SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2-(4-tertbutylbenzyl) propionaldehyde

EC No 201-289-8

CAS RN 80-54-6

Evaluating Member State: Sweden

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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2012

Before concluding the substance evaluation a Decision to request further information was issued on: 21 February 2014

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

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

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Part A. Conclusion

1. CONCERNS SUBJECT TO EVALUATION

The Substance, 2-(4-tertbutylbenzyl) propionaldehyde (EC number 201-289-8), here referred to also as “lysmeral”, was originally selected for substance evaluation in order to clarify concerns about:

- CMR (reproductive toxicity)
- Consumer use
- Wide dispersive use

During the evaluation additional concerns were identified:

- Endocrine disrupting properties for human health
- Endocrine disrupting properties for the environment

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

In 2019, the Scientific Committee on Consumer Safety stated in their opinion that they could not conclude on the safety of the substance in cosmetic products and that the evaluation under REACH will also need to be taken into consideration (SCCS, 2019).

In 2019, the opinion of the Risk Assessment Committee (RAC) to classify the Substance as Repr. Category 1B, H360Fd was adopted.

In 2019, a risk management option analysis (RMOA) was performed by the Swedish MSCA. The outcome was a proposal to identify lysmeral as a Substance of Very High Concern (SVHC), according to the REACH Article 57(c), for inclusion in the Candidate List, with the primary aim to exercise pressure on industry to substitute the Substance.

In 2021, lysmeral was identified as a SVHC, according to the REACH Article 57(c) and included in the Candidate List for eventual inclusion in the REACH Annex XIV (Authorisation list).

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
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	X

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

Not applicable.

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

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	
Clarification of hazard properties/exposure	X
Actions by the registrants to ensure safety, as reflected in the registration dossiers	

Since 2019, lysmeral has a harmonised classification as Repr. Category 1B, H360Fd. Lysmeral was also identified in 2021 as a SVHC, according to the REACH article 57(c), for reproductive toxicity.

Consequent to its classification as Repr 1B, uses of lysmeral are being restricted. Exposure of lysmeral to the environment and humans is expected to diminish as a result of its restricted uses and the availability of alternatives.

The Registrants have informed the evaluating MSCA that they intend to update the registration dossiers with a revised CSR by March 2022 (the entry into force date of the harmonised classification).

During the SEv, the evaluating MSCA identified additional concerns for potential endocrine disrupting properties for both, the environment and human health. The evaluating MSCA has concluded that the current data is inconclusive regarding ED properties for human health and that further information is needed to clarify the concern for potential ED properties for the environment. A second draft decision was sent to the Registrants to address potential ED properties for the environment.

However, the evaluating MSCA did not foresee that requesting further information to clarify the ED concern would lead to a significant improvement of the regulatory risk management for the Substance.

Therefore, the decision making was terminated and the SEv process concluded without further test requests. Hence the ED concerns are not clarified in this SEv.

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, 2-(4-tertbutylbenzyl) propionaldehyde (EC number 201-289-8), here referred to also as “lysmeral”, was originally selected for substance evaluation in order to clarify concerns about:

- CMR (reproductive toxicity)
- Consumer use
- Wide dispersive use

During the evaluation, additional concerns were identified:

- Endocrine disrupting properties for human health
- Endocrine disrupting properties for the environment

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Reproductive toxicity, Fertility and development	Concern confirmed. Lysmeral is classified as Repr. 1B, H360Fd.
Reproductive toxicity, Developmental neurotoxicity	Concern refuted. No developmental neurotoxicity (DNT) effects (structural or functional) were observed in the investigations of the DNT cohort in the EOGRTS with lysmeral.
Endocrine disruption Human health	Concern not resolved. The EOGRTS was requested to also address the potential ED properties of the Substance. The study did not confirm ED properties. However, as the EOGRTS was performed at low doses no definitive conclusion could be reached.
Endocrine disruption Environment	Concern not resolved. A Fish short-term reproductive toxicity screening study (FSTRA) was requested under SEv to address the potential ED properties in the environment. The results were inconclusive.
Use and exposure	Concern resolved DNEL derivation was revised by the Registrants. Information in the CSRs on the use and exposure will be revised by the Registrants.

7.2. Procedure

Lysmeral was included in the Community Rolling Action Plan (CoRAP) for Substance Evaluation (SEV) in 2012, by the competent authority of Sweden. The scope of the evaluation was the concern for reproductive toxicity (fertility and development) and endocrine disruptive properties for human health and the environment.

Based on the assessment of the available data, the evaluating MSCA concluded that further information was required to clarify the abovementioned concerns. In October 2013, SEV draft decisions with information requests were sent to the Registrants for comments (a

SEV draft decision to all registrants, and 2 SEV draft decisions targeted to specific registrants). In November 2013, the Registrants sent their comments on the draft SEV decisions.

In February 2014, the adopted SEV decisions were sent to the Registrants. A Fish Short Term Reproduction Assay (FSTRA), according to the OECD TG 229 and an Extended One-Generation Reproductive Toxicity study (EOGRTS), according to the OECD TG 443 were requested. In addition, information on the justification behind the derivation of DNELs was requested.

In 2014, the Registrants submitted a proposal for classification of lysmeral as Repr. Category 2. This proposal was withdrawn as new information on the reproductive endpoints was requested in a SEV decision.

In 2014, the screening of the potential PBT properties of the Substance was discussed by the ECHA PBT expert group (6th PBT expert group meeting in April 2014) and concluded after a written procedure in 2015.

In July 2017, the Registrants updated their registration dossiers with the information requested in the SEV decisions. The Registrants also provided new information on the mode of action for testicular toxicity.

In November 2017, the evaluating MSCA presented relevant data for assessment of potential ED properties of the Substance, to be discussed at the 10th advisory ED expert group (EDEG) meeting at ECHA. No conclusion was reached at the meeting regarding the possible ED properties of lysmeral.

In December 2017, the Registrants (re-)submitted a CLH dossier to ECHA with the proposal for classification of the Substance as Repr. 2, H361f.

In January 2019, the opinion to classify lysmeral as Repr. category 1B, H360Fd was adopted by the Risk Assessment Committee (RAC), with the date of March 2022 for entry into force of the harmonised classification of lysmeral.

In March 2021, a second SEv DD, requesting further information to clarify the concern for potential ED properties in the environment was sent to the Registrants for comments. A Fish sexual developmental test was requested in the draft decision. In April 2021, Registrants sent their comments on the draft SEV decision: they argued that because of its harmonised classification, uses and consequently emission to the environment of the Substance would decrease significantly.

In June 2021, lysmeral was identified as a SVHC, according to the REACH Article 57(c), for reproductive toxicity effects and included in the Candidate List for Authorisation.

In September 2021, the evaluating MSCA terminated the draft decision and concluded the SEv process without further information request to address the ED concerns, as no significant added regulatory risk management benefit was foreseen.

In January 2022, the Registrants informed the evaluating MSCA that they intend to update the registration dossiers, including the CSRs by March 2022 (informal communication).

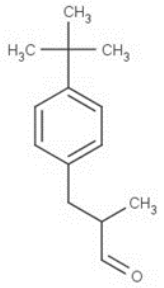
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	2-(4-tert-butylbenzyl)propionaldehyde
EC number:	201-289-8
CAS number:	80-54-6
Index number in Annex VI of the CLP Regulation:	605-041-00-3
Molecular formula:	C14H20O
Molecular weight range:	204.3 g/mol
Synonyms:	Lysmeral 2-Methyl-3-(4-tert-butylphenyl)propanal 4-tert-Butyl-a-methylhydrocinnamic aldehyde Lilial

Type of substance: Mono-constituent

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid
Density	0.941 g/cm^3 at 25 °C
Vapour pressure	ca. 0.25 Pa at 20 °C
Water solubility	33 mg/L at 20 °C
Partition coefficient n-octanol/water (Log Kow)	4.735
Flammability	Non flammable
Flash point	79°C at 101.3 kPa
Explosive properties	Non explosive
Oxidising properties	No

7.5. Manufacture and uses

7.5.1. Quantities

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

Lysmeral is mainly used as a fragrance ingredient.
The industrial uses include cleaning agents. Uses by professional and industrial workers include washing and cleaning agents. Consumer uses are mainly as fragrance in cleaning agents, air care products and cosmetics.

