10	Unpublished study report, 2013a
Rat (Crl:CD(SD) rats), n=20/sex/group Test material: 1,2-Benzenedicarboxylic acid, benzyl B7-9P-branched and linear alkyl esters (CAS no. 68515-40-2) Klimisch 1 (reliable)	Modified OECD TG 422. Oral exposure in feed for 98 days (males) and 65 days (females). Doses of 0, 375, 1500 or 6000 ppm, corresponding to approx. 25, 103, 389 mg/kg /day for	F1: Pup survival indices were not affected up to PND 21, and no Substance-related pup mortality was observed. Male and female pup weights were not statistically significantly affected in any group. AGD and nipple retention was also not affected by the Substance exposure, and no statistically significant Substance-related reproductive organ weight changes were recorded in the offspring on PND 21.	Unpublished study report, 2019

	females during pre-pairing and gestation, and 66, 250, 975 mg/kg/day during lactation, and for males 17, 67, 280 mg/kg/day throughout the testing period.	TSH levels in male pups (PND 21) were increased by 42% and 65%, in groups administered 1500 or 6000 ppm respectively, and in females pups by 21% (375 ppm), 48% (1500 ppm) and 39% (6000 ppm) but these changes were not statistically significant. No effects on T4 levels. Lack of thorough examination of AGD, nipple retention, reproductive organ weight makes it difficult to conclusively determine the developmental toxicity effects of the test compound. See also table 9 and 11	

In the **prenatal developmental study**, OECD TG 414, with mated females exposed to approximately 0, 16.7, 50 or 250 mg/kg bw/day from GD 6-20 (unpublished study report, 2013b) no adverse effects on the investigated reproductive or developmental parameters were seen. However, the top dose level chosen for this study (i.e. 3750 ppm corresponding to 250 mg/kg bw/day) was lower than top doses used in both the one-generation and the two-generation studies, and was therefore by the eMSCA not considered sufficiently high to address the concerns for the developmental effects on the Substance.

In this non-guideline **one-generation reproductive and developmental toxicity study** (unpublished study report, 2005), pregnant rat dams were exposed to 0, 750, 3750 or 7500 ppm of the Substance via feed from GD 6-PND 21, corresponding to 50, 250 or 500 mg/kg bw/day during the gestation period. Several reproductive endpoints in male offspring exposed to the Substance during development were adversely affected.

Even at the lowest tested dose of the Substance (750 ppm), some indications of adverse effect on the reproductive development were seen. On PND 1, male AGD was reduced by 3.6%, which was a significant effect both with and without adjusting for body weight (which in males was decreased by 4.5% on PND 1). On PND 21, the absolute weight of the Cowper's glands was reduced by 20% (even though body weight was only reduced by 4 % at this age). The reduction in relative weight of this organ was 16 %, however this was not statistically significant. Significant effects were seen on female AGD, which was reduced by 10% on PND 1 (when body weight was reduced by 3.4%). Males furthermore showed a significant 1 day delay in preputial separation (PPS) and a significant 5% increase in right relative testes weight on PND 75.

At the middle dose level (3750 ppm) pup body weights were reduced similarly to the low dose group (e.g. 3-4%), however more effects indicating reproductive toxicity were seen. Male AGDs in this group were reduced by almost 11% on PND 1 and by 6 % on PND 21. On PND 21, significant reductions of between 7-28% in the absolute weights of epididymis, prostate, Levator ani/bulbocavernosus muscle (LABC) and Cowper's glands were seen, while body weight at this age were reduced by 4%. A significant increase in the number of males with epispadias was also seen in this dose group. As in the lower dose group, female AGD on PND 1 was significantly reduced, and in males preputial separation was significantly delayed. Relative testes weights on PND 75 were significantly increased, as seen in the lowest dose group.

In the highest dose group (7500 ppm) pup body weights were reduced somewhat more than in the two lower dose groups (i.e. by 5-11%). The same indications of adverse reproductive effects that were seen in the low and middle dose group were also present at 7500 ppm, but in some cases more pronounced and with some additional endpoints affected. Male AGD on PND 21 was reduced more than in the 3750 ppm group, and a significant increase in areolas on PND 12 was also seen. PPS was delayed by 1.6 day, and significantly more animals showed epispadias and undescended testes on PND 21 compared to the control group. On PND 21 the absolute weights of testes, epididymis, prostate, LABC, Cowper's glands and pituitary were reduced by between 10-30%, while body weight on PND 21 was reduced by 11 %.

