SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Bis(2-ethylhexyl)adipate

EC No 203-090-1

CAS No 103-23-1

Evaluating Member State(s): Finland

Dated: 7 July 2021

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2020

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

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

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

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

Bis(2-ethylhexyl)adipate (the Substance) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected CMR (suspected reproductive and developmental toxicity)
- Consumer use
- High (aggregated) tonnage
- Wide dispersive use

During the evaluation endocrine disruption was identified as an additional concern based on the structural similarity to Bis(2-ethylhexyl) phthalate (DEHP) and the structural alerts identified for the adipate structure.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

There are no other on-going processes at EU-level for the Substance.

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 1. below.

Table 1. Conclusion of Substance Evaluation

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

Regarding human health, no hazards have been confirmed (no harmonised or self-classifications for any hazards). Therefore, no exposure assessment and risk characterisation regarding workers and consumers are required (REACH Annex I: 0.6.2./0.6.3.).

The eMSCA notes that exposure assessment of the Substance should be updated in case the available data warrants hazard classification of the Substance.

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

The eMSCA recommends that a proposal for harmonised classification and labelling should be done to reach a conclusion on the need for a harmonised classification for the reproductive toxicity endpoint.

Death of the developing organism as well as structural abnormalities are considered major manifestations of adverse effects on development. The findings of increased skeletal and visceral abnormalities, increased pre- and post-implantation loss and late intrauterine deaths raise a concern in the eMSCA. The findings of the GLP compliant PNDT study (OECD TG 414) in rats showed effects that are not necessarily attributable to secondary effects of maternal toxicity and may indicate treatment related adversity in the developing fetus.

Consequently, the eMSCA recommends an assessment of the impact of the findings in reproductive and developmental toxicity studies on classification for reproduction and development in line with CLP criteria (CLP Annex I). It should focus on:

- Adverse effects on sexual function and fertility in adult males and females and
- Adverse effects on development of the offspring

It is worth noting that at the registered tonnage band of the Substance (Annex X of REACH) an extended one generation toxicity study (EOGRTS) is a standard information requirement. However, neither an EOGRTS nor a two-generation reproductive toxicity study with the Substance are available in the registration dossier.

The eMSCA recognises that this standard information cannot be requested via the current Substance Evaluation. Therefore, we recommend ECHA to request this study under a Compliance Check.

Ideally, this data would be available in the dataset before any potential proposal for harmonised classification for the Substance (if still deemed necessary upon information availability).

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

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

Not applicable.

5.2. Other actions

Under Compliance check (Decision on 14 March 2013) a PNDT study (OECD TG 414) in the second species (rabbit) was requested. The request is related to the identified concerns.

In the course of the substance evaluation, the eMSCA identified a data gap in the standard information requirements. This data gap is related to the identified concern. A compliance

check should be initiated (under Article 41 of REACH), to request an EOGRTS which is a standard information requirement at Annex X of REACH.

The eMSCA notes that exposure assessment of the Substance should be updated in case relevant new experimental information becomes available warranting hazard classification of the Substance.

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

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

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Compliance check	tbd	ECHA
CLH	tbd	FI CA

Part B. Substance evaluation

7. EVALUATION REPORT

This evaluation report focuses primarily on the potential reproductive and developmental toxicity, which was initially identified as a potential concern for the Substance. In addition, the eMSCA identified endocrine disruption as an additional concern during the evaluation. These properties were identified as potential concerns because of the similarities to phthalate plasticisers and because the Substance is widely used as a replacement plasticer for phthalates, i.e., the exposure is expected to be widespread consumer exposure. There have also been some findings of adverse effects from toxicological studies in animals.

The experimental data included in the registration dossier for reproductive and developmental toxicity, endocrine disruption and for repeated dose toxicity were evaluated. Additionally, publicly available literature data, structure-based and e.g. ToxCast screening information were utilised.

The available information was used as the basis for the hazard assessment of the potential of the Substance to produce adverse effects on reproductive and developmental endpoints and reflected against the classification criteria of the CLP regulation for these properties.

No concerns analogous with the endocrine disruptive effects of well-known phthalates were identified with respect to the similarity in structure nor was the Substance found to have (anti)androgenic properties characteristic for phthalates. Therefore, the concern based on structural similarity with phthalates e.g. Bis(2-ethylhexyl)phthalate (DEHP) is clarified.

During the evaluation of the available information related to reproductive and developmental toxicity, a true effect of the Substance on reproduction and development could not be excluded.

Therefore, a concern for this endpoint remains.

The eMSCA does not request additional studies at this time under substance evaluation but recommends:

- Performing a compliance check to address the endpoint for reproductive and developmental toxicity,
- Drafting of a CLH proposal.

7.1. Overview of the substance evaluation performed

The Substance was originally selected for substance evaluation in order to clarify concerns about:

- Suspected CMR (suspected reproductive and developmental toxicity)

- Wide dispersive use

- Consumer use

- High (aggregated) tonnage

During the evaluation endocrine disruption was identified as an additional concern based on the structural similarity to Bis(2-ethylhexyl) phthalate (DEHP) and the structural alerts identified for the adipate structure.

Table 2. Evaluated endpoints

EVALUATED ENDPOINTS Endpoint evaluated	Outcome/conclusion
Reproductive and developmental toxicity	Concern confirmed. Further action required to clarify the hazard.
Exposure-related concerns	No hazards have been confirmed (no harmonised or self-classifications for any hazards). Therefore, no exposure assessment and risk characterisation regarding workers and consumers are needed (Annex I: 0.6.2./0.6.3.). The eMSCA notes that exposure assessment of the Substance should be updated in case the available data warrants hazard classification of the Substance.
Endocrine disrupting properties	Concern refuted. No further action.

7.2. Procedure

Reproductive and developmental toxicity, and the additional concern identified during the evaluation related endocrine disruption were evaluated by the eMSCA. Repeated dose toxicity was not evaluated unless the studies were combined with reproductive screening or offered value to the evaluation of the above-mentioned endpoints. The evaluation focused on the information provided in the registration dossier for the Substance as well as additional publicly available data. No additional endpoints were evaluated.

The Substance has been subject to a Compliance Check by ECHA under Article 41(3) of REACH in 2013. A pre-natal developmental toxicity study in rabbits via the oral route was requested according to standard data requirements for the applicable tonnage band with a deadline for submission of an updated dossier with the requested data in 2014. The information was submitted by the deadline.

The eMSCA initially selected the Substance as a candidate for inclusion to the CoRAP list in 2013 based on Article 45(5) of the REACH Regulation ((EC) No 1907/2006). The CoRAP evaluation was carried out from March 2020 until March 2021. The eMSCA identified a need to pursue further actions and conclude the Substance Evaluation with the recommendation for the drafting of a proposal for harmonised classification according to CLP, Annex I. The eMSCA further recommends opening a compliance check to address the reproductive toxicity endpoint.

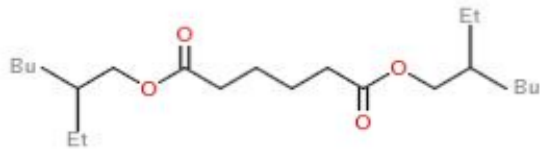
7.3. Identity of the substance

Table 3. Substance identity

SUBSTANCE IDENTITY	
Public name:	Bis(2-ethylhexyl) adipate
EC number:	203-090-1
CAS number:	103-23-1
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	C22H42O4
Molecular weight range:	370.6 g/mol
Synonyms:	1,6-bis(2-ethylhexyl) hexanedioate

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

Structural formula: **Figure 1. Structure of bis(2-ethylhexyl) adipate**



7.4. Physico-chemical properties

Table 4. Overview of physicochemical properties

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid
Vapour pressure	0.0000003 hPa at 20°C
Water solubility	0.0032 mg/l at 22 °C
Partition coefficient n-octanol/water (Log Kow)	8.94 at 25° C
Flammability	Not flammable
Explosive properties	Not explosive
Oxidising properties	Not oxidising
Granulometry	Not applicable
Stability in organic solvents and identity of relevant degradation products	No data available
Dissociation constant	Not applicable
Viscosity	13.7 mPa*s at 20 °C

