CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name:

2-(4-tert-butylbenzyl)propionaldehyde

EC Number: 201-289-8

CAS Number: 80-54-6

Index Number: --

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	2-(4-tert-butylbenzyl) propionaldehyde
EC number:	201-289-8
CAS number:	80-54-6
Annex VI Index number:	--
Degree of purity:	≥ 99 %
Impurities:	Impurities are not considered relevant for the classification and labelling of the substance.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	No classification	No classification
Current proposal for consideration by RAC	Classification Repr. 2, H361f Labelling GHS08 Wng, H361f,	Classification Repr. Cat.3, R62 Labelling Xn; R62
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Classification Repr. 2, H361f Labelling	Classification Repr. Cat.3, R62 Labelling

	GHS08 Wng, H361f,	Xn; R62

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.7.	Reproductive toxicity	GHS08, Repr. 2, H361f,			

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

No other endpoints have been discussed in this report.

Labelling: Signal word:
Warning

Pictogramms:
GSH08

Hazard statements:
H361f: Suspected of damaging fertility.

Precautionary statements:
No subject for Annex entry.

Proposed notes assigned to an entry: none

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Toxicity to reproduction – fertility	Repr. Cat. 3; R62			

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

No other endpoints have been discussed in this report.

Labelling: Indication of danger:
Xn- harmful

R-phrases:
R62 – Possible risk of impaired fertility

S-phrases:
S37 – wear suitable gloves

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

2-(4-tert-butylbenzyl)propionaldehyde (lysmeral) is not legally classified according to Regulation 1272/2008/EC (CLP) and Directive 67/548/EEC (DSD) and is not listed in the Annex I of 67/548/EC Directive and Annex VI of the CLP regulation. The dossier submitter has been in contact with the German MSCA since 2008 in order to discuss the content of the submitted CLH report proposal. In agreement with the German MSCA, BASF SE took the lead to submit the proposal for a CLH report for 2-(4-tert-butylbenzyl)propionaldehyde (lysmeral) to the ECHA.

1.5 Short summary of the scientific justification for the CLH proposal

Toxicity to reproduction

Current classification: no classification in Annex VI of CLP

Proposed classification: Repr. 2, H361f,(CLP) and Repr. Cat. 3; R62 (DSD)

Lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) has been found to induce testicular toxicity and spermatotoxicity when administered orally to rats and at higher dose levels to dogs. Infertility in rats due adverse effects of orally administered lysmeral on the male reproductive system has been confirmed in a feeding one-generation range-finding study. Based on clear evidences from experimental animals, it is considered appropriate to classify lysmeral for reproductive toxicity, i.e. adverse effects on fertility.

However, in determining the appropriate hazard category, the assessment of the relevance of the given hazard to humans needs to be taken into account.

Species specificity for lysmeral induced testicular toxicity has been observed. Adverse effects of lysmeral on the male reproductive system at a clearly defined threshold dose have been found in rats whereas no evidence for testicular toxicity was observed in the mouse and guinea pig. Considering non-rodent species, the dog has been shown to be susceptible towards lysmeral induced testicular toxicity. In contrast, short-term oral exposure to rabbits did not indicate a potential of lysmeral to induce testicular toxicity. Furthermore in rhesus monkeys, no indication of testicular toxicity, at doses causing testicular toxicity in the rats, was observed.

Based on the accordance in the testicular toxicity profile of lysmeral, p-tert-benzaldehyde (TBB), p-tert-butyltoluene (TBT) and the shared metabolite para-tert-butylbenzoic acid (TBBA), the formation of the systemic TBBA intermediate is the causative agent responsible for the mode of action for lysmeral induced testicular toxicity.

Species specificity for lysmeral induced testicular toxicity is reflected by species dependent differences in the conversion of lysmeral to TBBA in hepatocytes. TBBA formation in human hepatocytes is of low magnitude compared to rats and is comparable to levels found in the rabbit at toxicologically relevant doses, a species not sensitive to lysmeral induced testicular toxicity.

Due to its low odour threshold and the perception as rather unpleasant at toxicological relevant but unrealistic concentrations (1000 fold above the respective odour threshold), a prolonged human uptake of lysmeral resulting in doses as in animals is highly unlikely.

Because if the properties of lysmeral as fragrance material leading to palatability issues, substance administration needed to be performed via gavage or encapsulation. This represents a non-relevant

form of application with respect of realistic use patterns. Studies via the relevant route to humans (i.e. dermal) in rats showed no testicular toxicity up to the limit dose. On the basis of comprehensive conservative assumptions, a potential human lysmeral uptake would be of low magnitude when compared to doses leading to rat testicular toxicity

A clear evidence for a species specificity and, if at all, a lower human susceptibility concerning lysmeral induced testicular toxicity raises doubt about the relevance of the effect for humans. Furthermore, evident reproductive toxicity has been observed after substance administration via gavage or encapsulation, representing a non-relevant form of application with respect of realistic use patterns. A low odour threshold and unpleasant perception at toxicologically relevant but unrealistic concentrations are intrinsic properties of lysmeral, making a human lysmeral uptake above the clearly defined threshold dose for lysmeral induced testicular toxicity in rats highly unlikely. Therefore, a classification as substance to be suspected of damaging fertility, i.e. category 2 (H361f; regulation 1272/2008) and Repr. Cat3 R62 (67/548/EEC) is warranted**.**

Developmental toxicity has been observed at doses leading to evident maternal toxicity and is considered to be a secondary non-specific consequence of general systemic toxicity in the dams. Therefore, based on the present data, no classification concerning developmental toxicity is warranted.

1.6 Current harmonised classification and labelling

1.6.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No classification.

1.6.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No classification.