The relative weight of LABC on PND 21 was significantly reduced, whereas the relative weights of the other reproductive organs were not significantly affected, even though the relative weight of the Cowper's glands was reduced by 24%. On PND 75 absolute weights of prostates and LABC were still significantly reduced whereas testes weights were increased both as absolute and relative. Furthermore, relative weights of pituitary and adrenals were increased. Histopathology of males from the highest dose group additionally indicated dilatation of the lumen of the seminiferous tubules of the left testis in two males. This result deemed treatment-related.

The Substance caused adverse developmental effects on male reproduction already from the lowest tested dose, and not all the adverse effects were reversible, as e.g. testes weights were significantly affected in male pups from all three doses on PND 75. It is possible that the delayed preputial separation described above, observed in all three dose groups, could be due to delayed development (i.e. a lower body size at a given age), or to unusual control values. The observed effects on male AGD, increased nipple retention and reduced reproductive organ weights are all effects that have been seen for several other reproductive toxic phthalates (David 2006, Howdeshell et al., 2008).

The developmental effects described above may be due to anti-androgenic effects of this phthalate ester and it is however also possible that some of the significant effects could be due to either delayed development (i.e. a lower body size at a given age), or to unusual control values.

- The delayed PPS might be indicative of delayed development in the offspring.
- The number of animals with undescended testes in the exposed groups was not very large when compared to controls (2 animals in control group vs. 5 in high dose group), and the statistically significant difference could therefore be a chance finding.
- The biological significance of the observed reductions in female AGDs is at the moment unclear, as this is not a typical effect seen in females exposed to phthalate esters. When comparing the absolute female control AGD values (0.96+0.01 mm) in the one-generation study (unpublished study report, 2005), with those measured in the two-generation study (0.84+0.01 in F1 and 0.87+0.02 in F2) (unpublished study report, 2013a) the control values in the one-generation study seem very high, and this could be a possible explanation for the significant effect in all dose groups in the females from the one-generation study.

However, the observed:

- Significant decreases in male AGD on PND 1 and 21, both with and without adjusting for body weight,
- the significantly increased number of areolas in male offspring on PND 12
- and the reduction in absolute and relative reproductive organ weights (LABC and Cowper's glands on PND 21 and the increase in absolute and relative testis weight on PND 75)

All effects have previously been seen after exposure to anti-androgenic reproductive toxicants. See section 7.10 – assessment of endocrine disrupting properties.

A **two-generation reproductive toxicity study** (unpublished study report, 2013a) of the Substance has been provided by the registrant (table 10). The results are further discussed below and in section 7.9.7.3.

A detailed evaluation of the data from the two-generation study, showed that only very few developmental endpoints were affected. No effects on fecundity, pup sex ratio/litter, or on developmental landmarks were seen at any dose in either F1 or F2. AGD on PND 1 for both F1 and F2 male and female pups were equivalent across all groups. Nipple retention and presence of areola was not assessed at any time point during the study.

F1 pup body weights were significantly reduced in the highest dose group in the pre-weaning period, but not after weaning. At the scheduled necropsy for F1 pups on PND 21, male absolute brain, spleen, and thymus weights were significantly reduced at the highest

dose, but reproductive organs, testicular descent, AGD and malformations were not evaluated on PND 21. These endpoints are not mandatory in the OECD TG 416, but as they were evaluated and many of them were significantly affected in the previously conducted one-generation study (unpublished study report, 2005), as presented above, it would have been highly relevant if these endpoints had been included in the OECD TG 416 study, as they would have been interesting to have for comparison of the two studies.

Age and body weight at vaginal patency was unaffected by exposure in F1 female offspring. Preputial separation (PPS) in male F1 offspring appeared to show a non-monotonic dose-response relationship, however the observed differences in age at preputial separation were not statistically significant. Thus, males dosed up to 2500 ppm appeared to show delayed sexual maturation, of 1½ days later than controls, whereas males from the high dose group (5000 ppm) acquired PPS 1.5 day earlier than control males. These males also had a significantly lower body weight at acquisition of PPS than controls.

At the F1 male necropsy around PND 105, reproductive organ weights were not significantly affected. As already presented in section 7.9.7.1 (effects on fertility), the only statistically significant sign of reproductive toxicity of the Substance in this study was a dose-related increase in the percentage of abnormal sperm. The statistically significant increase in abnormal sperm was dose-dependent and seen in both F0 and F1 males, and thus the eMSCA considers that the effect was not likely to be a chance finding, but rather a fertility effect caused by exposure of the Substance.