7.5. Manufacture and uses

7.5.1. Quantities

Table 5. Aggregated tonnage per year

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

Table 6. Overview of reported uses per lifecycle stage

USES	Use(s)
Uses as intermediate	Use as an intermediate in the manufacture of chemicals and plastic products
Formulation	Formulation in open and closed systems of plastisols, plasticisers, lubricants and their additives, greases, PVC articles, fragrances, rubber products, tyres, inks, blending into solid matrices, cleaning and maintenance products, metalworking fluids, laboratory chemicals, medical devices, polymer preparations and compounds, paper and board treatment chemicals, construction chemicals, resins, water treatment chemicals and cosmetic products
Uses at industrial sites	Industrial use in open and closed systems for cleaning and maintenance, use as a processing aid/auxiliary chemical in cleaning uses, oil and gas field operations, lubricant use in open high temperature processes, use in the manufacture of other substances and products, industrial use of inks and coatings, metalworking fluids, functional fluids, production of electrical circuits, as a plasticiser and additive in the production of articles and polymer preparations, laboratory use, low energy manipulation of articles and matrices, use in mining operations, use as a fuel, adhesives and sealants.
Uses by professional workers	Uses in medical devices, in washing and cleaning (including high pressure processes), spraying, wiping, dipping, pouring and brushing applications, as a process aid in the use and manufacture of explosives, water treatment chemicals, low energy manipulation of polymer preparations, outdoor and indoor construction chemical use, stainless steel care products, lubricants waxes and greases, automatic laundry and dishwashing aid, application of coatings, adhesives and inks, oil and gas field drilling operations, furniture care, use of plastisols, laboratory use, fuel and fuel additive use, functional fluids, use in highspeed machinery, de-icing operations, use in plant protection/agrochemical products, hand/skin cleaners and disinfectants, animal care products, manufacture of cosmetic and personal care products, use of solvents, plastics and in welding-like operations.
Consumer Uses	Uses in fuels and as fuel additive, washing and cleaning products, automotive care products, lubricants, coatings, waxes, greases, plant protection and other agrochemical products, building and construction preparations, adhesives/sealants, paints, thinners and paint removers, fillers, putties, plasters, modelling clay, finger paints, polymer preparations, de-icing preparations, solvents, polishes, biocides, inks, cosmetics, functional fluids, air care products and medical devices
Article service life	Service life of plastic, rubber, paper, fabric, leather and textile articles, vehicles, machinery, mechanical appliances, electrical/electronic articles, packaging material for metal parts, greases and corrosion inhibitors, medical devices, lubricants and their additives, waxes, building materials, explosive articles and inks and coatings

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

There is currently no harmonised classification for the Substance.

7.6.2. Self-classification

- In the registration(s)*:*

The Substance is not classified for any hazardous properties in the registration dossier.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

The majority of notifiers do not classify the Substance. However, some self-classifications are notified among the aggregated self-classifications in the C&L inventory:

- Aquatic Acute 1 (H400)
- Aquatic Chronic 1 (H410), 2 (H411) and 4 (H413)
- Skin Irritation 2 (H315)
- Eye Irritation 2 (H319)
- Acute Toxicity 4 (H302 or H332)
- Carcinogenic 2 (H351)
- Reproductive Toxicity 2 (H361)

7.7. Environmental fate properties

Not evaluated.

7.8. Environmental hazard assessment

Not evaluated.

7.9. Human Health hazard assessment

The initial grounds for concern justifying the selection of the Substance for evaluation under CoRAP were wide dispersive use including consumer use, high aggregated tonnage as well as potential effects on reproduction, more specifically on the male reproductive system.

The Substance shares structural similarities as well as a similar metabolic pathway with DEHP, a substance causing adverse effects to reproduction with an anti-androgenic mode of action. The eMSCA wished to evaluate whether the data included in the registration dossier is robust enough for the evaluation of the reproductive toxicity endpoint.

The need for harmonised classification and labelling of the Substance is also evaluated for the reproductive toxicity endpoint.

7.9.1. Toxicokinetics

Not evaluated

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated

7.9.3. Sensitisation

Not evaluated

7.9.4. Repeated dose toxicity

Not evaluated

7.9.5. Mutagenicity

Not evaluated

7.9.6. Carcinogenicity

Not evaluated

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

Similarity to phthalates

The structural similarity between the Substance and the four phthalates subject to authorisation (DEHP, benzyl butyl phthalate (BBP), dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP)) is limited to the di-carboxylic acid structure present in both. The Substance possesses an ester moiety of 4 carbons in place of the alkylphenol structure characteristic to the aforementioned phthalates. The Substance does not possess the phenolic ring structure present in the phthalates, which gives rise to their estrogen and androgen receptor binding potential (Shengde, et al., 2013).

Both the Substance and DEHP share a common hydrolysis product of metabolism, ethylhexanol (2-EH). The effects of 2-EH have been evaluated in a Substance Evaluation by another Member State in 2007 (ECHA, 2017). The evaluation concluded that there is no indication that 2-EH would adversely affect fertility and sexual function nor is it considered responsible for causing effects on male fertility and reproduction typical for DEHP nor developmental effects.

Structural alerts of the Substance

The di-carboxylic acid structure of adipates has been associated with developmental effects (Shengde et al., 2013). The OECD QSAR Toolbox (v.4.2) DART Scheme v.1.0 gave a positive alert for the Substance based on the di-carboxylic acid derivatives structure (adipates). Adipic acid esters with a core structure of adipic acid (Figure 2) are the basis for the alert. The training set is further described in the below table. Adipic acid (EC No. 204-673-3) does not have a harmonised classification nor any self-classifications in ECHA's C&L inventory for reproductive toxicity or other chronic effects for human health. The database on which the structural alert for the Substance is based includes two publications focusing on structural features associated with developmental and reproductive effects as well as an EPA IRIS assessment.

The basis of the EPA IRIS assessment conclusions for these endpoints are the effects seen in a prenatal developmental toxicity (PNDT) study with the Substance (Unpublished study, 1988b) and effects observed in pups in a one-generation study (Unpublished study, 1988a). Extrapolation within the group of adipate structures is included as a basis for the alert. The seven substances included in the category are described in Table 7. below. The alert for developmental effects for other members of the adipic acid category appear to be based on the findings with the Substance described in the IRIS assessment as well as structural similarity to other cyclic and more relevantly acyclic carboxylic acids.

Figure 2. The scope of structural features of adipic acid esters, alert 22d of the DART Scheme (taken from OECD QSAR Toolbox v4.2)

Table 7. Training set of substances in the DART Scheme subcategory 22d

Substance	CAS RN	Reproductive toxicity	Developmental toxicity	Database
Dimethyl adipate	627-93-0	No relevant studies identified	Spectrum of developmental	Reference to teratogenic

			toxicity endpoints observed include structural malformations	potential of cyclic and acyclic carboxylic acids*
Diethyl adipate	141-28-6	No relevant studies identified	Spectrum of developmental toxicity endpoints observed include structural malformations	Reference to teratogenic potential of cyclic and acyclic carboxylic acids*
Dipropyl adipate	106-19-4	No relevant studies identified	Spectrum of developmental toxicity endpoints observed include structural malformations	Reference to teratogenic potential of cyclic and acyclic carboxylic acids*
Dibutyl adipate	105-99-7	No relevant studies identified	Spectrum of developmental toxicity endpoints observed include structural malformations	Reference to teratogenic potential of cyclic and acyclic carboxylic acids*
Diisobutyl adipate	141-04-8	No relevant studies identified	Spectrum of developmental toxicity endpoints observed include structural malformations	Reference to teratogenic potential of cyclic and acyclic carboxylic acids*
Bis(2-ethylhexyl) adipate (DEHA); i.e. the Substance	103-23-1	No relevant studies identified	Spectrum of developmental toxicity endpoints observed include structural malformations	EPA IRIS assessment (EPA, 1992): developmental toxicity database consisting of one 1-generation study and one teratogenicity study from 1988 both with rats**
Adipic acid	124-04-9	No relevant studies identified	No relevant studies identified	None

*Narotsky, M. et al, 1994; Di Carlo, F., 1990

** These studies are both included in the dataset for the current Substance Evaluation.

Of the training set of substances, REACH registration data exists in ECHA's registered substance database for the Substance, diisobutyl adipate and adipic acid. Data from dimethyl glutarate is used to read across to dimethyl adipate. For dipropyl adipate, diethyl adipate and dibutyl adipate there are no registration or no relevant experimental data available. For diisobutyl adipate only a screening level study is available with a NOAEL in pups of 300 mg/kg based on low pup weight and increased mortality at PND 4 in the high dose group (1000 mg/kg bw/day).

Effects in the one-generation study with the Substance included a decreased total mean litter weight and decreased pup weight gain and resulting in a NOAEL of 170 mg/kg bw/day for development. Several whole litter losses also occurred in treated groups, though without a clear trend in dose-response. The findings were however not reported as conclusive of developmental toxicity as adverse effects at the same dose level were also seen in parental animals; a NOAEL for systemic toxicity of 170 mg/kg bw/day was derived based on increased liver weights and reduced body weight gain in parental females.

In the PNDT study, some skeletal and visceral abnormalities were observed in the ureters and slightly reduced ossification in pups of the mid- and high-dose groups in the absence of significant maternal toxicity. In addition, there was an increased pre- and postnatal loss rate and two late intrauterine deaths in the high dose group. The effects were considered

by the Registrant as unrelated to the treatment. The findings from the two studies with the Substance are further described and discussed in the following sections of this report. In conclusion, the categorical alert for all adipate structures in the DART scheme does not appear to provide a strong basis to substantiate a definitive conclusion on the developmental toxicity of the Substance as such nor for the group of adipates as a whole.

7.9.7.1. Effects on reproduction and fertility

Similarities between the Substance and DEHP have been proposed as discussed above. However, similar findings characteristic to 'phthalate syndrome' as seen with DEHP such as antiandrogenic activity characterised by testicular toxicity, reduced semen quality, reduced fertility, decreased anogenital distance (AGD), nipple retention, hypospadias and cryptorchidism are not observed with studies in the available database with the Substance. Findings from *in vitro* assays studying (anti)androgenicity as well as other assays for endocrine disruption are summarised and discussed in section 7.10.2.

One-generation study (Unpublished study, 1988a)

A one-generation study (Unpublished study, 1988a) was conducted under GLP. The study followed no guideline but can be considered equivalent to OECD Guideline 415. In this study 15 male and 30 female Wistar rats per group were fed with 99% pure Substance in the diet from 10 weeks before mating and through mating (males) or until day 22 postpartum (females) at doses of 28, 170 and 1080 mg/kg bw/day (300, 1800 or 12 000 ppm). After 10 weeks the animals were mated to produce a single litter, which were reared to day 36 post-partum.