Table 7

USES	
Manufacture	Manufacturing of the substance
Formulation	Air care products Biocides (e.g. disinfectants, pest control products) Perfumes and fragrances Polishes and waxes Washing & cleaning products Cosmetics and personal care products.
Uses at industrial sites	Use for the manufacture of chemicals and as chemical intermediate Washing & cleaning products
Widespread uses by professional workers	Polishes and waxes and washing & cleaning products Formulation of mixtures and/or re-packaging Indoor use as processing aid and outdoor use as processing aid
Consumer Uses	Washing and cleaning products Air care products Cosmetics and personal care products Polishes and wax blends Biocides Perfumes and fragrances Coating products Fillers, putties, plasters, modelling clay Finger paints, inks and toners.
Article service life	Use of substance in scented articles Indoor use in long-life materials with low release rate

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (EC 1272/2008)							
Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Codes	Hazard statement codes		
605-041-00-3	2-(4-tert-butylbenzyl) propionaldehyde	201-289-8	80-54-6	Repr. 1B	H360Fd	-	-

7.6.2. Self-classification

In the registrations:

Acute Tox. 4	H302
Aquatic Chronic 2	H411
Skin Irrit. 2	H315
Skin Sens. 1B	H317
Repr. 2	H361
Repr. 1B	H360

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

Skin Sens. 1	H317
Acute Tox. 5	H313
Aquatic Chronic 3	H412

7.7. Environmental fate properties

Not assessed.

7.8. Environmental hazard assessment

Not assessed.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Metabolism of lysmeral has been studied *in vitro* and *in vivo*, following oral and dermal exposure, in several species.

In vivo oral exposure studies have identified metabolites of lysmeral in plasma, such as lysmerylic acid and tert-butyl benzoic acid (TBBA) and in urine, including TBBA, TBBA-glucuronide and tert-butyl benzoylhippuric acid (TBHA). Interspecies quantitative differences are reported, with TBBA as a main metabolite in rats, dogs and monkeys, but a minor metabolite in guinea pigs and mice. Excretion of the urinary metabolites, TBBA and TBHA has been compared in rat, mouse, guinea pig, dog and rhesus monkey

(registration dossier). Urine samples were analysed following oral administration of lysmeral for 5 days. Comparing the ratio between TBBA and TBHA showed that the main urinary metabolite in rats, dogs and monkeys was TBBA, whereas TBHA was predominant in mice and guinea pigs.

A comparative *in vitro* metabolism study with lysmeral is available (registration dossier). The study was performed using liver microsomes and hepatocytes from rat, mouse, guinea pig, dog, rabbit, rhesus monkey and human. The microsomes and hepatocytes were incubated with 10, 50 and 100 µM lysmeral. The highest amount of TBBA was detected in the rat hepatocytes, when compared to other rodent and non-rodent species. The levels of TBBA observed in human hepatocytes was approximately 4-fold lower than levels in the rat. Based on this comparative study, it was proposed by the Registrants that the level of formation of TBBA correlates with lysmeral toxicity observed in the sensitive species.

Two published studies of metabolism and excretion of lysmeral in humans are also available (Pluym et al., 2016; Scherer et al., 2016). In the first study, a biomonitoring method for quantitative exposure assessment of lysmeral was developed. Using this method lysmerol, lysmerylic acid, hydroxylated lysmerylic acid, TBBA and TBHA were identified as the major lysmeral metabolites. The method was then applied to urine samples of 40 adult volunteers and 4 of the metabolites were detected in most of the urine samples in the following order according to quantity excreted: TBBA >> lysmerol ≈ lysmeric acid> hydroxylated lysmerylic acid. In a subsequent clinical study, using the same method, metabolism and excretion of lysmeral was examined in 5 subjects dosed once oraly with 5.26 mg.

Urine was collected before and for 48h post administration and analysed for presence of the 4 metabolites: lysmerol, lysmerylic acid, hydroxylated lysmerylic acid and TBBA. After 48h TBBA, lysmerol, lysmerylic acid and hydroxylated lysmerylic acid represented on average 14.3, 1.82, 0.20 and 0.16% of the administrated dose, respectively. In total, these 4 metabolites represented 16.5% of the dose.

The evaluating MSCA notes that the existing data suggests differences in metabolism of lysmeral between species, with TBBA being generally the predominant metabolite in the sensitive species.

7.9.2. Acute toxicity and Corrosion / Irritation

Not assessed.

7.9.3. Sensitisation

Not assessed.

7.9.4. Repeated dose toxicity

Several repeated dose toxicity studies with lysmeral are available in rodents (rat, mouse and guinea pig) and non-rodents (dog, rabbit and monkey).

Studies in rodents: in an oral study male rats were treated with 50 mg/kg bw/d for 1, 2, 3, 4 and 14 days. Testicular toxicity was observed after a single exposure on day 1. Spermatotoxicity with additional systemic toxicity were seen up to day 14. In a subchronic 90-day oral study, rat were dosed with 2, 5, 25, and 50 mg/kg bw/d for five days a week. Toxicity was seen in the liver. Furthermore, a significant decrease in plasma cholinesterase activity ranging from 30%-70% and lower plasma cholesterol levels ranging from 40%-70% at 25 and 50 mg/kg bw/d in both genders was detected. In females treated with 25 and 50 mg/kg bw/d, elevated absolute and relative weights of adrenal glands and hypertrophy of the zona fasciculata were observed. Substance-related testicular toxicity such as spermatoceles in the epididymides and testicular atrophy was observed at 50 mg/kg bw/d. Disturbances of spermatogenesis and spermiogenesis, testicular increases in Sertoli cell-only tubules and increased surface density in Leydig cells were described along with a decreased density of spermatozoa, nucleated cells and spermatoceles in the epididymides of the high dose animals. In the 4-week recovery group, the same testicular

pathology was observed to a lesser extent. The NOEL was set at 5 mg/kg bw/d based on the decreased plasma cholinesterase activity at 25 mg/kg bw/d. NOAEL was set at 25 mg/kg bw/d based on the effects on testes and additional systemic toxicity at 50 mg/kg bw/d.

A supporting study in rats treated for 52 days confirmed the clinical chemistry and the testicular effects. Additionally, 3 subacute 5-day studies confirmed the effects on testis with the NOAEL 25-50 mg/kg bw/d. Studies in mice or guinea pigs treated orally with 100 mg/kg bw/d for 5 days did not show any general adverse effects or adverse effects on male reproductive organs.

In a dermal toxicity study rats were dosed for 5 days. No clinical signs of toxicity or evidence of skin irritation were observed. Slight body weight decrease and marked testicular atrophy was seen in the high dose group (2000 mg/kg bw/d). The NOAEL was set at 1000 mg/kg bw/d, based on testicular and systemic toxicity.

Non-rodents: studies in dogs confirm testicular toxicity and showed systemic effects including clinical chemistry parameters and liver toxicity. An overall NOAEL of 44.6 mg/kg bw/d based on the testicular toxicity was set for dogs. In a pilot study, lysmeral was administered to two male beagle dogs orally in subsequently increasing doses (47-564 mg/kg bw/d) for 9 weeks. Histological examinations revealed multifocal inflammation in the liver of the two animals. In parallel, these dogs showed mild atrophy in seminiferous tubules (necrosis of germ cells, multinucleated giant cells in tubular lumen). Further studies in beagle dogs were performed by administration of 4.4, 22.3 or 44.6 mg/kg bw/d to each 3 male and female dogs or 200 mg/kg bw/d to 3 female dogs for 90 days. In the former study, occasional diarrhoea at 22.3 or 44.6 mg/kg bw/d and vomiting at the high dose group were observed, but no other alterations and no findings from the latter study were reported.

In a study in male rabbits treated with 30, 100 and 300 mg/kg bw/d for 15 days. No general or testicular toxicity was seen. In a study on primates, using a limited number of animals, 2 males, oral administration of 100 mg/kg bw/d lysmeral for 5 days did not lead to any general adverse effects or testicular toxicity.

In conclusion, repeated dose toxicity studies with lysmeral consistently indicate testicular toxicity. Testicular effects are seen after a single exposure in rats. Thus, no duration threshold seems to exist for toxicity. A dose-threshold for testicular toxicity has been shown and rats are shown to be the most sensitive species, followed by dogs.

The evaluating MSCA notes that overall, studies in the species where testicular toxicity was not detected are performed at low doses, since no general toxicity was observed. In most of these studies too few animals were tested. Although other tested species, i.e. mouse, guinea pig, rabbit and monkey seem to be less sensitive to testicular effects, toxicity in these species cannot be excluded as the available data is limited.