Furthermore, the method that was used for the AGD assessment in this study was not as sensitive as the one used in the one-generation study. By investigating study report data on AGD from individual pups, it became clear that a measuring unit of 0.18 mm was used. In the one-generation study (unpublished study report, 2005), which was performed in the same laboratory, the measuring unit was 0.10 mm. This means that the AGD measurements performed in the one-generation study were clearly more precise than the ones used in the two-generation study, and using a less precise measuring unit inevitably reduces the chance of finding statistically significant differences between groups.

Finally a **modified OECD TG 422 study** in rats was performed (unpublished study report, 2019). As presented in section 7.9.4, no Substance-related mortality or clinical signs of toxicity were observed. Terminal body weights in males were 3.8% lower in the highest dose compared to controls, which was not a statistically significant difference. The female terminal body weights were 6% higher in high dose females than in controls but this difference was not statistically significant. Hence, in terms of effects on body weight, the eMSCA assessed that the tested doses did not cause adverse effects.

Pup survival indices were not affected up to PND 21, and no Substance-related pup mortality was observed. Male and female pup weights, measured on PND 1, PND 4 (pre- and post-cull), PND 13, and PND 21, were not statistically significantly affected in any group. No treatment-related macroscopic findings and no Substance-related clinical chemistry changes were observed in the pups.

AGDs on PND 4 were not affected in male or female pups and there were no indication that male offspring from higher dose groups had shorter AGDs than control males. The eMSCA however notes that the unit of AGD measurement was very imprecise, as the AGDs were only reported with 1 mm accuracy. Thus, males were ascribed an AGD of either 3.0, 4.0, 5.0, 6.0 or 7.0 mm. For comparison, when the AGD is measured at academic laboratories with great research experience in this area, the accuracy of the measurements is much larger[7]. The low accuracy used in the present study makes it very hard to identify subtle effects of test substance exposure.

Nipple retention was also not affected by the test compound exposure on PND 13, and no tendencies were observed. However, eMSCA notes that of the almost 500 male pups examined for nipples/areolae, no areolae were registered for any of the pups. This indicates

[7] As an example, studies performed at the Technical University of Denmark (DTU), Research Group for Molecular and Reproductive Toxicology the measuring unit is 0.17 mm, based on the units present in the measuring ocular of the microscope used for the AGD measurements. So in studies performed at DTU, a male pup can be ascribed an AGD of e.g. 3.5, 3.67, 3.84, 4.01 mm etc.

that the endpoint was not assessed with enough accuracy. As can be seen in well performed developmental studies from contract laboratories, as well as in the open literature, some biological variation always exists on the endpoint of nipple retention. Hence, some control pups are registered with one or even two areola, once in a while. The fact that none of the 500 pups assessed in this study had any nipples/areolae, indicates that the endpoint was not assessed correctly and therefore the results are not informative regarding the assessment of anti-androgenic properties of the Substance.

No statistically significant Substance-related reproductive organ weight changes were recorded in the offspring on PND 21. Approximately 18% and 21% lower weight (both absolute and relative) for the bulbourethral muscle was seen in male pups of dams fed diets containing 1500 and 6000 ppm, respectively. However, the variation in this endpoint was quite large, and in absence of any other changes in primary or secondary male sex organ weights the registrant concluded that this was not treatment related and was within normal biological variation.

A statistical analysis of the data performed by the eMSCA showed that these differences were not even close to attaining statistical significance (p= 30% & 47 % for the two dose groups respectively). Examining the single offspring data however indicated that for instance for the bulbourethral muscle, the weight of this organ in certain offspring was 10 times higher than the weight of the same organ in all the other siblings from the same litter. These outlier values were however uncritically included in the calculation of litter mean values, which contributed substantially to the high variability of the data. The eMSCA agrees with the conclusion from the study report that no adverse effects on the reproductive organ weight seemed to occur after developmental exposure to the test compound at the examined doses, but severely questions the reliability of the quality control performed for these data.

In male and female combined pups (PND 4), circulating T4 concentrations were not affected. However, a detailed analysis performed by the eMSCA showed more than one fourth of the values for single litters were below level of detection, and in the two higher dose group, there were twice as many litters with T4 values under the detection limit, than in controls. Hence employing an analysis method with a lower limit of detection would have improved the accuracy of the results substantially, and might have yielded different results.

On PND 21, no changes in thyroid weight were observed and T4 levels were also not affected. These were however measured post fixation, which somewhat reduces the sensitivity of the measurement, compared to wet weight measurements. However, no effects were seen in the adult thyroid gland weight either.