Effects in parents

Body weight gain and body weight were slightly (~9%) reduced in the females of the high dose group. There was no effect on either male or female fertility, gestation length or pre-coital interval in any dose group. Absolute and relative liver weights were increased by 18-20% in the 12000 ppm group. No microscopic changes were detected in the reproductive tract of the parents.

Effects in pups

There were four whole litter losses, but none in the control group. One litter was lost in the 300 ppm group, two in the 1800 ppm group and one in the 12000 ppm group. The litter losses were considered unrelated to the treatment by the study author and the losses had no dose response. Mean pup weight and total litter weight were reduced during post-partum days 1-36 in the high dose group. Also, the mean litter size was slightly reduced in the high dose group. Excluding the litter losses there was no effect on survival. No macroscopic changes were detected in the pups up to 18 days old.

The study concluded that DEHA had no adverse effect on rat fertility. The eMSCA agrees with this conclusion.

Ovarian toxicity in repeated dose studies (Wato, 2009)

Ovarian toxicity was studied in 2- and 4-week repeated-dose studies (and in a fertility study) in rats (Wato, 2009). Histopathological examination of ovaries of groups of 10 female SD rats dosed by gavage with 200, 1000 and 2000 mg/kg bw/day was performed in the study.

Mean estrous cycle length was statistically significantly reduced (-16.3%, $p<0.05$) in the 200 mg/kg bw/day animals of the 4-week study compared to controls (4.2 versus 4.9 days). However, the estrous cycle lengths of the 200 mg/kg bw/day animals of the 4-week study are comparable to those of control animals used in the 2-week study, where the mean estrous cycle length was 4.2 days. No changes in the estrous cycle lengths of the females in either study were observed in the other dose groups. The eMSCA considers that it could be useful to evaluate the significance of the finding by comparison to historical control data due to the small group size used in the study. In contrast to the lower estrous cycle length of low dose animals in the 4-week study, the mean estrous cycle length of the

2000 mg/kg animals of the 4-week study was increased by a mean of 1 day compared to controls (corresponding to 20%) with a high intergroup variability (+/-1.9 days) and no statistical significance. There was no change in estrous cycle length at 1000 mg/kg. No dose response relationship was seen for effects in the estrous cycle lengths and in the absence of individual animal data, it is not possible to evaluate the possible effect of outliers.

Relative ovary weights were reduced by 25.6% compared to controls in the 2000 mg/kg bw/day group of the 2-week study. The finding was not dose-dependent and occurred only in the high-dose group. There were no similar findings in the 4-week study. There was no reduction in body weight observed that would correlate with reduced ovary weights. Absolute ovary weights were not provided in the publication. Increased mild and moderate large follicular atresia was noted in the histopathological examination of animals dosed at 1000 mg/kg bw/day and above in both studies in a dose- and duration dependent manner. Additionally, follicular cysts were observed in the high dose group of the 2-week study and the 1000 mg/kg and above groups of the 4-week study. A marked decrease in formed corpus luteum was observed at 1000 mg/kg and above in both studies.

Taken together, the reduced ovary weights, dose- and time-dependent increase in follicular atresia and follicular cysts as well as decreased currently formed corpus luteum can be regarded as treatment related findings. The significance of these findings with regard to fertility and reproductive outcomes were evaluated in the fertility study accompanying the repeated dose studies by Wato et al. (2009). The non-guideline fertility study can be compared to a screening level study but is not methodologically comparable to the OECD TG 421 reproductive/developmental toxicity screening study nor the OECD TG 414 PNDT study. The fertility study utilised dose levels of 200, 1000 and 2000 mg/kg bw/day by gavage to 10 female SD rats per dose group. Females were dosed at 10 weeks of age for 2 weeks prior to mating with untreated males, throughout mating until GD 7. Females were sacrificed on GD 14 and uterine contents as well as reproductive organs were examined macroscopically and microscopically.

Body weights of the high dose animals at day 15 of treatment were significantly lower than controls (269g +/- 19 and 245g +/-14, $p<0.01$) with a slight but statistically significant ($p<0.05$) decreased food consumption at day 7 of treatment in high dose females. Findings from the fertility study included an implantation rate decrease (68.9 +/- 19.5% compared to 91.7 +/- 9.3% in controls) in the high dose group. Post-implantation loss rate was also increased from 1000 mg/kg and above (4.5- and 3.5-fold respectively, although with high variability in the 1000 mg/kg group). Pre-implantation loss rate was significantly increased ($p<0.01$) in the high dose animals at 31.1 +/- 19.5% (compared with 8.3 +/- 9.3% in controls). A decrease of 22% in number of live embryos was observed in the high dose group compared to controls with a high group variability in values (+3,3% to -47.4%). The fertility index of high dose females was reduced to 80% corresponding to 8/10 pregnant females. All other dose groups achieved a fertility index of 100%. Individual litter data would be necessary to evaluate whether the effect occurred in multiple or single litters. Mean estrous cycle length was statistically significantly increased ($p<0.05$ and $p<0.01$ respectively) in the fertility study at 1000 mg/kg (+4.4%) and 2000 mg/kg (+17.4%) compared to control. This finding is in contrast with the reduction of estrous cycle length seen in the 4-week study at 200 mg/kg bw/day which may be attributable to outliers rather than a true effect on estrous cyclicity.

Subacute oral toxicity- enhanced OECD TG 407 (Miyata, 2006)

Similar findings of follicular atresia and disturbance of the estrous cycle as found by Wato et al. (2009) were also observed in a 28-day oral toxicity study in rats. The study was performed according to the 1999 draft protocol for an enhanced OECD TG 407 by Miyata and colleagues (2006). In this study, 10 male and 10 female SD rats per group were dosed via oral gavage at 40, 200 and 1000 mg/kg bw/day for 28 days. No effects on hormone levels or spermatological parameters (sperm morphology and sperm count) were found. The estrous stage of the estrous cycle was prolonged to 4 and 10 days (until the day of sacrifice) in two females of the high dose group. The prolonged stage of estrous observed is commonly attributable to aged acyclic rats and is not a common finding in young adult

animals usually utilised in the OECD TG 407 (OECD, 2009). The age of the SD rats used in the study was not stated in the publication. However, it may be assumed that the protocol was followed, and young adult animals were used as the finding was limited to two animals in the study. An increase in ovarian follicular atresia was noted in 4 animals of the high dose group, including those animals displaying prolonged estrous. Serum thyroid stimulating hormone (TSH), thyroxin (T4), triiodothyronine (T3), testosterone, follicle-stimulating hormone (FSH), luteinising hormone (LH), and estradiol levels were unaltered by the treatment. It is not possible to evaluate the potential effect of the observed extended estrous stage on ovulation and fertility in the OECD TG 407 study. An increase in premature follicular degeneration could be expected to correlate with increased irregular estrous cycles and a decrease in successful ovulation manifested in reproduction studies as longer duration to successful mating and lower fertility index.

In the one-generation study similar to OECD TG 415 (Unpublished study, 1988a) described above, where animals were dosed for a 10-week pre-mating period neither female fertility nor pre-coital interval were affected. Furthermore, no microscopic changes were observed in the reproductive organs.

Prenatal developmental toxicity in rats (effects to fertility)

In the PNDT study (unpublished study, 1988b) with Wistar derived rats the number of females (24 mated females per dose group were used) with live fetuses at termination and mean number of corpora lutea were unchanged by treatment at all dose levels (up to 1080 mg/kg bw/day). Pre-implantation loss was slightly increased in the high dose group at 19.1% compared to 13.5% in controls. A small non statistically significant increase in post-implantation loss as well as two late intrauterine deaths (both findings in the high dose group) also occurred in treated animals.

Prenatal developmental toxicity in rabbits (effects to fertility)

No effects on maternal reproductive or fertility parameters observed at doses up to 160 mg/kg bw/day.

Conclusions on fertility and reproduction

Although no effects on fertility were seen in the one-generation reproductive toxicity study (Unpublished study, 1988a), other studies (Miyata, 2007 and Wato, 2009) showed effects on the rat ovaries, such as follicular atresia as well as irregularities in the oestrus cycle length. One contributing factor to the inconsistent findings may be the use of different rat strains; while the one-generation study used Wistar rats, the two repeated dose studies utilised Sprague-Dawley rats.

In the non-guideline fertility study by Wato (2009) with rats, pre-implantation loss rate was significantly increased ($p<0.01$) in the high dose (2000 mg/kg bw/day) animals at 31.1 +/- 19.5% (compared with 8.3 +/- 9.3% in controls). Implantation rate was also decreased (68.9 +/- 19.5% compared to 91.7 +/- 9.3% in controls) in the high dose group.

These findings are not supported by those of the one-generation study (Unpublished study, 1988a) where animals were dosed for a 10-week pre-mating period and neither female fertility parameters nor pre-coital interval were affected.

Fertility seems to be unaffected by treatment with the Substance.