7.9.5. Mutagenicity

Not assessed.

7.9.6. Carcinogenicity

Not assessed.

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

7.9.7.1. Fertility

There is clear evidence for toxicity of lysmeral to reproduction. Lysmeral has been shown to induce testicular toxicity and spermatotoxicity in rats and at higher dose levels in dogs. Infertility in rats due to adverse effects of lysmeral on the male reproductive system has

been confirmed in feeding studies. Several acute and repeated dose toxicity studies show testicular toxicity in mammals following oral exposure (detailed under 7.9.4). Based on evidence from experimental animals, lysmeral is classified for reproductive toxicity, as Repr. 1B, H360Fd.

Species dependencies in susceptibility to lysmeral has been observed in the existing studies. Lack of effects in mice, guinea pigs, rabbits and monkeys were reported. However, existing data is inconclusive on species sensitivity (also see section 7.9.1). Differences in the metabolic conversion of lysmeral to TBBA (shown to cause similar testicular toxicity) has been proposed as a possible mechanism for these species differences in sensitivity. TBBA has a highly similar toxicity profile to lysmeral and is classified as Repr. 1B for fertility.

To address the concern for reproductive toxicity and ED potential of lysmeral an Extended one-generation reproductive toxicity study (EOGRTS), according to the OECD TG 443 with extension of the cohort 1B to produce the F2 generation, inclusion of the developmental neurotoxicity (cohorts 2A and 2B) and developmental immunotoxicity (cohort 3) was requested.

A range finding study was performed initially to select the doses for the main study. Rats were treated with lysmeral formulated in alginate-based microcapsules via diet at 230, 750 and 2300 ppm, corresponding to a dose range of 2.3-34.7 mg/kg bw/d. In the F0 males systemic toxicity consisting of decreased body weight, haematology and increased liver weight was seen at the mid and high dose (9.1/7.4 and 27.5/25.1 mg/kg bw/d, pre/postmating). Testicular and epididymal effects were seen at the high dose where mean motile sperm was accounted to 25% and abnormal sperm to 72%. Maternal toxicity at the high dose (30.6-34.7 mg/kg bw/d) resulted in reduced body weight and haematology. Toxicity in reproductive parameters including decreased fertility indices (40%) and increased mean implantation losses (17.7±23.57 versus 3.8±6.85 in controls) were seen at the high dose (25.1-34.7 mg/kg bw/d). No reproductive effects were observed at and below 7.4-11.9 mg/kg bw/d.

Based on the outcome of the range finding study doses for the main EOGRTS were set at 75, 230 and 750 ppm, corresponding to the nominal dose levels of 1, 3 and 10 mg/kg bw/d. At the mid and low dose, 3 and 1 mg/kg bw/d, no adverse effects were reported in any generation. At the high dose (10 mg/kg bw/d), in females of the first parental generation (P0), body weight and body weight gain were slightly reduced (mostly below 10%). Absolute and relative liver weights were increased in presence of minimal to slight liver histopathology. Absolute and relative ovary weights were significantly reduced statistically in a dose dependent manner. The weight decrease (absolute 97.067 mg; relative 0.045%) was below the historical control range values (absolute 109.542–130.320 mg; relative: 0.046–0.056%). This change was judged by the authors as “not treatment-related and attributed to physiological differences in the phases of the sexual cycle”. This reasoning is not clear to the evaluating MSCA, since the values were means compared to the mean values from the control animals. According to the OECD Guidance Document 43 (OECD 2008) in the rat, ovarian weight does not fluctuate during the oestrus cycle, and changes should be considered adverse. It is also stated that the function of the ovary shifts during the oestrus cycle so histopathology can reveal a variety of effects. The nature and the magnitude of the histological lesions enable to determine whether there is a concomitant effect on reproductive performance. The evaluating MSCA notes that the reported changes in the ovary weights were small and there were no corresponding changes in ovarian histopathology.

In the first parental generation (P0) males, the only systemic toxicity reported was prolonged prothrombin time at the high dose. In the F0 males, the mean percentage of abnormal sperm in the cauda epididymidis was increased significantly (6.3±0.6 vs 9.8±13.2 in controls and at 10 mg/kg bw/d) and was above the historical control in the high dose animals. This change was regarded as treatment-related by the study authors. The authors argue there were no findings in the F1 offspring males or any indication of impaired fertility in the F0 or F1 generation and thus regarded this effect as not of “toxicological relevance”. In the second parental generation (P1), body weight and body

weight gain were reduced in the high dose males and females. Liver weights were increased. Reproductive parameters including oestrous cycles, mating index, gestation index and birth index were not affected.

The evaluating MSCA concludes that lysmeral is a reproductive toxicant, based on the available repeated dose toxicity studies. The EOGRTS was performed at doses below the NOAEL for reproductive effects and thus did not provide information on reproductive effects.

7.9.7.2. Development

In the EOGRTS, the pup body weight of the F1 and F2 offspring was reduced at the high dose by 14-16% throughout the lactation period and did not recover until weaning. The pup weights were about 10% below the control values at PND 21. Absolute and relative liver weights were increased in presence of histopathology in males and females. In the F2 generation at the high dose pup mean body weight and body weight gain were decreased throughout the lactation period.

Changes were also observed in the Anogenital distance (AGD) in both F1 and F2 pups at the high dose. F2 male and female pups had statistically significant reduced mean AGD (4%) and were at the lower limit of the historical control values. The AGD index (AGD (mm)/cubic root of pup weight (g)) was increased in the high dose F1 males and females with no statistical significance (Table 10). The NOAEL for systemic toxicity was set to 3 mg/kg bw/d based on reduced pup body weights.

The evaluating MSCA notes that adverse effects on development were observed in the EOGRTS. These included decreased body weights and liver toxicity in the offspring.

7.9.7.2.1. Developmental neurotoxicity

An inhibitory effect of lysmeral on the peripheral acetylcholinesterase (AChE) activity was reported in the repeated dose toxicity studies in rats. In these studies, lysmeral was shown to inhibit plasma, erythrocyte and liver AChE. However, the extent and impact of this inhibition consequent to pre- or postnatal exposure on the function of the nervous system was not clarified.

In the EOGRTS study, AChE activity was measured at PND 4, 22 and 76. At PND 4 in the high dose males, AChE activity was decreased in serum (12%), erythrocytes (28%), diaphragm (8%) and cortex (6%). At PND 22 in the high dose females, AChE activity was decreased in hippocampus (mean 9%). At PND 76 in the high dose females, AChE activities were decreased in serum (46%), erythrocytes (9%), diaphragm (9%) and cortex (6%). In the mid dose females, AChE activities were decreased in serum (27%). The Registrants judged these changes as treatment related, but not adverse at PND 4 and potentially adverse at PND 76.

Neurobehavioral testing at PND 24 revealed lower maximum amplitudes in the auditory startle response test of the high dose F1 males. The maximum amplitude was below the placebo-control values in each part of the measurement, but statistically significant in 2 of total 5 measurements. This led to an overall lower average maximum amplitude in these animals. However, in comparison to the corresponding vehicle control data and high dose F1 females the placebo control values were unusually high, which the authors suggest may providean explanation for this effect.

According to the US EPA Guidance (2000), inhibition of AChE in the central nervous system is an indicator of an adverse effect. Reductions in neural AChE activity may not always be accompanied by overt clinical signs because, e.g., the critical functions of those specific neurons may not be sufficiently evaluated to detect related changes or tolerance may have developed. The time at which potential functional effects are evaluated may also contribute to an apparent lack of concordance between functional effects and the neurochemical effects, i.e. cholinesterase inhibition. Based on such factors, it is difficult to determine the degree of cholinesterase inhibition that will cause specific physiological or behavioral

changes. Data showing such a decrease are appropriate for use as a critical effect for the derivation of reference doses.

The evaluating MSCA agrees that observed changes in the AChE activity should be used for setting the developmental NOAEL to 3 mg/kg bw/d.

7.9.7.2.2. Prenatal developmental toxicity

Several prenatal developmental toxicity studies, according to the OECD TG 414, with lysmeral are available. These studies show developmental effects such as reduced fetal weights and incomplete ossification. In a prenatal developmental toxicity study with the Substance, increased postimplantation loss, decreased fetal body weights and increased skeletal variations were reported at 45 mg/kg bw/d. Developmental effects were seen in presence of maternal toxicity, i.e., reduced body weights, increased liver weights and peripheral acetylcholinesterase inhibition.