TSH levels in male pups were increased by 42% and 65% in groups administered 1500 or 6000 ppm respectively, but the changes were not statistically significant (according to statistical analysis performed by eMSCA). In females pups TSH increases of 21%, 48% and 39% were seen in the groups administered 375, 1500, or 6000 ppm respectively, and these changes were not statistically significant either. In the study report, the TSH changes were considered Substance-related but not adverse due to no corresponding or associated effects in T4 concentration and no changes in thyroid weight.

In the view of the eMSCA, the most likely explanation for the non-consistency of thyroid effects in the offspring, is that the doses of the Substance, which were transferred through the maternal milk during the lactation period, were not high enough to elicit a measurable response as seen in the parental animals. The parental animals received the Substance directly in the feed and through a longer time period and therefore the effects on the thyroid system were clearer than in the pups. Indication of TSH increases were however seen in the offspring, but the indirect exposure probably resulted in only moderately increased TSH concentrations, which in turn could keep the circulating T4 levels at a concentration that was not different from control animals.

7.9.7.3. Summary and discussion of reproductive toxicity

The two-generation study (unpublished study report, 2013a) showed some signs of adverse effects on fertility with increase in abnormal sperm cells seen after exposure to the Substance in both F1 and F2 males (described and discussed in section 7.9.7.1)

Developmental toxicity on male reproductive development was not observed in the two-generation study. The one-generation study (unpublished study report, 2005) on the other hand showed several adverse developmental effects, on male sexual development as described in section 7.9.7.2. The modified OECD TG 422 (unpublished study report, 2019) was requested in order to clarify and further investigate effects on fertility and development. The studies have been discussed separately in the previous sections, and below the data are discussed together, to provide combined assessment of reproductive toxicity of the Substance

The two-generation study (unpublished study report, 2013a) and the one-generation study (unpublished study report, 2005) on the Substance have been performed in the same laboratory, using similar group size, the same rat strain, and a comparable purity of the Substance. Despite of this, the discrepancies in the results between the two studies seem large at first sight. However, the two studies differ in the tested dose levels, the investigated end-points and the methodologies used.

In comparison, the requested modified OECD TG 442 study (unpublished study report, 2019) was conducted using a larger group size (n=20/sex/dose group) as requested in ECHA's Final Decision Letter[4]. This group size was considered to be sufficiently high to properly address the concerns regarding possible developmental toxicity and/or endocrine disrupting mode of action of the Substance. The doses used were similar to the dose ranges of the one- and two-generation studies, but unfortunately the highest tested dose was lower than the one suggested by ECHA (7500 ppm suggested; 6000 ppm used). This meant that even at the highest tested dose, no adverse effects on body weight were observed, indicating that this top dose was not high enough.

The eMSCA has included the following considerations in the evaluation of the available data:

- Many of the adverse developmental effects of the Substance seen in the one-generation study (presence of areolas, significantly decreased absolute and relative weight of LABC and Cowper's glands on PND 21 and increased absolute and relative testis weight on PND 75) were mainly present at the highest tested dose of 7500 ppm (500 mg/kg bw/day). However, this dose was not included in the two-generation study nor in the modified OECD TG 422 study.
- Additionally, many of the reproductive endpoints, which were significantly affected in the one-generation study (nipple retention on PND 13 and assessment of reproductive organ weights, penile malformations and AGD on PND 21) were simply not investigated in the two-generation study. In the requested modified reproductive study, these endpoints (nipple retention on PND 13, AGD on PND 4 and reproductive organ weights on PND 21) were included but no significant effects were observed in doses up to 6000 ppm.
- Sperm morphology, which was significantly affected in the two-generation study was not assessed in the one-generation study. In order to address the concern regarding male fertility, dosing of the males was prolonged to cover one complete spermatogenesis cycle in parental animals in the requested modified OECD TG 422 study. However semen parameters, including sperm morphology, mortality and sperm count, were not affected

The actual discrepancies in reproductive toxicity findings between the three studies, i.e. where the same endpoints have been studied at similar doses, but different results have been obtained:

- Male AGD on PND 1; reduced by 4-11% (50-250 mg/kg) in one-generation study but unaffected in two-generation study (50-333 mg/kg) and the modified OECD TG 422 study (25-389 mg/kg)
- Female AGD on PND 1; reduced by 10-17% (50-250 mg/kg) in one-generation study but unaffected in the two-generation study (50-333 mg/kg) and the modified OECD TG 422 study (25-389 mg/kg)
- Timing of preputial separation; significantly delayed by 1.0 day (50-250) in one-generation study but not significantly affected at any dose in the two generation

study, even though a delay of 1.5 day was seen at 167 mg/kg and an advancement of 1.5 day was seen at 333 mg/kg. This endpoint was not evaluated in the study report of the modified OECD TG 422 study.