7.9.7.2. Effects on development

Developmental toxicity in rats

In a GLP compliant rat PNDT study (Unpublished study, 1988b) comparable to OECD TG 414 groups of 24 mated female Wistar derived Alpk:APfSD rats were fed diets containing 0, 300, 1800 or 12000 ppm of the Substance (corresponding to achieved doses of approximately 28 mg/kg, 170 mg/kg and 1080 mg/kg) from days 1-22 of gestation. The females were killed on day 22 of gestation and their uteri were examined for live fetuses and intrauterine deaths. The fetuses were examined for external abnormalities, sexed, eviscerated and subjected to skeletal examination. Blood samples were not collected.

At the highest dose there was a small but statistically significant change (p=0.01) in the body weight gain of maternal animals, particularly at the beginning of dosing from days 1-3 (5.2 grams on day 3 compared to 12.6 grams in controls). The rate of weight gain in dams was statistically significantly lower than that in controls for the remainder of the study (p=0.01). The high dose dams failed to reach maternal bodyweight gain rates comparable with the control, low- and mid-dose animals by the end of dosing, although the final total weights of high dose dams on day 22 (382.8g corresponding to a 33.9% increase) was not statistically significantly different from the control or low- and mid-dose groups at the end of the dosing period ((396.9g corresponding to a 37.3% increase in controls), 386.6g and 401.9g, respectively). A statistically significant reduction in food consumption in high dose dams from days 2-18 was seen with a corresponding statistically significantly lower total food consumption of 565g compared to 603.5g in controls as well as a reduction in food consumption. There was no indication of maternal toxicity in the lower doses and the number of females with live fetuses at termination and mean number of corpora lutea were unchanged by treatment at any dose level. Pre-implantation loss was slightly increased in the high dose group at 19.1% compared to 13.5% in controls. This change was not dose-dependent or statistically significant. A dose-dependent but not statistically significant increase in accentuated reticular pattern was noted in the macroscopic examination (0, 2, 3 and 4 dams in the control, low-, mid-, and high-dose groups, respectively). No microscopic examination of organs is required by the guideline for dams.

There was no significant effect at any dose on fetal weight, litter weight, gravid uterus weight or number of external abnormalities. Two late intrauterine deaths occurred in two separate dams of the high dose group (0 in controls). This was not statistically significant nor considered treatment related in the study. The number of fetuses showing variants was significantly increased in the high dose group (32.1% of pups compared to 24.5% in controls). Six major abnormalities (in five fetuses) were seen in treated groups (Table 8) and eight in control animals (of which seven consisted of multiple minor skull defects in one litter). There was an increase in visceral abnormalities in the mid- and high dose groups; kinked ureters in 1800 and 12000 ppm group and dilated ureter in the 12000 ppm group. These findings were within historical control range and were considered unrelated to the treatment. However, when combined they showed a slight dose-dependent increase. More major defects were observed in controls than in treated animals in total. However, the number of fetuses with minor defects (Table 9) increased in a dose-dependent manner (24.8, 29.7, 34.9 and 49.4% in controls, low-, mid- and high dose groups respectively. The increase was statistically significantly higher (p<0.01) in the mid- and high dose groups. Skeletal variants and pes score were increased in the high dose group only; with slightly poorer ossification at several sites in pups of the 1800 and 12000 ppm groups. These included partial ossification or unossified cervical vertebrae, partially ossified occipital and unilateral parietals, bipartite sternebrae, thoracic vertebrae and transverse processes and unossified odontoid and calcaenum. Partial ossification of the parietals of the skull was dose-dependently increased with treatment (1800ppm and 12000ppm). Non ossified centra of the 3rd-7th cervical vertebrae were also higher in the mid- and high dose groups.

Table 8. Summary of numbers of fetuses showing major fetal defects in the teratogenicity study

Nature of defect	Control incidence	300 ppm	1800 ppm	12000 ppm
Major skeletal				
3rd and 7th (left) ribs not ossified				1 pup
Several minor skull defects	7 pups of one litter			
1st rib (right) partially ossified			1	

Total major skeletal defects	**2.5%**	**0**	**0**	**0.4%**
Major external and visceral				
Situs inversus totalis				1 pup
Left adrenal, kidney and ureter absent	1 pup			
Umbilical hernia				1 pup
Small right kidney		1		
Cyst attached to liver		1		
Total major external and visceral defects	**0.4%**	**0.8%**	**0**	**0.8%**

Table 9. Summary of minor fetal defects and variants in the teratogenicity study

Nature of defect	% control incidence	300 ppm	1800 ppm	12000 ppm
Total minor external and visceral defects	2.5%	3%	3.2%	1.2%
Total minor external and visceral variants	24.5%	26.2%	29.1%	32.1%*
Total minor skeletal defects	24.8%**	29.7%	34.9%*	49.4%**
Total skeletal variants	95.7%	97.7%	96.4%	100%**

* statistical significance to $p<0.05$
** statistical significance to $p<0.01$

The slight increase in increased pre-implantation loss (19.1% and 13.5% in controls) in the high dose group receiving 12000 ppm of the Substance in diet although not statistically significant could be considered treatment related as the value does not fall within historical control values of the laboratory. The post-implantation loss rate observed in treated animals (5.8% in the high dose animals and 4.1% in controls) was not remarkable and is within historical control range. However, these findings of pre- and post-implantation loss combined with the 2 late intrauterine deaths observed only in the high dose group could be indicative of a treatment related effect. In conclusion, the Substance induced slight fetotoxicity in the two highest dose groups but also some slight maternal toxicity at the highest dose. The NOEL for developmental effects was determined to be 300 ppm.

Together with the visceral variants of the ureter, the total skeletal defects and variants indicate slight fetotoxicity but may partly be related to a slight maternal toxicity at the highest dose level. The maternal effects in the high dose group (1080 mg/kg bw/day) were slight and not indicative of severe toxicity (slight but statistically significant reduction in bodyweight gain during gestation and slightly decreased food consumption). However, some degree of delayed and incomplete ossification may be secondary to maternal toxicity.

Developmental toxicity in rabbits

In a GLP compliant PNDT study according to OECD TG 414 (Unpublished Study, 2014) New Zealand white rabbits were investigated by exposing groups of 22, 27, 23 or 21 mated rabbits to the Substance in feed. The respective groups were control, 40, 80 and 160 mg/kg. The dose levels were selected based on a range finding study where the rabbits

exhibited considerable toxicity; at 300 mg/kg two females were killed in extremis and at 1000 mg/kg one female showed an abortion.

In the main study, all animals surviving to day 29 post-coitum were subjected to an examination postmortem and external, thoracic and abdominal findings were recorded. A laparohysterectomy was performed on the surviving females of the group. The uteri, placentae and ovaries were examined and the numbers of fetuses, early resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded and corrected body weights (changes) were calculated. The fetuses were weighed and examined for external and visceral malformations and variations and sexed by internal examination. Soft cephalic tissue examinations were done for about half of the fetuses of all groups and skeletal examination for all fetuses.

No maternal toxicity was observed in any of the dose groups. Average compound intake was 36 mg/kg, 70 mg/kg and 145 mg/kg.

No developmental toxicity was observed in the 40, 80 or 160 mg/kg bw/day groups. There were no effects on the external, visceral or skeletal fetal morphology up to 160 mg/kg bw/day. The NOAEL for developmental toxicity was established as being at least 160 mg/kg bw/day which corresponds to 145 mg/kg bw/day based on the average food consumption.

Investigation of antiandrogenicity following pre- and postnatal exposure (Dalgaard, 2003)

Antiandrogenic effects of the Substance during the pre- and postnatal period were screened *in vivo* by Dalgaard et al. (2003) following some elements of the basic study design of the draft OECD TG 426. The study included a dose-range finding study of 8 pregnant Wistar rats per group dosed by gavage at 0, 800 and 1200 mg/kg bw/day from GD 7 to day 17 after parturition and a main study with 20 dams per group dosed at 0, 200, 400 and 800 mg/kg bw/day also from GD 7 to 17 postpartum. The high dose of 800 mg/kg was selected following significantly decreased (-25% compared to controls) maternal weight gain during GD 7-21 as well as increased perinatal and postnatal death at 1200 mg/kg in the range-finding study. One male and one female pup from each litter in the main study were retained after weaning, and sexual maturation, hormone and sperm analysis and histopathology at adulthood were investigated. The remaining pups were sacrificed at PND 21. Functional, behavioural, neuropathological and brain weight investigations according to the test guideline were not included in the study. Litter numbers per dose group were slightly smaller than those recommended by the TG (15-18 versus the recommended 20 per group). Gestation time was slightly but statistically significantly increased at 1200 mg/kg (23.2 +/- 0.4 versus 22.4 +/- 0.5 in controls) in the dose-range finding (DRF) study. A decrease in maternal body weight gain from GD 7-21 at 1200 mg/kg was found in the DRF study, but postpartum body weights were similar between groups. The lower gestational body weights followed by normal postpartum body weights compared to controls could be explained by the significantly lower birth weights of pups at 1200 mg/kg (-10.6% in males and -18.6% in females compared to control group pup birth weights). Lower birth weights of -7.8% (males) and -12.1% (females) was seen also in mid-dose pups (800 mg/kg) compared to controls, showing a dose-response although not statistically significant at the mid-dose level. Persisting lower pup weights were also recorded in the 800 and 1200 mg/kg groups of the DRF study on PND 3 (19.4% (m) and 18.9% (f) lower than controls at 800 mg/kg and increasing to 36.6% (m) and 34.4% (f) compared to controls at 1200 mg/kg). Perinatal loss followed a dose-response and was significantly increased by 42% at 1200 mg/kg albeit with a very high standard deviation of +/- 45.6%. At 800 mg/kg a perinatal loss of 15.1% was observed versus 4.6% in controls. Perinatal loss is defined in the study as the number of implantations minus the number of live pups at PND 13 divided by the number of implantations. Hence, also some postnatal deaths are potentially included in these figures additional to post-implantation loss and early neonatal mortality. Raw data is not available and potentially affected single litters are not discussed in the paper. The increase seen at 1200 mg/kg is nevertheless significant. None of the maternal animals displayed clinical signs in the study. Gestation time was significantly increased in the main study by an average of 1 day compared to control at 800 mg/kg (23.2 days versus 22.3 days). This finding is in line with the slightly

increased gestation time observed in the range-finding study at 1200 mg/kg although without a dose-response relationship.