During the discussions in the RAC for classification of lysmeral for reproductive toxicity, these studies were taken into account. No correlation was found between the individual maternal weight loss and the respective pup weights. Therefore, RAC considers the effects on pup body weights not secondary to maternal toxicity and thus relevant for classification. The evaluating MSCA agrees with this conclusion.

7.9.8. Hazard assessment of physico-chemical properties

Not assessed.

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

In the SEv decisions sent to the Registrants in 2014, information on the justification behind the derivation of the critical DNELs was requested. The CSRs were updated in 2017.

In the updated registrations, DNELs for workers and consumers and general public for the long-term systemic effects are derived (CSRs June 2017). According to the information in the registrations, long-term DNELs for the systemic effects are derived from the NOAEL=5 mg/kg bw/d from the oral repeated dose toxicity studies, based on reproductive toxicity effects.

To derive the DNELs for local effects after short-term or long-term dermal exposure, data for skin sensitization were used. In the human skin sensitization studies, i.e., human repeated insult patch test, no sensitisation reactions were observed after repeated application of 4125 µg/cm². Skin sensitisation reactions of a single subject were reported after application of approx. 30000 µg/cm². Quantitative animal data from several Local Lymph Node Assays (LLNA) using different vehicles were also considered. The EC3 values (i.e., the concentration of test chemical required to induce a 3-fold increase in lymph node cell proliferation) ranged from 3 % to 14% (743-3478 µg/cm²) in these studies. Using a weight of evidence approach, the No expected sensitization induction level (NESIL) for lysmeral was set at 4100 µg/cm² by the expert panel of the Research Institute for Fragrance Materials (IFRA 2015). This was the basis for the recommended concentration limits for lysmeral in final products, i.e., IFRA standard of the international fragrance association.

For derivation of DNELs for the general population for the systemic effects after long-term dermal exposure the NOAEL=5 mg/kg bw/d from the repeated dose toxicity studies is used.

No DNELs for the short-term local effects via the oral or inhalation route were derived for workers.

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

Lysmeral has self-classifications as skin irritant and skin sensitiser, Skin Sens. 1 and 1B.

Lysmeral has a harmonised classification for reproductive toxicity, fertility, and development, Repr. 1B, H360Fd.

7.10. Assessment of endocrine disrupting (ED) properties

Additional concerns for potential endocrine disrupting properties for human health and for the environment were identified.

Publicly available information suggests *in vitro* oestrogenic activity, both agonistic and antagonistic of lysmeral. Available studies suggest that *in vitro*, lysmeral or its metabolite tert-butylbenzoic acid (TBBA) binds to and modulates the activity of the human and fish oestrogen receptor, respectively.

In vivo studies show toxicity of lysmeral to male endocrine organs/endpoints, including testes and sperm parameters, concurrent with infertility. Available data suggested species-dependency in lysmeral-induced testicular toxicity (see section 7.9.4). No toxicity to the female endocrine endpoints was reported in the existing studies.

The *in vitro* (anti)oestrogenic activity together with the toxicity observed in male animals raised a concern for possible ED properties of the Substance, for both human health and the environment. In the first SEv decision sent in 2014, an Extended One-Generation Reproductive Toxicity Study (EOGRTS) in rats and a Fish Short Term Reproduction Assay (FSTRA) screening study were requested to address the ED concerns. The registration dossiers were updated with these studies in 2017. The available data relevant for lysmeral ED assessment, including the abovementioned new studies, were discussed at the ECHA advisory ED Expert Group meeting in 2017 (10th ED EG meeting). No conclusion was reached at the meeting regarding possible ED properties for human health. The experts advised to follow up the screening study in fish with an OECD TG 234 or an OECD TG 240 to enable a conclusion on possible ED properties for the environment.

7.10.1. Endocrine disruption – Environment

7.10.1.1. In vitro data

In an *in vitro* study using the fish rainbow trout (rt) oestrogen receptor, 4-tert-butylbenzoic acid (TBBA), a main metabolite of lysmeral, was shown to bind the rt oestrogen receptor (Tollefsen and Nilsen, 2008). In another study, TBBA was shown to have both agonistic and antagonistic activity in vitellogenin (VTG) production, at high and low micromolar concentrations, respectively (Tollefsen et al., 2008). This was observed in primary rt hepatocytes when cells were co-exposed with 17b-oestradiol. No effect on VTG production was observed after treatment with TBBA alone.

7.10.1.2. In vivo data

Based on the *in vitro* data, a Fish Short Term Reproduction Assay (FSTRA), according to the OECD TG 229, was requested in the first SEv decision to address the concern for potential ED properties in aquatic species.

The FSTRA was performed using Fathead minnow with the nominal concentrations 0.0195, 0.0625 and 0.200 mg/L. For dose selection, an acute LC50 of 2 mg/L derived for Zebrafish was used. The highest dose corresponded to 10% of the LC50. The measured concentrations of the (parent) substance were about 50% of the nominal doses. However, when measured concentrations of the oxidation product (lysmeric acid) was added, concentrations were within 20% difference from the nominal doses. In this study, no general or reproductive toxicity was reported at any dose. No adverse effects were

observed on survival, growth, appearance, behaviour, secondary sex characteristics or fecundity.

VTG levels were measured in the blood plasma of male and female fish. In males, mean VTG values were significantly increased at 0.0625 and 0.200 mg/L (Table 9). Median values were increased at all doses, compared to the controls by one order of magnitude. The individual VTG concentrations varied by as much as 5 orders of magnitude in the same treatment group, resulting in high coefficients of variation (% CV). A plausible reason for these variations was not identified.

The evaluating MSCA notes no systemic toxicity was reported in this study and testing higher concentrations may have been needed. Furthermore, the high individual variation in VTG levels within each treatment group adds to the uncertainty of the results. The VTG concentrations were increased significantly, by several orders of magnitude in absence of general toxicity. The difference between the controls and the two highest dose groups was statistically significant with both the Jonckheere-Terpstra trend test and the Wilcoxon test with Bonferroni-Holm adjustment. According to the OECD TG 229, VTG measurements should be considered positive if there is a statistically significant increase in males ($p<0.05$), at least at the highest dose tested and in the absence of signs of general toxicity. Accordingly, the evaluating MSCA interprets the data as positive for VTG induction.

Table 9

Plasma vitellogenin levels (mg/L) in male Fathead Minnow, p <=0.05					
Nominal Conc (mg/L)	Mean measured conc (mg/L)	Mean measured conc (mg/L) lysmeric acid	Mean	Median	%CV
0 (control)	0	0	2418	2217	106%
0 (solvent)	0	0	20322	3013	190%
0.0195	0.0092	0.0147	153041	52868	154%
0.0625	0.0383	0.0326	**980843**	**76560**	177%
0.200	0.117	0.0813	**38652**	**44200**	72%

The observed VTG induction in the male fish was consistent with the *in vitro* data, indicating oestrogenic potential of the lysmeral metabolite TBBA. The evaluating MSCA concluded that further information was needed to address possible adverse effects *in vivo* and to determine if the Substance acts as an ED in the environment.

A second SEv draft decision, with a request for a Fish Sexual Development Test (FSDT), according to the OECD TG 234, was prepared and sent to the Registrants for comments. In their comments on exposure of the Substance to the environment, the Registrants argued that the volumes of the Substance will decline considerably in the foreseeable future, as the Substance has been added to Annex VI of the CLP with the classification Repr. 1B and will be added to Annex II (prohibited substances) of the EU Cosmetic Regulation cosmetics-prohibited-subs - ECHA (europa.eu). Subsequently, the Substance will be banned in any kind of cosmetic products. The harmonised classification will further restrict the concentrations of the Substance in home care products for the general population to below 0.3%.

The evaluating MSCA re-assessed the hazard data and performed a mode-of-action and weight of evidence analysis. Also, the possible benefit of addressing the ED potential of the substance to improve its regulatory risk management was analysed. This resulted in no significant benefit for the regulatory risk management and SEv was terminated without further information request (see sections 7.10.3, 7.12 and 7.13).