- Absolute and relative weight of testes; was significantly increased on day 75 (50-250 mg/kg) in the one-generation study but was not significantly affected on day 105 (50-333 mg/kg) in the two-generation study. Testes weights were not significantly affected on PND 21 in the modified OECD TG 422 study.

As discussed previously, the less sensitive AGD measuring procedure used in the two-generation study and in the modified OECD TG 422 study compared to the one-generation could explain why no effect was seen on male AGD in those studies, as using a larger measuring unit inevitably reduces the likelihood of finding statistically significant differences between groups. As previously pointed out, the significant effect on female AGD in the one-generation study could be due to an unusually high control value.

Additionally, a quantitative count of retained nipples/areolas in males was included in the test design of the modified OECD TG 422 study, as this is considered to be more sensitive than evaluation of presence/absence only (OECD, 2013). A possible reduction in male AGD and/or an increase in nipple/areolae retention in males would be regarded as an adverse effect that could support identification of the Substance as a developmental toxicant, but due to the outcome of the requested study, this concern was not substantiated.

The timing of preputial separation differed quite much between the one- and two-generation studies (i.e. day 44.1-47.1 in the two-generation study and day 40.7-42.3 in the one-generation study), making the comparison between the studies and the interpretation of the combined results rather difficult. Furthermore, as discussed previously the effect on delayed PPS seen in the one-generation study might be an effect of delayed development and not necessarily an endocrine mediated defect.

Finally, it could be due to biological variation that the relative right testes weights increased by around 5 % at the two lower doses in the one-generation study, but not significantly affected in the two-generation study or the modified OECD TG 422 study. In the two-generation study, the relative right testes weights actually also increased by 5.0% in the 2500 ppm dose group and by 3.9% in the 5000 ppm dose group. In the modified OECD TG 422 study, the relative testes weight (data only reported as paired testes weight) were increased by 3.8% in the lowest dose group (375 ppm), 1.8% in the 1500 ppm dose group and 7.5% in the highest dose group (6000 ppm). While none of these differences were statistically significant, they indicate a tendency towards a similar effect as the one seen in the one-generation study. However, since they were not statistically significant, they were not assessed as being adverse in the present context.

Based on the results of modified OECD TG 422 study, the eMSCA has identified several shortcomings related to the study design and performance, which puts many of the observed results into question. Lack of thorough examination of AGD, nipple retention, reproductive organ weight and offspring T4 levels on PND4, makes it difficult conclusively to determine clear adverse effects on male sexual development after exposure of the Substance, at doses up to 6000 ppm (367-411 mg/kg/day). Furthermore, the eMSCA finds that since no adverse effects were seen on systemic toxicity (terminal body weight was decrease by 4% in males and increased by 6% in females), higher doses should have been employed in order to properly assess the reproductive and developmental toxicity of the Substance.

In conclusion, the described discrepancies in results between the one-generation study (unpublished study report, 2005), the two-generation study (unpublished study report, 2013a) and the modified OECD TG 422 study (unpublished study report, 2019) may be explained by methodological differences between the studies, and particularly the use of a higher dose (500 mg/kg bw/day) in the one-generation study only. As described previously, the Substance has common metabolites with the repro-toxic phthalates BBP and DINP. Based on this fact, and the above presented comparison of the one- and two generation studies, the eMSCA found that the absence of reproductive effects in the two-generation study could not clearly negate the adverse reproductive effects seen in the one-generation study. The results from the sub-acute repeated dose toxicity study (unpublished

study report, 1999) further indicated that high doses of the Substance may adversely affect the male reproductive system. However, as the requested modified OECD TG 422 study did not find clear effects on the development of the male reproductive system, the concern for toxicity to reproduction cannot be substantiated. Therefore, the eMSCA considers that further testing should not be required, although the study performance was associated with several deficiencies as discussed in sections 7.9.7.1 - 7.9.7.3

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

The concern regarding reproductive toxicity related to male sexual development, as well as the concern for toxicity to fertility, cannot be substantiated taking all of the available data into account. The initial ground of concern was raised due to adverse effects on male reproductive development observed in the one-generation study (unpublished study report, 2005), including reduced AGD, increased presence of areolas, decreased absolute and relative weights of LABC and Cowper's glands on PND 21, and increased absolute and relative testis weight on PND 75, as well as adverse effects on sperm parameters seen in both F0 and F1 generation males the two-generation study (unpublished study report, 2013a). However, in the requested modified OECD TG 422 study (unpublished study report, 2019), the concern for adverse effects on fertility and male reproductive development were not substantiated. The NOAEL for reproductive toxicity in the F0 generation and developmental toxicity in the F1 generation pups was in the study report determined to be 6000 ppm (highest dose group tested) since there were no toxicologically significant changes observed in the endpoints assessed in the study. The eMSCA agrees with the NOAEL setting, but as discussed in section 7.9.7, several shortcomings related to the study design and performance were identified, which puts some of the observed results into question.