In the main study, pup birth weights were slightly reduced at 800 mg/kg (-3.1% (m) and -4.2% (f)) and remained lower than controls on PND 3 (-19.1% (m) and -16.7% (f)) and PND 13 (-14.6% (m) and -14.1% (f) compared to controls). The body weights of adult F1 males (PND 21) of the high dose group was 11.4% lower than controls. Perinatal loss was increased from 11.2% (+/- 9.7%) in controls to 23% (+/-27.2%) at 400 mg/kg and 22.7% (+/- 26.2%) at 800 mg/kg. The increase was not statistically significant; however, the mean number of losses was numerically twice that seen in control in the mid- and high dose animals. The mean perinatal loss values have high standard deviations, indicating high variability between dams/litters. It is however not possible to evaluate whether the increase was due to single litters from the presented data. The perinatal loss values were calculated in the study including all losses up to PND 13 which potentially captures some of the postnatal death figures in addition to post-implantation losses, uterine deaths and early postpartum deaths of pups. A dose-related increase in postnatal deaths was observed, with a significant increase in 400 and 800 mg/kg animals at 9.7% and 9.5%, respectively. Although the incidence of postnatal death was numerically similar in the mid- and high-dose pups, there was an increase in affected litter number by dose level (2/16 in controls, 1/16 at 200 mg/kg, 4/15 at 400 mg/kg and 7/18 at 800 mg/kg suggesting that the finding could be treatment related.

No antiandrogenic effects were observed in the main study at sexual maturation (PND 21) in male F1 offspring of dams dosed up to 800 mg/kg bw/day. The Substance did not affect anogenital distance, nipple retention, sexual maturation, hormone levels (testosterone, serum LH or prolactin in F1 males on PND21), sperm parameters (motility and count), endocrine organ weights or histopathology of the reproductive system in F1 males of the main study. Additionally, anogenital distance, nipple retention, testis, epididymides, prostate and seminal vesicle weights were unaffected by treatment with the Substance in the DRF study up to 1200 mg/kg bw/day.

No antiandrogenic effects were observed neither in the main study nor in the dose-range finder at sexual maturation (PND 21) in male F1 offspring of dams dosed up to 800 and 1200 mg/kg bw/day, respectively. The slight increase in postnatal deaths in an increasing number of litters by dose, increased perinatal loss as well as low pup weights at birth and persistent at PND 3 and 13 in the main study were consistent with that seen in the dose-range finding study indicating a potential concern for developmental toxicity. F1 females were not followed into sexual maturation and adulthood and there was no post-weaning exposure of male pups.

The study does not raise any concerns with respect to endocrine disruption of the Substance, in particular (anti)androgenic effects males up to sexual maturity. The study was disregarded by the registrant from the REACH registration dataset due to unreliability. However, the eMSCA considers that the study can be used as supporting information for developmental toxicity due to the reduced pup body weights and increase perinatal loss and postnatal deaths seen in the main study at 800 mg/kg bw/day and consistent with findings in the dose-range finding study.

IP injection of adipates during gestation

Singh et al (1973) exposed five pregnant rats to seven different adipates diethyl, dipropyl, di-isobutyl, di-n-butyl, di-2-ethyl hexyl (the Substance) and dicyclohexyl adipate via an intraperitoneal injection. The Substance was administered in three different doses: 1, 5 or 10 ml/kg. The administrations were done on gestation days 5, 10 and 15. The rats were killed on day 20 of gestation, one day prior to parturition and the fetuses were examined for visceral and skeletal malformations or variations. The following parameters were investigated: embryonic-fetal toxicity (resorptions and stillbirths), gross external malformations of fetuses, skeletal and visceral malformations, fetal size (weight) and viability. The number of resorptions varied from 1.6% to 20%; for the Substance the resorption rate varied between 5.3 and 7.0%. Gross malformations varied between 0% and 3.8% from lowest to highest dose. Similarly, from lowest to highest dose the Substance caused 3.6%, 9.4% or 7.1% skeletal malformations. Visceral malformation

rates were 0% (1 ml/kg), 3.2% (5 ml/kg) or 4.0% in the 10 ml/kg dose group. No control group values were reported.

Conclusions on developmental toxicity

In rats, the Substance caused slight maternal toxicity in the high dose group throughout the gestation, manifested by a decreased body weight gain and slightly decreased food consumption, and slight but dose related foetotoxicity in the mid- and high-dose groups demonstrated by visceral defects (kinked and dilated ureter) and also skeletal abnormalities such as poor ossification in a PNDT study (Unpublished study, 1988b). An increase in pre-implantation loss was also observed in dams of the high dose group (1080 mg/kg bw/day) (+19.1% vs +13.5%).

In the OECD TG 414 study in rabbits (Unpublished, 2014), no toxicity was seen in the P animals or the fetuses. In the dose range finding study it was reported that at 300 mg/kg rabbits showed clinical signs, reduced body weight gain or body weight loss and reduced food consumption. Therefore, the highest dose was set at a rather low level at 160 mg/kg (nominal) corresponding to an achieved dose of 145 mg/kg bw/day which did not cause maternal toxicity in the main study. This selection of such low dose levels for the main study causes some concern regarding missing a potential effect on fetuses as the maternal animals did not exhibit any signs of toxicity. However, in the fertility and development studies with rats a similar maternal NOAEL of 170 mg/kg bw/day was obtained with developmental effects noted in offspring at this dose level. This suggests that rats may be a more sensitive species for the developmental effects of the Substance.

The study conducted by Singh et al. (1973) seemed to show that adipates have some tendency to increase skeletal and visceral malformations, but because the study was conducted with such a low number of animals (5) and because the administration route (ip) and schedule (three days of gestation) were unconventional, it is not possible to draw any conclusions from this study.

Pre- and postnatal exposure of rats through sexual maturation of the Substance did not give rise to any effects which could be attributed to an (anti)androgenic mode of action at doses up to 800 mg/kg bw/day in the study by Dalgaard (2003). The effects on development could indicate treatment related developmental toxicity. These included a slight increase in postnatal deaths in an increasing number of litters by dose, increased perinatal loss as well as low pup weights at birth, persistent at PND 3 and 13.

Death of the developing organism as well as structural abnormalities are considered major manifestations of adverse effects on development. The increase in skeletal and visceral abnormalities, increase in pre- and post-implantation loss and late intrauterine deaths observed at doses significantly below a limit dose in the PNDT study with rats (Unpublished study, 1988b) raise a concern in the eMSCA. Some of the skeletal variants observed in the study can be considered common fetal variants and hence without toxicological relevance. However, the major finding of situs inversus totalis and unossified 3rd and 7th ribs in one pup each of the high dose group, partial ossification of the 1st rib in 1 pup of the mid-dose group as well as the dose-dependent increase in total minor skeletal defects (in 49.4% of high dose pups) and total minor external and visceral variants cannot be excluded as treatment related but may be at least in part excarbated by general maternal toxicity (lower body weight gain and food consumption) in the high dose group.

Although the study conducted by Singh et al (1973) was a non-guideline study with a non-physiological administration route using only 5 animals dosed for only three days of gestation, the findings of increased skeletal and visceral malformations may support the findings from the teratogenicity study with rats. However, the study protocol has severe deficiencies and thus provide little value as supportive evidence for the interpretation of developmental toxicity.

Although a PNDT study has also been conducted with rabbits, the dose levels selected for the study may have been too low to elicit an effect on development. The rat may also be considered a more sensitive species to the effects of the Substance. No conflicting

information is available, where effects would not have been seen in rats following prenatal exposure.

In a one-generation study with the Substance (Unpublished study, 1988a), four whole litter losses were observed; one in the low-dose group at 300mm, two in the mid-dose group at 1800ppm and one at the high dose group of 12000ppm. None occurred in the control group. The authors considered the litter losses to be unrelated to the Substance treatment and the losses were not dose related. In this study mean pup weight and total litter weight were reduced during post-partum days 1-36 in the high dose group. Mean litter size was slightly reduced in the high dose group.

The study by Dalgaard (2003) can also be used as supporting information with regard to developmental toxicity. In the study, the Substance was administered to rats at doses up to 800 mg/kg bw/day. Findings of slight increase in postnatal deaths in an increasing number of litters by dose, increased perinatal loss as well as low pup weights at birth, persistent at PND 3 and 13 were recorded.