7.10.2. Endocrine disruption - Human health

7.10.2.1. In vitro data

A study using a human cell-line suggests (anti)oestrogenic activity for lysmeral (Charles and Darbre, 2009). In this study using the oestrogen-responsive human breast cancer cells MCF7, lysmeral was shown to (i) inhibit binding of oestradiol to the oestrogen receptor (ER) alpha and beta at 3 000 000-fold molar excess, (ii) increase expression of the oestrogen-responsive genes (both a transfected reporter gene and an endogenous gene) and (iii) increase cell-growth. The latter was suggested to be through an ER-mediated mechanism. A small oestrogen antagonistic effect on proliferation was observed, when cells were co-treated with 17b-oestradiol and lysmeral. The study was performed without metabolic activation. Lack of metabolic activation in this study can be regarded as a limitation as lysmeral is effectively metabolised *in vivo* (section 7.9.1).

In vitro studies to examine interaction of lysmeral with the androgen receptor are not available. In a study using primary rat Leydig cells, no effect on basal or gonadotrophin-induced testosterone production was observed when cells were treated with lysmeral.

The evaluating MSCA notes that the available *in vitro* data indicate potential (anti)oestrogenic activity for the substance.

7.10.2.2. In vivo data

There is clear evidence that lysmeral is a reproductive toxicant, predominantly toxic to male fertility, through induction of testicular toxicity, as shown in some mammalian species. Testicular toxicity induced by lysmeral has been observed in rats and dogs, but not in mice, guinea pigs, rabbits, or monkeys.

In a short-term study male rats were treated with 50 mg/kg bw/d for 1 to 14 days. Notably, testicular toxicity was observed already after a single exposure on day 1. In a 90-day subchronic study rats were treated with 2, 5, 25 and 50 mg/kg bw/d. Spermatoceles in epididymides and testicular atrophy was observed at 50 mg/kg bw/d. Also, disturbances of spermatogenesis and spermiogenesis, testicular increase in Sertoli cell-only tubules and increased surface density in Leydig cells were described along with a decreased density of spermatozoa, nucleated cells and spermatoceles in the epididymides. In the 4-week recovery group, the same testicular pathology was observed, but to a lesser extent. In females elevated weights of adrenal glands and hypertrophy of the zona fasciculata was reported at 25 and 50 mg/kg bw/d. Studies in mice and guinea pigs treated with 100 mg/kg bw/d for 5 days did not show any general toxicity or adverse effects on male reproductive organs.

Studies in dogs also show testicular toxicity. A NOAEL of 45 mg/kg bw/d based on testicular toxicity was set for dogs. In one study, lysmeral was administered to two male dogs in a dose range of 47-564 mg/kg bw/d for 9 weeks. These dogs showed atrophy in seminiferous tubules. In other studies, in dogs administration of 4, 22 or 45 mg/kg bw/d to 3 males and females and 200 mg/kg bw/d to 3 females for 90 days no other findings than diarrhoea and vomiting was reported. In rabbits treated with lysmeral at 30, 100 and 300 mg/kg bw/d for 15 days no general or testicular toxicity was reported. In a study on two male primates administration of 100 mg/kg bw/d lysmeral for 5 days showed no general or testicular toxicity.

The evaluating MSCA notes that lysmeral-induced toxicity shows species-dependency. However, some of the studies in the species where testicular toxicity was not detected were performed at a too low dose range. Also, in most of these studies too few animals were tested. Thus, the difference in toxicity to lysmeral between species may be quantitative rather than qualitative.

Testicular toxicity reported in the repeated dose toxicity studies added to the concern for potential ED properties of lysmeral. However, information was insufficient to conclude. To address the ED concern and additional concerns related to reproductive toxicity (section 7.9.7) an Extended One-Generation Reproductive Toxicity Study, according to the OECD

TG 443, was requested in the SEv decision issued in 2014. The requested study was with extension of the cohort 1B to produce F2 generation and inclusion of the developmental neuro- and immunotoxicity cohorts.

The registrations were updated with the EOGRTS in 2017 (more detailed assessment of this study in section 7.9.7). In a preceding range-finding study rats were treated at a dose range of about 2-35 mg/kg bw/d (measured doses). Lysmeral uptake in males was measured to a mean of 2.8/2.3, 9.1/7.4 and 27.5/25.1 mg/kg bw/d (pre-/postmating) and in dams to 3.3-3.6, 10.6-11.9 and 30.6-34.7 mg/kg bw/d during premating and gestation. In the first-generation males decreased body weight, haematology and increased liver weight was seen at the mid and high dose. Testicular and epididymal effects were seen at the high dose, where mean motile sperm was 25% and abnormal sperm 72%. Decreased fertility indices (40%) and increased mean implantation losses (17.7±23.6 versus 3.8±6.8 in controls) was also seen at the high dose group animals. No reproductive effects were observed at and below about 7-12 mg/kg bw/d.

Based on the outcome of the range-finding study doses for the EOGRTS were set to the nominal doses 1, 3 and 10 mg/kg bw/d. Measured doses were about 1.4, 4.5 and 15 mg/kg bw/d. At the mid and low dose no treatment-related adverse effects were reported in any generation. In the high dose P0 males the only reported systemic effect was prolonged prothrombin time. In these animals the mean percentage of abnormal sperms in the cauda epididymidis was statistically significantly increased (9.8±13.2 vs 6.3±0.6 in controls) and was above the historical control. This change was regarded treatment related. Reproductive parameters including mating, fertility and birth indices were not affected.

The evaluating MSCA notes that the high dose was set below the reproductive LOAEL from the range finding study (about 25 mg/kg bw/d), which implied a lack of data on ED parameters such as oestrous cycles, nipple retention, anogenital distance and vaginal opening at doses that cause reproductive toxicity (above 15 mg/kg bw/d). In respect to the selection of doses, the Registrants argued that attention was given to the fact that the high dose in the range-finding study produced an impairment of the male ability to reproduce. Only 4 out of 10 mating pairs were able to produce living offspring and the litter size in those fertile pairs was only 36% of the control. Using dose levels closer to 25 mg/kg bw/d (than the effective doses of about 15 mg/kg bw/d) in males would have increased the risk that the goal set to ensure production of a high enough number of pups was not met. The Registrants pointed out that the EOGRTS request was with additions to the standard protocol (measurement of AChE), which generated the demand for a higher number of offspring. This demand was only met by increasing the number of F0 mating pairs. These additions further increased the pressure to make sure that enough offspring were produced. Higher dose levels would have created a high uncertainty in the study design and would have required to use a triplicate number of F0 animals in the EOGRTS, if a similarly impaired fertility as in the range finding study was considered.

The evaluating MSCA acknowledged the reasoning given by the Registrants regarding the dose selection, considering the effects of the substance on fertility. However, low dose levels are considered as a limitation of the study, as possible effects on ED-sensitive parameters at higher doses could not have been detected.

In the EOGRTS, the pup body weight of the F1 and F2 offspring was reduced up to 16% at the high dose throughout the lactation. Changes were also observed in the Anogenital Distance (AGD) (Table 10). F2 male and female pups had significantly reduced mean AGD (4%) and were at the lower limit of the historical control values. However, the AGD index (AGD (mm)/cubic root of pup weight (g)) did not indicate a change in these animals. According to the OECD Guidance Document 150 (OECD 2018) AGD is influenced by the body weight and therefore, normalisation using the cube root of body weight is recommended. A statistically significant change in AGD that cannot be explained by the size of the animal indicates an adverse effect. The evaluating MSCA noted that the decrease in the AGD in the F2 pups was not seen after normalisation.

Table 10

ANOGENITAL DISTANCE (AGD) AND ANOGENITAL INDEX (AG INDEX) VALUES IN THE F1 AND F2 PUPS. * (P≤0,05)				
Dose (mg/kg bw/d)	**0**	**1**	**3**	**10**
F1 Mean AGD m/f	3.08/1.48	3.1/1.48	3.08/1.47	3.01/1.47
F2 Mean AGD m/f	3.08/1.55	3.01/1.54	3.05/1.54	**2.97*/1.49***
F1 Mean AG index m/f	1.62/0.79	1.62/0.79	1.63/0.79	1.67/0.83
F2 Mean AG index m/f	1.61/0.83	1.57/0.82	1.60/0.82	1.64/0.83

In an EOGRTS, changes in the ED-sensitive endpoints indicate possible endocrine disruption. These endpoints include weight and histopathology of the endocrine organs, oestrous cyclicity and pup parameters, such as nipple retention, AGD, vaginal opening and periputial separation. In the study with lysmeral examination of these endpoints showed no changes.