In conclusion, based on the presented results in section 7.9.7, it is concluded that no further information should be requested as the eMSCA considers that the outcome of the requested modified OECD TG 422 study (unpublished study report, 2019) did not substantiate the concern for reproductive toxicity. The eMSCA also does not consider the adverse effects observed to be sufficiently severe to trigger further regulatory action at present.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Environmental toxicity data is not exhaustively reviewed and thus only limited information on this is included in this document.

The modified OECD TG 422 study (unpublished study report, 2019) may indicate some thyroid disruption due to changed thyroid levels and changes in thyroid histopathology in mammals (see section 7.9.4). Given the high degree of conservation among species of the thyroid system, it would be desirable to test for thyroidal modalities in the environment in order to clarify thyroid-disrupting properties of the Substance either for the environment but also since non-mammalian data might support a future human concern. However, the possibilities of testing for thyroid effects in guideline studies are still scarce due to limited sensitivity for thyroid endpoints.

Further testing for thyroidal modalities in the environment and in mammals may be a more reasonable approach once highly advanced tests revealing thyroid disrupting properties and MoAs are developed and validated. The eMSCA thus considers that further testing related to thyroid disruption in the environment would not be appropriate for now.

7.10.2. Endocrine disruption - Human health

In the opinion of the eMSCA there was a concern regarding reproductive toxicity of the Substance, due to adverse developmental effects seen in the one-generation study (unpublished study report, 2005) as described in section 7.9.7. In the course of the evaluation, an additional concern regarding endocrine disrupting properties was identified.

In the adopted decision issued by ECHA on 19 May 2017[4], a modified OECD TG 422 study was requested in order to clarify the identified concerns of anti-androgenic mode of action and thyroid mode of action. Many of the endpoints relevant for endocrine disruption, was also discussed in the previous section regarding toxicity to reproduction (section 7.9.7.)

Anti-androgenic mode of action

A concern for was identified since exposure to anti-androgenic endocrine disruptors during development generally causes effects on the male reproductive system (Kay *et al*., 2014, Howdeshell *et al*., 2008, Wilson *et al*., 2008) that are similar to the effects observed with the Substance (reduced AGD, increased nipple retention, altered sexual maturation and decreased weight of several reproductive organs) (unpublished study report, 2005). Additionally, the Substance is expected to be metabolised to amongst others monoisononyl phthalate and monobenzyl phthalate. These metabolites are also the metabolites of BBP and DINP, phthalates which are both recognized as possessing an anti-androgenic mode of action (ECHA, 2013), primarily related to effects on steroidogenesis. Also, it was demonstrated by Ema *et al*., (2003), that monobenzyl phthalate (CAS RN 2528-16-7) in itself reduces AGD in foetal male rats which further supported the concern for anti-androgenic mode of action of the Substance leading to adverse effects on the developing reproductive system.

Anti-androgenic effects on male reproductive development were not observed in the two-generation reproductive toxicity study (unpublished study report, 2013a). However, due to the lower doses used and the selection of only few investigations relevant to anti-androgenic mode of action, the eMSCA considered that the data from the two-generation reproductive toxicity study did not contradict the anti-androgenic effects observed in the one-generation toxicity study (unpublished study report, 2005).

In order to increase the sensitivity of the Modified OECD TG 422 requested by ECHA, several modifications such as quantitative count of retained nipples/areolas in males were included. Effects on male reproductive organs of prepubertal rats (testis, epididymis, seminal vesicle, ventral prostate, LABC, bulbourethral glands) are known to be sensitive to compounds with an endocrine mode of action, and therefore requested as additional endpoints to the TG 422 study.

However, exposure to the Substance did not substantiate the concern of anti-androgenic activity. AGD and nipple retention were not affected in male or female pubs. Additionally, there was no indication of shorter AGDs in male offspring in higher dose groups compared to control and no tendencies were observed regarding nipple retention. Furthermore, no statistically significant Substance-related changes in reproductive organ weight were seen in offspring on PND 21. A non-significant decrease in the bulbourethral muscle weight was reported in male pups of dams in the 1500 and 6000 ppm dose groups (~18% and ~21% decrease, respectively).