The eMSCA notes that a two-generation study or an extended one-generation study have not been conducted with the Substance. Therefore, information on effects through sexual maturation (PND 90 in the EOGRTS), effects on sexual maturation, organ weights and histopathology of all organs and tissues in F1 animals at adulthood, haematology and clinical chemistry in F1 animals at adulthood, growth and clinical signs in F1 animals up to adulthood are lacking from the dataset. The EOGRTS is a standard requirement for substances registered at tonnages exceeding 1000 tons per annum, and the eMSCA considers that it would provide value to the evaluation of effects to reproduction and development of the Substance.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated

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

Not evaluated

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

The findings of the PNDT study in rats showed effects that are not necessarily attributable to secondary effects of maternal toxicity and may indicate treatment related adversity in the developing fetus. In particular, death of the developing organism as well as structural abnormalities are considered major manifestations of adverse effects on development. The findings of increased skeletal and visceral abnormalities, increased pre- and post-implantation loss and late intrauterine deaths raise a concern in the eMSCA. These findings warrant further reflection in relation to the classification criteria of CLP for reproductive toxicity category 1B or 2.

Therefore, the eMSCA recommends an assessment of the impact of the findings on reproduction and development in line with CLP criteria (CLP Annex I). It should focus on:

- Adverse effects on sexual function and fertility in adult males and females and
- Adverse effects on development of the offspring

Ideally, an EOGRTS would be available prior to an Annex I dossier under CLP.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Not evaluated

7.10.2. Endocrine disruption - Human health

The molecular structure of the Substance has been compared to that of the phthalate substance DEHP. Studies have been conducted to assess whether similar findings of anti-androgenicity and male reproductive toxicity as seen with DEHP would be seen also with the Substance subject to evaluation.

DEHP as well as other phthalates produce symptoms of 'phthalate syndrome' in exposed rats due to the suppression of fetal androgen action. The effects characteristic of this are relevant for humans, e.g. inhibition of fetal testosterone production, reduction of male anogenital distance, increased permanent nipple retention in male offspring, increased incidence hypospadias and cryptorchidism, delayed onset of puberty, reduced semen quality and testicular changes, and are also seen in humans.

The Substance lacks the characteristic alkylphenol structure that is present in the phthalates. Structural similarity with DEHP is limited to the di-carboxylic acid structure which is present in both substances. The Substance shares a common metabolite 2-ethylhexanol with DEHP and so also the effects of this metabolite have been studied.

Studies for (anti)androgenicity, (anti)estrogenicity, thyroid mediated effects and effects on steroidogenesis of the Substance have been conducted and are summarised in the following text and tables.

(anti)androgenicity

Antiandrogenic effects of the Substance during the pre- and postnatal period were screened *in vivo* by Dalgaard et al. (2003), see Section 7.9.7.2. No antiandrogenic effects were observed in the study at sexual maturation (PND 21) in male F1 offspring of dams dosed up to 800 mg/kg bw/day. The Substance did not affect anogenital distance, nipple retention, sexual maturation, hormone levels, sperm parameters, endocrine organ weights or histopathology of the reproductive system in F1 males of the main study. The findings are consistent with those from the dose-range finding study where anogenital distance, nipple retention, testis, epididymides, prostate and seminal vesicle weights of pups were also unaffected by treatment of dams at doses up to 1200 mg/kg bw/day. Please refer to the section on developmental toxicity (Section 7.9.7.2.) for a review of non-endocrine related findings from the study.

Borch et al. (2004) gavaged pregnant Wistar rats during gestation and lactation with vehicle, DEHP (300 or 750 mg/kg bodyweight per day), DINP (750 mg/kg bodyweight per day), and DEHP (750 mg/kg bodyweight per day) in combination with the Substance (400 mg/kg bodyweight per day), or DEHP (300 mg/kg bodyweight per day) in combination with DINP (750 mg/kg bodyweight per day). DINP and DEHP reduced testicular testosterone production *ex vivo* and testosterone levels in testes and plasma of male fetuses at gestation day 21. Also plasma LH levels in male fetuses were elevated. Neonatal anogenital distance was reduced and the number of nipples at postnatal day 13 increased in DEHP-exposed male offspring. Serum inhibin B levels were significantly reduced in DEHP-exposed prepubertal male offspring, and in a few adult males. The Substance did not show any modulating effects on the endocrine effects of DEHP.

The effects of DEHP and five of its metabolites including the common metabolite with the Substance, 2-ethylhexanol, on rat testis were investigated *in vivo* and *in vitro* (Sjöberg et al., 1986). The study found that mono-2-ethylhexyl phthalate (MEHP), the primary metabolite of DEHP caused a significant increase in spermatocyte and spermatid degeneration *in vivo* and germ cell detachment from primary rat testicular cell cultures in vitro. MEHP is not formed in the metabolism of the Substance. The common metabolite 2-ethylhexanol did not cause similar effects *in vitro* or *in vivo* as seen with MEHP.

The *in vitro* effect of the Substance on AR activity was studied by Krüger et al. (2008) using a chemically activated luciferase gene expression (CALUX) bioassay in transient transfected Chinese Hamster Ovary (CHO-K1) cells (AR-CALUX). The Substance did not produce agonist or antagonist effects in the androgen receptor gene (AR-CALUX) assay at non-toxic concentrations up to 1×10^{-4} M. AhR transactivity was also investigated in the same paper. A similar luciferase gene expression assay to that used in the AR assay was

used utilising recombinant mouse Hepa1.12cR cells (AhR-CALUX). No effects on AhR activity in the AhR-CALUX assay were observed at the same concentrations used in the AR assay.

The Substance was not active in any of the 16 assays (Table 10) for androgen receptor mediated effects screened for in the US EPA EDSP21 program (US EPA, 2020). These include, among others, assays for androgen receptor agonism and antagonism, AR ligand binding, receptor co-activation, transcription factor activity and reporter gene expression.

Table 10. EDSP21 data of androgen receptor activity assays with the Substance (US EPA, 2020)

Assay	Outcome	Organism and cell origin	Intended target family
ATG_AR_TRANS_up	Inactive	Human liver, HepG2	Nuclear receptor
OT_AR_ARELUC_AG_1440	Inactive	Chinese hamster ovary (CHO)	Nuclear receptor
OT_AR_ARSRC1_0480	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_AR_ARSRC1_0960	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_AR_BLA_Agonist_ratio	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_AR_BLA_Antagonist_ratio	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_AR_LUC_MDAKB2_Agonist	Inactive	Human breast, MDA-kb2	Nuclear receptor
TOX21_AR_LUC_MDAKB2_ Antagonist_10nM_R1881	Inactive	Human breast, MDA-kb2	Nuclear receptor
TOX21_AR_LUC_MDAKB2 _Antagonist_0.5nM_R1881	Inactive	Human breast, MDA-kb2	Nuclear receptor
TOX21_AR_LUC_MDAKB2_Agonist_ 3uM_Nilutamide	Inactive	Human breast, MDA-kb2	Nuclear receptor
ACEA_AR_agonist_80hr	Inactive	Human prostate, 22Rv1	Nuclear receptor
TOX21_AR_BLA_Antagonist _viability	Inactive	Human breast, MDA-kb2	Cell cycle
TOX21_AR_LUC_MDAKB2_ Antagonist_10nM_R1881_viability	Inactive	Human breast, MDA-kb2	Cell cycle
ATG_AR_TRANS_dn	Inactive	Human liver, HepG2	Nuclear receptor
TOX21_AR_LUC_MDAKB2_ Antagonist_0.5nM_R1881_viability	Inactive	Human breast, MDA-kb2	Cell cycle
TOX21_AR_LUC_MDAKB2_Agonist _3uM_Nilutamide_viability	Inactive	Human breast, MDA-kb2	Cell cycle

None of the *in vitro* or *in vivo* studies for (anti)androgenicity indicate a concern for adverse effects of the Substance resulting from perturbation of androgen-dependent pathways.

(anti)estrogenicity

Estrogenicity of the Substance has been tested in an immature type uterotrophic assay in rats (Park et al., 2007) according to a method generally equivalent to that described in OECD TG 440 (described by Jordan et al. (1983)). Doses up to 1000 mg/kg/day were administered by subcutaneous injection to 5 groups of 6 20-day old female SD rats for 3 consecutive days. Uterus, vagina and ovary weights were recorded and livers, thyroid, pituitary and adrenals were taken for histopathological evaluation after the scheduled kill 24 hours following the final administration. FSH and LH concentrations in blood were

unchanged by treatment. BrdU immunohistochemistry gave no indication of increased cell proliferation in liver and uterine cells of treated animals. The relative organ weights of the kidney and ovary were slightly increased ($p<0.05$) in the high dose group animals compared to controls. However, the average change was slight (1,3% (-4.1 to 6.7%) increase in kidney weight and 4% (-10 to 18%) in ovarian weight). Due to the low statistical power of the study and absence of individual animal data, it is not possible to conclude on the significance of the high variability seen in ovary weights of the high dose group. No histopathological changes were seen in the uterine muscle thickness, number of endometrial glands or endometrial height in the treated groups. The uterine weight was unaltered by treatment and the Substance did not show estrogenicity in this study.

The Substance did not produce any effect on estrogen receptor transactivation in the assay by Ghisari and Bonefeld-Jorgensen (2009) utilising a human breast cancer cell line (MVLN) with estrogen response element-luciferase reporter gene vectors at concentrations from 1 x 10^{-10} to 5 x 10^{-5} M.