The evaluating MSCA concludes that *in vivo* data for lysmeral indicate species dependency and dose threshold for testicular- and reproductive toxicity. No consistent pattern of effects indicating ED properties, specifically (anti)oestrogenic activity, is observed in the existing data to link the *in vitro* ED indications to the testicular toxicity of the Substance. As discussed above, there are limitations in the *in vivo* data. Importantly, in the EOGRTS the tested dose range may have been too low to detect ED effects, as the highest dose was below the LOAEL for reproductive toxicity and this dose produced minimal systemic effects. As the Substance causes infertility, low doses were chosen to enable sufficient pup production for conduct of the full study.

7.10.2.3. Mode-of-Action for reproductive toxicity

A Mode-of-Action (MoA) analysis for lysmeral-induced testicular toxicity was provided by the Registrants in 2017. The proposed MoA indicates that toxicity of lysmeral is mediated via its metabolite tert-butylbenzoic acid (TBBA). According to this MoA, TBBA forms conjugates with Coenzyme A (CoA). TBBA-CoA conjugate accumulation leads to disruption of the intracellular pools of CoA, consequent disruption of lipid synthesis and testicular toxicity (Figure 1). Lipids, in particular complex lipids such as sphingolipids, ceramides and phosphatidylcholines are essential in spermatogenesis and disturbances in lipid biosynthesis has been linked to testicular toxicity and male infertility. The biosynthesis of complex lipids, in turn, is dependent on CoA (reviewed by Naquet et al., 2020). Disturbances in CoA homeostasis by TBBA-CoA accumulation is presented as causal for testicular toxicity of lysmeral.

The proposed MoA has been supported by *in vitro* and *in vivo* data, which show a correlation between kinetics of TBBA-CoA conjugate accumulation, disruption of lipid synthesis and testicular toxicity. Formation of TBBA-CoA has been shown *in vitro* in hepatocytes, where these conjugates accumulate. In rat hepatocytes, TBBA and lysmeral were rapidly and dose dependently transformed to TBBA-CoA conjugates and accumulated to stable levels. Using primary rat hepatocytes decreased acetyl-CoA levels and inhibition of hepatic lipogenesis by TBBA has also been shown.

Figure 1. Proposed Mode-of-Action for lysmeral induced testicular toxicity.

Comparative toxicokinetics studies show higher levels of TBBA formation in rat hepatocytes compared to other rodent, non-rodents and human hepatocytes (section 7.9.1). These studies support that the level of formation of TBBA correlates with lysmeral toxicity in the susceptible species. Also, comparative analysis of urinary metabolites in different species shows higher level of TBBA in rat compared to other species. Species dependency of lysmeral-induced testicular toxicity correlates with the kinetics of the clearance of TBBA-CoA conjugates. Lower and transient concentrations due to a rapid decrease is shown in the non-sensitive species like mouse and rabbit.

The evaluating MSCA notes that the available *in vitro* comparative metabolism studies have limitations. Generally, from the comparative *in vitro* metabolism studies quantitative conclusions cannot be drawn. Instead, such studies can be used to draw qualitative conclusions - i.e., whether similar metabolites can be found in two different species. In the provided study, basic information such as incubation time, viability of hepatocytes, source of human hepatocytes (pooled sample or not) and whether hepatocytes were used fresh or cryopreserved have not been included. There is no information on number of technical and biological replicates. This is of special importance when evaluating the human data and the inter-individual variability regarding metabolism.

In support of the provided MoA, the Registrants also provided metabolome analyses (registration dossier). In two studies, rats were treated for 28 days with 15 and 45 mg/kg bw/d lysmeral. Blood samples were taken after 7, 14 and 28 days. For comparison, the related alcohol, lysmerol, at 10 and 50 mg/kg bw/d or meta-lysmeral at 150 and 450 mg/kg bw/d were tested. Mass spectrometry was used for profiling of metabolites including the steroid hormones and catecholamines. By comparing 204 different metabolites it was shown that most metabolites that were commonly changed were lipids, fatty acids and fatty acid related metabolites. Plasma levels of all these metabolites were decreased compared to levels in the untreated animals. In contrast, meta lysmeral which does not cause testicular toxicity showed a different metabolite pattern for complex lipids. The study did not show any consistent change of steroid hormones, including androstenedione, testosterone, progesterone, 11-deoxycorticosterone or corticosterone as a result of lysmeral treatment.

This MoA was discussed in the meeting of the RAC in 2018 for harmonised classification of lysmeral. In their opinion (January 2019), RAC considered the proposed MoA plausible, but not sufficient to preclude relevance for humans. Regarding the species-specificity of lysmeral-induced toxicity, RAC indicated that even though some quantitative differences have been shown between rats and humans to dismiss these effects for humans and downgrade the classification to Category 2, RAC would have needed stronger (mechanistic) evidence.

The evaluating MSCA agrees with the RAC opinion that the proposed MoA for testicular toxicity of lysmeral seems plausible. The MoA is partly supported by experimental data (also see section 7.10.2.5). Further investigation is needed to define the steps following TBBA-CoA conjugate formation, e.g. in different tissues (hepatocytes and testes) to clarify the mechanisms of toxicity.

7.10.2.4. Analogue substances

Lysmeral is structurally similar and belongs to the group of alkyl aldehyde substances. Additional substances in the group include EC 242-016-2: 3-(4-tert-butylphenyl)propionaldehyde (bourgeonal) and EC 203-161-7: 3-p-cumenyl-2-methylpropionaldehyde (cyclamal). These substances share a similar structure and toxicity profile. Like lysmeral, bourgeonal and cyclamal cause testicular- and reproductive toxicity.

A five-day oral study (non-guideline, GLP) with bourgeonal shows testicular and epididymal toxicity in rats treated with 100 or 250 mg/kg bw/d. A more recent reproductive and developmental toxicity screening study (OECD TG 422) with bourgeonal is also available (registration information on ECHA website Substance Information - ECHA (europa.eu). In this study, rats were treated via gavage with 0.5, 1 or 5 mg/kg bw/d for 28 days. Bourgeonal did not cause any mortality or clinical signs in males or females at any dose.

There were no macroscopic or microscopic findings, alterations in organ weights or reproductive effects. Based on this study, the NOAEL for general and reproductive toxicity was set to 5 mg/kg bw/d, the highest dose tested. The evaluating MSCA notes that the study showed no effect up to the highest tested dose, which was also below the LOAEL observed in the earlier study. Thus, a too low dose may have been tested.

Also, cyclamal is a reproductive toxicant in rats. In a one-generation reproductive toxicity study, rats were treated with 0, 25, 75 or 150 mg/kg bw/d cyclamal via gavage. In males, changes in reproductive organs were observed at and above 75 mg/kg bw/d. Infertility was reported at 150 mg/kg bw/d. No effect on ED-sensitive parameters in pups, such as sexual maturation or nipple retention was reported. Furthermore, no effects on oestrous cyclicity, mating and fertility parameters were observed in females. Pregnancies occurred in all the treated females and all pregnant dams delivered litters, when mated with untreated males at the high dose. In contrast, treated males at this dose level produced only one pregnancy (1/24) after mating with untreated female rats, indicating a predominant adverse effect on testes and male fertility by cyclamal. In rabbits treated for 14 days with up to 300 mg/kg bw/d no effect on male reproductive organs or spermatogenesis was reported (registration information on ECHA website).

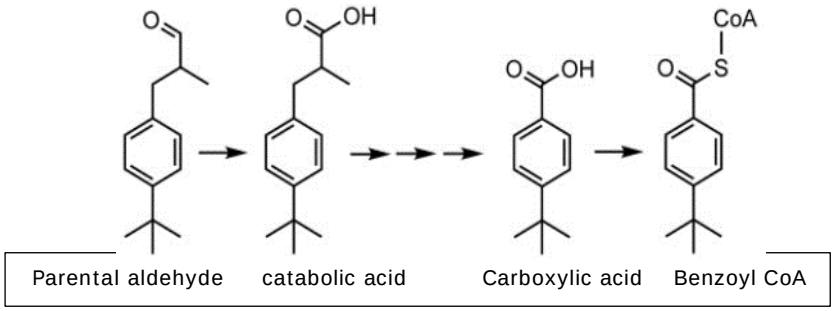


Figure 2. The metabolic pathway leading to formation of the suggested toxic tert-butylbenzoyl-CoA conjugate (TBBA-CoA) from lysmeral. Lysmeral, lysmeric acid and TBBA cause similar effects on male reproductive organs in rats (Laue et al., 2017).