As discussed in section 7.9.7., the eMSCA has identified several shortcomings related to the study design and performance, such as lack of thorough examination of AGD, nipple retention and reproductive organ weight. In the view of the eMSCA, higher doses should have been used as no systemic effects were seen on systemic toxicity (terminal body weight was decreased by 4 % in males and increased by 6% in females). Considering the deficiencies identified, it puts many of the observed results into question, which makes it difficult to conclusively determine the endocrine disrupting potential related to anti-androgenic activity of the Substance. Although this concern is not fully clarified, the eMSCA will not require further information as the requested study was modified to include several sensitive endpoints according to the decision issued by ECHA.

Thyroid mode of action

Thyroid toxicity, e.g. thyroid follicular hyperplasia, has been found for phthalates with backbone lengths of C6-C8 (Bhat *et al*., 2014, Howarth *et al*., 2001, Poon *et al*., 2007, Hinton *et al*., 1986). Also for DIDP, possible effects on thyroid glands have been described (ECHA, 2013). However, it is unclear whether thyroid toxicity is related to phthalates with specific backbone lengths only. The Substance contains constituents with backbone lengths of C7-9 and thus has constituents within the chain length range for which thyroid effects have been observed in other phthalates. Therefore, the eMCSA raised the concern that the

Substance may cause the same type of effects on the thyroid as observed for other structurally related phthalates.

Thyroid glands were weighed in adult F1 offspring in the two-generation reproductive toxicity study (unpublished study report, 2013a) on the Substance, and here a non-significant 10% increase in thyroid weight was seen. Effects on the thyroid glands were not evaluated in the 21-days repeated dose toxicity study unpublished study report, 1999), nor in the non-guideline one-generation reproductive and developmental toxicity study (unpublished study report, 2005) on the Substance.

For the Substance, only limited data were originally available on thyroid toxicity, which could be related to endocrine disruption of the thyroid hormone axis, and the eMSCA considered that further studies on the Substance were needed to elucidate whether this chemical has thyroid disrupting properties.

Following the identification of a concern for thyroid toxicity, measurements of thyroid hormone levels of males, dams and pups (T4 at PND 4 and 21, PND 21 in dams and at termination in males) were justified and included in the study design of the requested modified OECD TG 422 study. Dose-related changes in thyroid hormone levels were measured (increased TSH levels and decreased T4 levels) in the parent animals, but no effects on thyroid gland weight was affected in either parental males or females. Histopathology of the thyroid gland was affected in the high dose group of both sexes. This was seen as an increase in the incidence and severity of follicular cell hypertrophy.

Regarding circulating T4 levels in male and female pups on PND 4 and 21, no effects were reported as well no observed changes in thyroid weight. TSH levels on PND 4 were not affected but on PND 21, TSH levels in male pups were increased in groups administered 1500 ppm or above (1500 ppm: 42% increase, 6000 ppm: 65% increase) and in females, increases in TSH levels were seen in the doses of 375 ppm or above (375 ppm: 21%, 1500 ppm: 48%, 6000 ppm: 39%).

As discussed in section 7.9.7., the eMSCA finds that when all the thyroid data from this study is analysed together, it clearly shows adverse effects on the thyroid hormone system after 65-68 days of exposure to a dose of 6000 ppm. Therefore, the eMSCA does not agree with the conclusion from the study report that the changes were non-adverse and adaptive. The amount of Substance transferred through the maternal milk during the lactation period may be limited (or insufficient to induce adverse effects) which might explain the absence of effects observed in the offspring. In the view of eMSCA, higher doses of the Substance should have been selected for testing in the parental animals in order to properly assess whether adverse effects related to disruption of the HPT axis could be found in the offspring.

According to the study report of the modified OECD TG 422 study, dietary exposure of 1500 ppm was considered the NOAEL for systemic toxicity of the F0 generation. The eMSCA agrees with the NOAEL setting, but finds that the critical effect was thyroid hormone system disrupting effects and not systemic toxicity. The NOAEL for reproductive toxicity in the F0 generation and developmental toxicity in the F1 generation pups was in the study report determined to be 6000 ppm, since there were no toxicologically significant changes observed in the endpoints assessed in the study. The eMSCA agrees with the NOAEL setting, but has identified several shortcomings related to the study design and performance, which puts many of the observed results into question; i.e. lack of thorough examination of offspring T4 levels on PND 4 and disputable interpretations of statistical analyses of parental TSH and T4 concentrations (further discussed in section 7.9.7). Therefore, it is difficult to conclusively determine the endocrine disrupting potential related to thyroid disruption of the Substance, although a substance that induces histopathological changes in the thyroid gland would pose a hazard to human thyroid hormone insufficiency in adults as well as pre- and post-natal neurological development of offspring, according to the ECHA/EFSA guidance on endocrine disruptors.