Hashimoto and colleagues (2003) studied the estrogenic activity of the Substance and several other plasticisers *in vitro*. The E-screen assay measures the proliferation of human breast cancer cells (MCF-7) as a marker of estrogenicity. The Substance did not increase proliferation of MCF-7 cells in the assay up to concentrations up to 2 x 10^{-3} g/ml ($p > 0.05$), showing no indication of estrogenic activity.

The Substance did not induce estrogen receptor mediated luciferase gene expression in tissues of transgenic male ER-luc reporter gene (INS7) mice 8 hours following intraperitoneal administration or 14 hours following oral administration with a single dose of 30 and 100 mg/kg of the Substance (ter Veld et al., 2008). Tissues studied for luciferase activity included the liver, testis, kidney, adrenal, brain, pituitary, femur and tibia.

The Substance has been screened in the US EPA EDSP21 program (US EPA, 2020). The existing assays consist of 27 estrogen receptor targeted assays (Table 12.), 16 assays for androgen receptor mediated effects (Table 11.), 8 on the thyroid and 2 steroidogenesis assays (Table 13.). Of all screening endpoints, only one gave a positive response; namely the TOX21_Era_BLA_Antagonist_ratio assay which produced an AC_{50} value of 10.89 µM for the Substance. The receptor activity assay measures the ability of a substance to inhibit estrogen receptor alpha signaling pathways (ToxCast, 2019). The assay detects GAL4 b-lactamase reporter gene induction using a human kidney derived cell line measuring antagonism of reporter gene by the detection of a loss of fluorescence intensity.

Table 11. EDSP21 data of estrogen receptor activity assays with the Substance (US EPA, 2020)

Assay	Outcome	Organism and cell origin	Intended target family
ACEA_ER_80hr	Inactive	Human breast cancer, T47D	Nuclear receptor
ATG_ERE_CIS_up	Inactive	Human liver, HepG2	Nuclear receptor
ATG_ERa_TRANS_up	Inactive	Human liver, HepG2	Nuclear receptor
NVS_NR_bER	Inactive	Bovine uterus membrane	Nuclear receptor
OT_ER_ERaERa_0480	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ER_ERaERa_1440	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ER_ERbERb_0480	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ER_ERbERb_1440	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ERa_EREGFP_0120	Inactive	Human cervix, HeLa	Nuclear receptor

OT_ERa_EREGFP_0480	Inactive	Human cervix, HeLa	Nuclear receptor
TOX21_ERa_BLA_Agonist _ratio	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_ERa_BLA_Antagonist _ratio	Active	Human kidney, HEK293T	Nuclear receptor
TOX21_ERa_LUC_VM7_ Agonist	Inactive	Human ovary, VM7	Nuclear receptor
TOX21_ERa_LUC_VM7 _Antagonist_0.5nM_E2	Inactive	Human ovary, VM7	Nuclear receptor
TOX21_ERa_LUC_VM7_ Antagonist_0.1nM_E2	Inactive	Human ovary, VM7	Nuclear receptor
TOX21_ERb_BLA_Agonist _ratio	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_ERb_BLA_Antagonist _ratio	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ER_ERaERb_0480	Inactive	Human kidney, HEK293T	Nuclear receptor
OT_ER_ERaERb_1440	Inactive	Human kidney, HEK293T	Nuclear receptor
TOX21_ERa_BLA_Antagonist _viability	Inactive	Human kidney, HEK293T	Cell cycle, cytotoxicity
TOX21_ERa_LUC_VM7_ Antagonist_0.5nM_E2_viability	Inactive	Human ovary, VM7	Cell cycle, cytotoxicity
ATG_ERa_TRANS_dn	Inactive	Human liver, HepG2	Nuclear receptor
ATG_ERE_CIS_dn	Inactive	Human liver, HepG2	Nuclear receptor
ACEA_ER_AUC_viability	Inactive	Human breast, T47D	Cell cycle, cytotoxicity
TOX21_ERa_LUC_VM7_ Antagonist_0.1nM_E2_viability	Inactive	Human ovary, VM7	Cell cycle, cytotoxicity
TOX21_ERb_BLA_Agonist_ viability	Inactive	Human kidney, HEK293T	Cell cycle, cytotoxicity
TOX21_ERb_BLA_Antagonist_ viability	Inactive	Human kidney, HEK293T	Cell cycle, cytotoxicity

Although antagonism of ER can give an indication of a potential mechanism leading to an adverse effect in an intact organism, no conclusive *in vitro* or *in vivo* findings indicating anti-estrogenicity of the Substance have been observed to support the single in vitro screening assay finding. The in vivo data include a one-generation study (Unpublished study, 1988a), uterotrophic assay (Park et al., 2007) and an in vivo luciferase gene expression test with transgenic mice (Ghisari and Bonefeld-Jorgensen, 2009).

Thyroid and steroidogenesis

Table 12**. Summary of EDSP21 thyroid and steroidogenesis assays with the Substance (US EPA, 2020).**

Assay	**Outcome**	**Organism and cell origin**	**Intended target family**
ATG_THRa1_TRANS_up	Inactive	Human liver, HepG2	Nuclear receptor
TOX21_TSHR_Agonist_ ratio	Inactive	Human kidney, HEK293T	G-protein-coupled receptor
TOX21_TSHR_Antagonist_ ratio	Inactive	Human kidney, HEK293T	G-protein-coupled receptor
TOX21_TR_LUC_GH3_ Agonist	Inactive	Rat pituitary gland, GH3	Nuclear receptor
TOX21_TR_LUC_GH3_ Antagonist	Inactive	Rat pituitary gland, GH3	Nuclear receptor

TOX21_TR_LUC_GH3_ Antagonist_viability	Inactive	Rat pituitary gland, GH3	Cell cycle, cytotoxicity
ATG_THRa1_TRANS_dn	Inactive	Human liver, HepG2	Nuclear receptor
TOX21_TSHR_wt_ratio	Inactive	Human kidney, HEK293T	G-protein-coupled receptor
TOX21_Aromatase_ Inhibition	Inactive	Human breast MCF-7	CYP
TOX21_Aromatase_ Inhibition_viability	Inactive	Human breast MCF-7	Cell cycle, cytotoxicity

There were no indications of effects of the Substance to aromatase activity nor thyroid hormone (ant)agonism in the assays available in the EDSP21 database for the Substance.

Effects on immune response

Kim et al. (2014) investigated the effects of androgen-disrupting chemicals on innate immunity by testing eight candidate androgen disrupting chemicals (ADCs) for their influence on macrophages by measuring nitric oxide (NO) production and cell viability. The results showed that treatment with a mixture of lipopolysaccharide and hexachlorobenzene increased NO production in RAW 264.7 cells, a murine macrophage cell line. Compared to exposure to a negative control, exposure to the Substance, benzylbutyl phthalate (BBP), testosterone (TTT), or permethrin decreased NO production. The Substance, BBP, and TTT inhibited NO production in an inducible nitric oxide synthase-dependent manner.

It is difficult to form any conclusions on the role of a possible reduction of nitric oxide production via a potential decreased expression of iNOS mRNA and protein and the possible role on macrophage response on whole organisms from this *in vitro* assay alone.

7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

Endocrine disruption was identified as a potential concern for the Substance based on the structural similarity to DEHP and the structural alerts identified for the adipate structure.

(Anti)androgenicity

The *in vivo* studies for (anti)androgenicity consisting of an anti-androgenicity study following OECD TG 426 and a steroidogenesis study testing the modulation potential of the Substance on effects of known phthalates revealed no cause for concern for endocrine disruption via these modalities. There were no similarities in effects with the Substance to those seen with some phthalates such as DEHP. The Substance was not active in any of the 16 assays for androgen receptor mediated effects screened for in the US EPA EDSP21 program (US EPA, 2020). These include, among others, assays for androgen receptor agonism and antagonism, AR ligand binding, receptor co-activation, transcription factor activity and reporter gene expression.

(Anti)estrogenicity

Anti(estrogenicity) was studied *in vivo* in an uterotrophic assay generally following OECD TG 440 and in an *in vivo* luciferase gene expression test. No effects suggestive of estrogenicity were observed.

From the database of in vitro assays as well as the 27 assays for (anti)estrogenicity in the US EPA EDSP21 program (US EPA, 2020) only one gave a positive response, namely the TOX21_Era_BLA_Antagonist_ratio assay which measures inhibition of estrogen receptor alpha signaling pathways.

Overall, the *in vivo* and *in vitro* data do not give rise to a concern for (anti)estrogenicity of the Substance.

Thyroid and steroidogenesis

There were no indications of effects of DEHA to aromatase activity nor thyroid hormone (ant)agonism in the assays available in the EDSP21 database for the Substance. No cause

for concern was identified for these modalities during the review of the *in vivo* data included in the current Substance Evaluation. However, a comprehensive review of all data was not carried out focusing on thyroid or steroidogenesis mediated effects as this was not in the scope of the current evaluation.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

Regarding human health, no hazards have been identified, therefore no exposure assessment and risk characterisation regarding workers and consumers are needed (REACH Annex I: 0.6.2./0.6.3.).

The eMSCA notes that exposure assessment of the Substance should be updated in case relevant new experimental information becomes available warranting hazard classification of the Substance.

7.12.1.1. Worker

Because the Substance does not have any hazard classifications, no exposure scenarios have been included in the registration dossier and the chemical safety report for the Substance. Based on the available information in the dossier, the Substance has two main uses: plasticiser and lubricant use.