A publicly available study of aromatic aldehydes, including lysmeral, cyclamal and bourgeonal proposes a common MoA for these substances, mediated via their shared metabolite TBBA (Laue et al., 2017). There is clear evidence for TBBA-induced testicular toxicity. TBBA has a harmonised classification as Repr. 1B H360F. Testicular toxicity caused by TBBA is qualitatively identical to that caused by lysmeral. Quantitatively, TBBA is more potent than lysmeral, based on the LOAEL of 8 mg/kg bw/d compared to 25 mg/kg bw/d for lysmeral.

In the study by Laue et al., 2017, it was hypothesized that these aldehydes are metabolized to benzoic acids, such as TBBA as key toxic principle and that CoA conjugates are formed from such acids. Laue et al. performed structure activity relationship studies on the formation of CoA conjugates, either directly from these substances and their related chemicals or their benzoic acid metabolites. The study shows that substances that cause reproductive toxicity in rats formed alkyl-benzoic acids. Compounds metabolized to alkyl-benzoic acids led to accumulation of benzoyl-CoA conjugates at high and steady levels, whereas CoA conjugates of most other xenobiotic acids were only transiently detected. The correlation between this metabolic fate and the toxicity suggested that accumulation of the alkyl-benzoyl-CoA conjugates could impair male reproduction by affecting CoA-dependent processes, required for spermatogenesis. In support of this, derivatives which show no reproductive toxicity in rats did not form benzoic acid metabolites and the corresponding CoA conjugates (Table 11).

Table 11

LEVELS OF TBBA-CoA IN RAT HEPATOCYTES TREATED WITH 50µM OF EACH SUBSTANCE (INDICATED AS % DETECTED IN CELLS TREATED WITH LYSMERAL).				
Substance Name	**EC number**	**LOAEL male reprotoxicity (mg/kg bw/d)**	**Benzoyl-CoA**	
			4h	**22h**
Lysmeral	201-289-8	25	100	100
Lysmeric acid	-	50	90	94
Lysmerol	259-996-2	50	62	62
Meta-lysmeral	263-580-6	>450	18	24
Buorgeonal	242-016-2	>5≤100	115	143
Cyclamal	203-161-7	75	64	66
TBBA	202-696-3	8	110	98

The evaluating MSCA notes that the available data from these analogue substances support a similar MoA for the group, where toxicity is mediated via their common metabolite TBBA. Provided evidence supports that formation of the benzoyl-CoA conjugate is likely a key step for induction of male reprotoxic effects, based on correlation of elevated benzoyl-CoA levels and testicular toxicity shown for these substances and their non-toxic derivatives.

7.10.2.5. Weight-of-evidence analysis and conclusion

According to the OECD Guidance Document 150 a Weight-of-Evidence (WoE) analysis of all the existing data should be performed to conclude on the ED properties of a substance. The evaluating MSCA performed a WoE analysis of the MoA for testicular toxicity caused by lysmeral. The ED, specifically (anti)oestrogenic, MoA and the alternative MoA, namely TBBA-CoA conjugate accumulation were assessed. Assessment of the information from the registration dossiers and publicly available literature, including the identified limitations in the studies are described in the above sections.

In support of an ED MoA, the *in vitro* mechanistic information, based on a study in the human MCF cell-line, suggests (anti)oestrogenic activity (see section 7.10.2.1). However, no *in vivo* mechanistic information is available to further support an (anti)oestrogenic MoA for lysmeral. In the EOGRTS, no effects were observed to further substantiate (anti)oestrogenic activity. The evidence for *in vivo* adversity is mainly based on testicular effects and infertility in males. Although testicular toxicity is an “ED-sensitive” effect, it is not a diagnostic parameter and further evidence is needed to support that it is linked to an ED MoA. Currently, there is no further evidence in the data set to support an ED MoA, e.g. no toxicity to female endocrine organs or ED-sensitive endpoints in the pups from the EOGRTS investigations.

Regarding the consistency of the data, the evaluating MSCA notes that testicular toxicity is consistently observed in studies in rats and dogs, but not in several other tested species. Toxicity across several species is generally expected if caused by an endocrine MoA, e.g., interaction of the Substance or its metabolites with the oestrogen receptor. Furthermore, testicular effects consistently show a threshold in studies in the sensitive species. Toxicity caused by an ED MoA may or may not show threshold. For the proposed alternative MoA, dose threshold and species differences could be expected, due to differences in the metabolic machinery and the kinetics of the clearance of TBBA-CoA in different species.

Regarding the temporality of the effects, in the existing studies, testicular toxicity is observed concurrent with liver toxicity. Concomitant liver and testicular toxicity is in line with the key events in the alternative MoA, that proposes accumulation of the TBBA-CoA

conjugates, which is toxic to liver and the hepatic process of lipogenesis. In the available studies, the indicated timeframe for TBBA-CoA conjugate formation and accumulation is shown to be rapid. This is consistent with the timing of the induction of the testicular toxicity, which is manifested within 24 hours. Such a rapid occurrence of testicular toxicity, e.g., testicular atrophy is generally not observed for ED mediated toxicity.

7.10.2.5.1. Biological plausibility

The biological plausibility of a MoA is deemed based on the broader current scientific knowledge. It is currently well-established that toxicity to male reproductive parameters, including testicular toxicity, can be caused by substances with (anti)oestrogenic activity. However, it is also well-established that a number of non-endocrine mechanisms may cause testicular toxicity. Testicular toxicity, per se, does not indicate an (anti)oestrogenic MoA in the absence of additional ED sensitive effects *in vivo*. Hence, the plausibility of an ED MoA for lysmeral-induced testicular toxicity in the absence of additional *in vivo* effects is deemed low.

The biological plausibility of the alternative MoA, namely testicular toxicity via disruption of CoA homeostasis and lipid biosynthesis is supported in the current knowledge. Xenobiotic-CoA conjugates have been shown to interfere with lipid metabolism and deplete the CoA pools (Darnell et al., 2013 and 2015; Lassila et al., 2015). CoA has been shown to play a key role in many catabolic and anabolic biochemical reactions, including lipid metabolism. The intracellular CoA pool in different tissues has been shown to be highly regulated and responsive to a variety of signals, including disturbances in the turnover of the CoA conjugates (Brass 2002; Naquet et al., 2020)

The link between disruption of Lipid biogenesis and testicular toxicity is also well-established. Complex lipids such as very-long-chain-polyunsaturated fatty acids (VLCPUFA) or ceramides, sphingolipids and phosphatidylcholines containing these PUFAs are present in high amounts in sperm and play an important role for spermatogenesis. These lipids are considered to stabilize cellular membranes with high curvature, such as the rims of the sperm head and provide membrane flexibility needed for efficient sperm formation. Defects in enzymes relevant for synthesis of such lipids have been shown to be associated with testes toxicity and male infertility. This has been shown in transgenic mice with disrupted enzymes such as FADS2 desaturase, ELOVL2 elongase or LPAAT3 acyltransferase (Zadravec et al., 2011; Iizuka-Hishikawa et al., 2017). Disruption of these enzymes is associated with decreases in polyunsaturated fatty acids (PUFAs) and result in disturbances of polyunsaturated ceramide, sphingolipid and phosphatidylcholin formation. In the Elovl2 -/- mice, both Sertoli cells and the Leydig cells appeared normal, whereas primary spermatocytes degenerated, formed multinucleated giant cells and a complete arrest of spermatogenesis was observed. The data imply, that ELOVL2 synthetized VLCPUFAs are essential components for normal completion of spermatocyte cytokinesis, accumulating in sphingolipids with these VLCPUFAs. For the other knock-out mice, normal spermatozoa formation was disturbed, and these animals produced no offspring.

7.10.2.5.2. Remaining uncertainty and conclusion

The identified limitations in the available *in vitro* and *in vivo* data have been discussed in the above sections. The main uncertainty regarding an ED MoA is lack of *in vivo* mechanistic data to support (anti)oestrogenic properties. Moreover, the evidence provided on the alternative MoA further weakens the weight of evidence for an ED MoA for lysmeral-induced testicular toxicity.

The evaluating MSCA concludes that data on lysmeral-induced testicular toxicity is not sufficient to support an ED-mediated MoA of the Substance.

7.10.3. Conclusion on endocrine disrupting properties

7.10.3.1. Human health

Lysmeral has been shown *in vitro* to bind to the human ER, transactivate ER-responsive genes and enhance/inhibit proliferation of an oestrogen-responsive cell-line dose-dependently. The metabolite of lysmeral, TBBA has been shown to bind to the fish ER and to act agonistically and antagonistically (dose-dependently) in VTG production.