Further studies investigating in depth the thyroid endpoints would be advantageous but, for now, not reasonable to request as the eMSCA considers that the currently available test designs are associated with limited sensitivity for revealing effects on the HPT axis.

7.10.3. Conclusion on endocrine disrupting properties

In conclusion, based on the available data of the Substance, the most pronounced effect of the Substance is reported on the thyroid system. The Substance seems to have some endocrine disrupting abilities related to thyroid disruption as TSH levels were elevated and in parental animals, T4 levels were lowered.

The concern for thyroid disruption is currently not followed up since the available test designs only cover thyroid endpoints with limited sensitivity. Therefore, the eMSCA does not consider it reasonable to require further testing of the potential thyroid disrupting properties.

Based on the newest *in vivo* data, the Substance does not seem to have other clearly shown activities in the endocrine system at the doses tested. However, in the recent modified OECD TG 422 study the eMSCA identified several deficiencies related to the study design, examinations and performance and unfortunately, the doses selected did not induce general toxicity in the highest dose group. Higher doses should have been employed in order to properly assess the reproductive and developmental toxicity of the Substance. Therefore it is not currently possible to fully exclude the additional concern that the substance is an endocrine disruptor. The OECD TG 422 study did not substantiate the findings on endocrine related endpoints (reduced AGD, increased nipple retention, altered sexual maturation and decreased weight of several reproductive organ weights) as observed in the performed one-generation reproductive and developmental toxicity study with the Substance. Despite the outcomes of the modified OECD TG 422 study, the available *in vivo* data cannot fully remove the concern for endocrine disruption due to indications of disruption of the HPT axis reported in this study. However, the concern for thyroid disruption is currently not followed up since the available test designs only cover thyroid endpoints with limited sensitivity and no tests to further clarify the concern are feasible and proportionate at this point.

7.11. PBT and VPVB assessment

Not evaluated by eMSCA.

7.12. Exposure assessment

Not evaluated by eMSCA.

7.13. Risk characterisation

Not evaluated by eMSCA.

8. References

Table 13

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Title	**Author**	**Publication / source details**	**Year**
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OECD TG 416 *Two-Generation Reproductive Toxicity Evaluation of Santicizer® 261A (S-261A) Administered in the Diet to CD® (Sprague Dawley) Rats*	Confidential	Unpublished study report	2013a
OECD TG 414 *Developmental Toxicity Evaluation of Santicizer 261A (S-261A) Administered in the Diet to CD ® (Sprague Dawley) Rats. Testing laboratory*	Confidential	Unpublished study report	2013b
Modified TG 422 *Modified Oral (Dietary) Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test in the Rat (OECD 422, adopted 29 July 2016 modified according to ECHA Final Decision dated 19 May 2017)*	Confidential	Unpublished study report	2019

Abbreviations

AC	Article category
AGD	Anogenital distance
BBP	Butyl benzyl phthalate (CAS RN 85-68-7, EC number 201-622-7)
BW	Body weight
CCH	Compliance check
CMR	Carcinogenic, mutagenic or toxic to reproduction
CSA	Chemical safety assessment
DEHP	Di(2-ethylhexyl)phthalate (CAS RN 117-81-7, EC number 204-211-0)
DHNUP	1,2-Benzenedicarboxylic acid, di-C7-11-branched and linear alkyl esters (EC 271-084-6, CAS RN 68515-42-4,)
DINP	Diisononylphthalate (EC 249-079-5, CAS RN 28553-12-0,)
DIDP	Di-isodecyl phthalate (primarily C10, EC number 271-091-4, CAS RN 68515-49-1)
ECHA	European Chemicals Agency
ED	Endocrine disruptor
EFSA	European Food Safety Authority
eMSCA	evaluating Member State
ERC	Environmental release category
GD	Gestational day
HPT	Hypothalamic Pituitary Adrenal
LABC	Levator ani/bulbocavernosus muscle
LOAEL	Lowest Observed Adverse Effect Level
MIE	Molecular initiating Event
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PC	Product category
PND	Postnatal day
PPM	Parts Per Million
PPS	Preputial separation
PROC	Process category
RCR	Risk characterisation ratio
T4	Thyroxine
TC CL	Technical Committee on Classification and Labelling
TG	Test guidance
TSH	Thyroid stimulating hormone