Based on the information given for the plasticiser use process categories (PROCs) there are the following exposure scenarios:

PROC 7: Industrial spraying

PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities

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

PROC 14: Tabletting, compression, extrusion, pelletisation, granulation

PROC 15: Use as laboratory reagent

Lubricant use:

PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities

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 15: Use as laboratory reagent

PROC 17: Lubrication at high energy conditions in metal working operations

For the plasticiser use probably the greatest exposure potential is during industrial spraying and during roller application and brushing through aerosols entering the respiratory

pathway. The conditions of lubricant use and use at high energy metal working are unknown but if manual processes are involved, there can be potential for dermal exposure.

7.12.1.2. Consumer

Studies have been published in which consumer exposure is quantified by determining various metabolites of the Substance in blood. Nehring et al. (2019) investigated exposure in six volunteers who had consumed food wrapped in commercial PVC-cling film containing the Substance and in two small populations without known exposure to the Substance (44 pregnant Brazilian women and 32 German adults). In the cling film experiment, all three metabolites were found in all post exposure urine samples, with mono-5-carboxy-2-ethylpentyl adipate (5cx-MEPA) being most prominent (0.30–10.2 µg/l), followed by 5OH-MEHA (0.12–4.31 µg/l) and mono-2-ethyl-5-oxohexyl adipate (5oxo-MEHA) (0.12–2.84 µg/l). In the Brazilian and German samples, exposure to the Substance was detected in 43 and 9% of all samples, with 5cx-MEPA as the most prominent metabolite.

Fromme et al. (2016) measured di-isononyl cyclohexane-1,2-dicarboxylate (DINCH), di(2-ethylhexyl) terephthalate (DEHT), the Substance, acetyl tri-n-butyl citrate (ATBC), and trioctyl trimellitate (TOTM) plasticizer levels in indoor air and dust samples from 63 daycare centers in Germany. The Substance was present in indoor air with median values of 34 mg/m3. In the dust, a median value 49 mg/kg of the Substance was found.

Fromme et al.(2007) investigated diet samples of 27 female and 23 male healthy subjects aged 14–60 years who collected daily duplicate diet samples over 8 consecutive days. Overall, 11 phthalates were measured in the duplicates by gas or liquid chromatograph/mass spectrometer methods. Urinary levels of primary and secondary phthalate metabolites were also detected.

The median (95th percentile in parenthesis) daily intake via food was 2.4 (4.0) µg/kg b.w. for DEHP, 0.3 (1.4) µg/kg b.w. Di-nbutyl phthalate (DnBP), 0.6 (2.1) µg/kg b.w. Di-isobutyl phthalate(DiBP) and 0.7 (2.2) µg/kg b.w. for the Substance.

7.12.2. Environment

Not evaluated.

7.12.3. Combined exposure assessment

Not evaluated

7.13. Risk characterisation

Regarding human health, no hazards have been identified, therefore no exposure assessment or risk characterisation regarding worker and consumer exposure is needed, Annex I: 0.6.2./0.6.3.). The eMSCA notes that the exposure assessment and risk characterisation of the Substance should be updated in case relevant new experimental information becomes available warranting any hazard classification.

7.14. References

Borch et al. 2004, Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reproductive Toxicology 18:1 53-61

Dalgaard, M. et al. 2003, Di(2-ethylhexyl) adipate (DEHA) induced developmental toxicity but not antiandrogenic effects in pre- and postnatally exposed Wistar rats. Reproductive toxicology 17: 163-170

Di Carlo, F., Structure-activity relationships (SAR) and structure metabolism relationships (SMR) affecting the teratogenicity of carboxylic acids. Drug Metabolism Reviews, 22(5), 411-449 (1990)

ECHA, REACH registration dossier for bis(ethylhexyl) adipate, ECHA, (2020)

ECHA, 2017, Substance evaluation conclusion and evaluation report for 2-ethylhexan-1-ol

EPA, 1992, Integrated Risk Information System (IRIS) Chemical Assessment Summary for Di(2-ethylhexyl)adipate; CASRN 103-23-1. Accessible at: https://iris.epa.gov/static/pdfs/0420_summary.pdf

Fromme, H. et al., 2007, Occurrence and daily variation of phthalate metabolites in the urine of an adult population. International Journal of Hygiene and Environmental Health 210(1) 21-33

Fromme, H. et al., 2016, Non-phthalate plasticizers in German daycare centers and human biomonitoring of DINCH metabolites in children attending the centers (LUPE 3). International Journal of Hygiene and Environmental Health 219(1) 33-39

Ghisari, M. and Bonefeld-Jorgensen, EC. 2009, Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicology Letters 189: 67-77

Hashimoto, Y. et al., 2003, Estrogenic activity of tissue conditioners in vitro. Dental Materials, 19: 341-346

Jordan, VC and Gosden, B., 1983, Differential antiestrogen action in the immature rat uterus: a comparison of hydroxylated antiestrogens with high affinity for the estrogen receptor. Journal of Steroid Biochemistry, 19(3), 1249-1258.

Kim et al. 2014, Diverse Influences of Androgen-Disrupting Chemicals on Immune Responses Mounted by Macrophages, vol 37: No 3: 649-656

Krüger, T., Long, M. and Bonefeld-Jorgensen, EC., 2008. Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor, Toxicology 246: 112-123

Miyata, K. et al. 2006, Subacute oral toxicity study of di(2-ethylhexyl)adipate based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". Arch Toxicol, 80: 181-186.

Narotsky, M. et al. 1994, Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Fundamental and Applied Toxicology 22, 251-265.

Nehring, A. et al., 2019. Determination of human urinary metabolites of the plasticizer di(2-ethylhexyl) adipate (DEHA) by online-SPE-HPLC-MS/MS. Journal of Chromatography B 1124, 239-246

OECD, 2009, Series on testing and assessment, Guidance document number 106, histologic evaluation of endocrine and Reproductive tests in rodents

Park, KD. et al., 2007, Immatured Type Uterotrophic Assay for Estrogenicity Evaluation of DEHA. Kor. J. Env. Hlth., 33(5), 379-385

Shengde, W. et al., 2013, Framework for identifying chemicals with structural features associated with the potential to act as developmental or reproductive toxicants. Chem.Res.Toxicol. 26(12), 1840-1861

Singh, 1973, Embryonic-fetal toxicity and teratogenic effects of adipic acid esters in rats. Journal of Pharmaceutical Sciences, 62:10, 1596-1600

ter Veld, MGR., et al., 2008. Food-associated estrogenic compounds induce estrogen receptor-mediated luciferase gene expression in transgenic male mice. Chemico-Biological Interactions 174: 126-133

Unpublished study report 1988a, Di(2-ethylhexyl)adipate: Fertility study in rats

Unpublished study report 1988b, Di(2-ethylhexyl)adipate: Teratogenicity study in the rat

Unpublished study report 2014, Prenatal developmental Toxicity study of bis(2-ethylhexyl)adipat in Rabbits by Dietary Administration

US EPA ToxCast dashboard, 2019. Accessed on 25.02.2020: https://comptox.epa.gov/dashboard/chemical_lists/TOXCAST

US EPA, 2020. Accessed on 11.3.2020: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=103-23-1#invitrodb-bioassays-toxcast-data

US EPA, 1992, Integrated Risk Information System (IRIS) Chemical Assessment Summary for Di(2-ethylhexyl)adipate; CASRN 103-23-1. Accessed on 2.3.2020: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0420_summary.pdf

Wato, E. et al., 2009, Collaborative work on the evaluation of ovarian toxicity 9) Effects of 2- or 4-week repeated dose studies and fertility study of di(2-ethylhexyl)adipate (DEHA) in female rats. J. Toxicol. Sci. 34(I) 101-109

7.15. Abbreviations

ADC	Androgen Disrupting Chemical
AhR	Aryl hydrocarbon Receptor
AR	Androgen Receptor
BBP	Benzyl butyl phthalate
CA	Competent Authority
CYP	Cytochrome P540
CLP	Classification, Labelling and Packaging
CoRAP	Community Rolling Action Plan
C&L	Classification and Labelling
DBP	Dibutyl phthalate
DEHA	Bis(2-ethylhexyl) adipate
DEHP	Bis(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthlalate
DINP	Diisononyl phthalate
DMEL	Derived Minimum-Effect Level
DNEL	Derived No-Effect Level
DRF	Dose Range Finding
ECHA	European Chemicals Agency
EDSP21	Endocrine Disruption Screening Program
ED	Endocrine Disruptor
ER	Estrogen Receptor
eMSCA	Evaluating Member State Competent Authority
F0	Parental Generation
F1	Filial Generation, first
GD	Gestational day
MEHP	mono-2-ethylhexyl phthalate
MSCA	Member State Competent Authority
NO	Nitric Oxide
OECD	Organisation for Economic Co-operation and Development
PND	Postnatal Day
PROC	Process Category
QSAR	Quantitative Structure-Activity Relationship
SEv	Substance Evaluation
SVHC	Substance of Very High Concern
TG	Test Guideline
TTT	Testosterone
US EPA	United States Environmental Protection Agency
2-EH	2-ethylhexanol
5CX-MEPA	mono-5-carboxy-2-ethylpentyl adipate
5OXO-MEHA	mono-2-ethyl-5-oxohexyl adipate