In vivo data for lysmeral indicate adverse effect on male reproductive function, through induction of testicular toxicity. Reproductive toxicity caused by lysmeral shows species-dependency and a clear dose threshold. Additional effects on endpoints indicative of an (anti)oestrogenic MoA for lysmeral could not be observed *in vivo*, including in an EOGRTS. No pattern of change in the ED sensitive endpoints to support an ED MoA was observed. The EOGRTS was inclusive, as it was performed at too low doses. Although a hormone-sensitive endpoint, testicular toxicity is not diagnostic for an ED MoA, as it can be caused by various alternative mechanisms. Testicular toxicity by itself, in the absence of additional *in vivo* evidence for endocrine activity is not conclusive on an ED MoA for lysmeral.

As detailed under section 7.10.2.3, an alternative MoA for testicular toxicity of lysmeral was provided by the Registrants. This MoA proposes that testicular toxicity caused by lysmeral is mediated via its metabolite TBBA. Upon production TBBA forms conjugates with Acetyl CoA. Accumulation of this conjugate leads to disruption of CoA dependent cellular processes, including lipid biosynthesis, disruption of which can lead to testicular toxicity. This MoA is supported in part by experimental evidence and is judged as plausible, which weakens the weight of evidence for an ED MoA for the Substance.

Although the available information does not support a ED-mediated testicular toxicity of the Substance, the evaluating MSCA concludes that the concern on ED properties remains unresolved as the EOGRTS was not performed at high enough doses.

7.10.3.2. Environment

Lysmeral has been shown *in vitro* to bind to the human ER, transactivate ER-responsive genes and enhance/inhibit proliferation of an oestrogen-responsive cell-line dose-dependently. The metabolite of lysmeral, TBBA has been shown to bind to the fish ER and to act agonistically and antagonistically (dose-dependently) in VTG production.

The available *in vivo* data on the potential ED properties of the Substance in the environment is not conclusive. The FSTRA screening test suggests that lysmeral may cause VTG induction in fish. However, it is not possible to conclude on potential ED properties in the environment without further testing. The evaluating MSCA did not issue another decision with further test requests to clarify the concern for potential ED properties, for the reasons explained in section 7.13.

7.11. PBT and vPvB assessment

The potential PBT properties of lysmeral were screened. The evaluation was discussed by the ECHA PBT expert group at the 6th PBT expert group meeting in April 2014 and concluded after a written procedure in 2015. Lysmeral was considered to screen for bioaccumulation, to fulfil the T criterion based on the classification as Repro cat. 2 but did not meet the P criterion. Thus, Lysmeral was not considered to meet the PBT/vPvB criteria of REACH Annex XIII. The hazard outcome document was published on ECHAs website in May 2016, PBT assessment list - ECHA (europa.eu).

7.12. Exposure assessment

Exposure (or emission) to the environment of lysmeral occurs mainly via (1) use of cosmetics and home care products and (2) manufacturing of the Substance. As a result of the harmonized classification of lysmeral, its uses are restricted, and its exposure is expected to diminish.

(1) Use of cosmetics and home care products

The use of lysmeral will decline, consequent to its Repr. 1B classification as lysmeral will be banned from cosmetic products. According to the Registrants, this would correspond to a 20-30% decline in lysmeral emission to the environment. Home care products containing lysmeral at concentrations above 0.3% will also be banned. The Registrants specified several uses of lysmeral >0.3%, which are expected to disappear, e.g., washing & cleaning products containing up to 0.74%, biocidal products up to 1.28% and air care products up to 10%. However, products containing <0.3% can still be used. Lysmeral at concentrations lower than 0.3% can be found in various products, the use of which can continue.

The evaluating MSCA notes that although a reduced use of lysmeral in home care products is expected, the extent of this reduction is not fully known. In informal communication, the Registrants indicated that most of the big producers of cosmetics and home care products are globally acting companies, many of them are expected to remove the Substance from their products to act sustainably and to avoid very cost- and labour-intensive reformulation trials of the existing fragrance mixtures in use. Registrants have also been informed by some downstream users, that they plan to substitute the Substance globally in all consumer goods. For this reason, it is estimated that a large portion of the home care products with <0.3% lysmeral will also be impacted.

(2) Manufacturing

The Registrants indicated that a decline in the use in cosmetics and home care products is in turn expected to lead to a decrease in production and consequently in environmental emission by manufacturing. The evaluating MSCA agrees that total emission to the environment during production of lysmeral is expected to decline, as its uses are restricted.

In their comments to the SEV draft decision regarding the exposure of the environment during the manufacturing, the Registrants indicated that the substance is classified cat 3 (strongly water polluting) according to the German Water Protection Directive. For this reason, the manufacturing process is already subject to risk management measures, which minimizes the probability of the chemical being released unintentionally to the environment.
Notably, an actual decline in the use of lysmeral is supported in a recent human biomonitoring study, which shows a decreasing trend over time in the exposure to lysmeral, from 2000 to 2018 (Scherer et al., 2021).

The evaluating MSCA notes that currently there is no quantitative measure of the remaining exposure, e.g., as a result of use of products containing less than 0.3% lysmeral.

7.13. Risk characterisation

Consequent to its classification the information on uses and exposure of lysmeral should be revised in the registrations. The Registrants have informed the evaluating MSCA that they intend to update the registration dossiers, including a revised version of the CSR, by March 2022 (informal communication).

In regard to the regulatory risk management for the Substance, identification as SVHC according to the article 57(f) as an ED for the environment, in addition to 57(c), could lead to higher priority for inclusion of lysmeral in the REACH annex XIV (Authorisation list). If included in the Authorisation list, an application for authorisation would be with low likelihood of success, due to no apparent socio-economic advantage. It should also be considered that less toxic fragrance alternatives are already available. Thus, identification as SVHC for ED for the environment could impose stricter regulatory measures for lysmeral. However, continued evaluation of potential ED effects and (if positive outcome) the following SVHC process will take several years. Until then a further reduction in exposure to lysmeral is foreseen, thus creating the risk of wasting resources for a highly uncertain or limited benefit for regulatory risk management.

Taken together, the evaluating MSCA has considered the hazard data, expected exposure and expected added regulatory benefit of requesting further studies. Weight-of-evidence analysis of the available hazard data does not support the ED MoA for lysmeral. Moreover, the uses and consequently exposure of the Substance is expected to decrease substantially. Therefore, a significant improvement of the risk management measures for lysmeral consequent to possible identification as SVHC, for ED properties for the environment was not foreseen.

7.14. References (not present in the registration dossiers)

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7.15. Abbreviations

AChE	Acetylcholinesterase
AGD	Anogenital Distance
CAS	Chemicals Abstract Service
CLP	Classification Labelling Packaging
CNS	Central Nervous System
CoA	Coenzyme A
CoRAP	Community Rolling Action Plan
CSR	Chemical Safety Report
CV	Coefficient of variation
DD	Draft Decision
DNEL	Derived No Effect Level
DNT	Developmental neurotoxicity
EC	European Commision
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EG	Expert Group
eMSCA	evaluating Member State Competent Authority
EOGRTS	Extended one-generation reproductive toxicity study
EPA	Environmental Protection Agency
ER	Estrogen Receptor
FSDT	Fish Sexual Development Test
FSTRA	Fish Short-Term reproduction Assay
GLP	Good Laboratory Practice
GHS	Globally Harmonized system
IUPAC	International Union of Pure and Applied Chemistry
LD	Lethal Dose
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
MoA	Mode of Action
MSC	Member State Committee
NESIL	No Expected Sensitization Induction Level
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
OECD	The Organisation for Economic Co-operation and Development
PBT	Persistent Bioaccumulative Toxic
PNS	Peripheral Neuronal System
PUFA	Poly Unsaturated Fatty Acid
RAC	Risk Assessment Committee
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation of Chemicals

RMM	Risk Management Measures
RMOA	Risk management option analysis
rt	rainbow trout
SCCS	Scientific Committee on Consumer Safety
SEv	Substance Evaluation
SVHC	Substance of Very High Concern
TBB	p-tert-butyl-benzaldehyde
TBBA	p-tert-butylbenzoic acid
TBHA	Tert-butylhippuric acid
TBT	Tert-butyltoluene
VLCPUFA	Very-long-chain-polyunsaturated fatty acids
VTG	Vitellogenin
WHO	World Health Organisation
WoE	Weight-of-Evidence