1.7 Current self-classification and labelling

1.7.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification

Repr. 2, H361f

Labelling

GHS08, H361f, Warning

1.7.2 Current self-classification and labelling based on DSD criteria

Classification

Repr. Cat.3, R62

Labelling

Xn

R: 62

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

2-(4-tert-butylbenzyl) propionaldehyde (lysmeral) shows adverse effects on testes and fertility, i.e. reproductive toxicity. Harmonized classification and labelling for CMR and respiratory sensitisation is a Community-wide action under article 36(1) of CLP. 2-(4-tert-butylbenzyl) propionaldehyde (lysmeral) is currently not listed in Annex I of 67/548/EC Directive and Annex VI of the CLP regulation. Repeated-dose toxicity and toxicokinetic data are presented for information as they provide relevant data for assessment of reproductive toxicity but no classification is discussed and proposed for these endpoints and other hazard classes.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

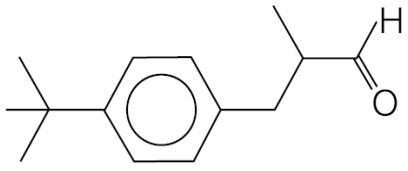
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	201-289-8
EC name:	2-(4-tert-butylbenzyl)propionaldehyde
CAS number (EC inventory):	80-54-6
CAS number:	80-54-6
CAS name:	Benzenepropanal, 4-(1,1-dimethylethyl)-.alpha.-methyl-
IUPAC name:	3-(4-tert-butylphenyl)-2-methylpropanal
CLP Annex VI Index number:	--
Molecular formula:	$C_{14}H_{20}O$
Molecular weight range:	204.31 g/mol

Structural formula:



1.2 Composition of the substance

In our manufacturing process Lysmeral Extra is obtained as racemic mixture (1:1) of the two enantiomers (2S)-3-(4-tert-butylphenyl)-2-methyl-propanal and (2R)-3-(4-tert-butylphenyl)-2-methyl-propanal.

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
• 2-(4-tert-butylbenzyl)propionaldehyde • EC no.: 201-289-8	--	>99 — <= 99.5 % (w/w)	--

Current Annex VI entry: No classification

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Non specified impurities	--	>=0.5 — < 1 % (w/w)	--

Current Annex VI entry: No classification

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	--	--	--	--

Current Annex VI entry: Not applicable.

1.2.1 Composition of test material

Not applicable.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	liquid	Ullmann (2003)	
Melting/freezing point	< -20°C	BASF AG (1991)	
Boiling point	279.5°C at 1013 hPa	BASF AG (1999)	
Relative density	0.94 at 20°C	Ullmann (2003)	
Vapour pressure	0.0025 hPa at 20°C	BASF AG (1999)	
Surface tension	based on chemical structure, no surface activity is predicted.	Expert judgement	Estimated
Water solubility	33 mg/l at 20°C	Givaudan-Roure (1995)	
Partition coefficient n-octanol/water	4.2 at 24°C	Givaudan-Roure (1995)	
Flash point	118°C	BASF_SDS (2006) (Validity 4 (not assignable))	
Flammability	Flammability upon ignition derived from flash point. The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	Expert judgement	Estimated
Explosive properties	Non explosive	Expert judgement	Estimated
Self-ignition temperature	250°C	BASF_SDS (2006) (Validity 4 (not assignable))	
Oxidising properties	No oxidizing properties	Expert judgement	Estimated
Granulometry	Substance is marketed or used in a non solid or granular form	Expert judgement	Estimated
Stability in organic solvents and identity of relevant degradation products	Stability of the substance is not considered as critcal	Expert judgement	Estimated
Dissociation constant	Substance does not contain any ionic structure	Expert judgement	Estimated
Viscosity	3 mm2/sec at 23°C (static)	BASF_SDS (2006) (Validity 4 (not assignable))	

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) is used as fragrance in a wide number of industries. It shows an intensive, radiant, floral odour with a typical lily-of-the-valley note. As a component of fragrance mixtures the main uses include cosmetic/personal care products and washing/cleaning products. Lysmeral may also be included as fragrance substance in air care products, biocidal products, coatings and paints, fillers/plasters, ink/toners, polishes/wax blends and scented articles (clothes, eraser, toys, paper articles, CD).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this report.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Quantitative data on the toxicokinetics of lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) are available from experimental animals (rat, mouse, rabbit, guinea pig, dog and rhesus monkey) and humans. Based on its physico-chemical properties, i.e. water solubility (33 mg/l at 20°C), partition coefficient log Pow (4.2 at 24°C), molecular weight (204 g/mol) and vapour pressure (0.25 Pa at 20°C), lysmeral is considered to have a high bioavailability via the oral route and a limited bioavailability via the inhalation route. After acute and repeated oral and dermal administration of lysmeral to experimental animals and humans there is clear evidence of systemic absorption. However, in humans only very limited percutaneous absorption of lysmeral is observed especially when compared to the rat.

After semiocclusive dermal application of [β-^{14}C]-lysmeral (14.7 µCi or 11.37 mg test substance in 70% ethanol on 10 cm^2 back skin) on 3 human volunteers for 6 hours, a mean of 1.4% (range 0.8 - 2.4%) of the applied dose was excreted in urine within 24 hours, whereas radioactivity was below the detection limit in urine samples of later time points and in all faeces and blood plasma samples (Huntingdon Research Centre, 1994). Taking into account, that the chosen vehicle promotes dermal penetration of the applied test substance, these data indicate very limited percutaneous absorption of lysmeral in humans.

In the rat, distribution predominately to the liver has been observed after dermal administration and can be assumed for the oral route as well. A detailed in vivo study on the metabolism of lysmeral is not available.

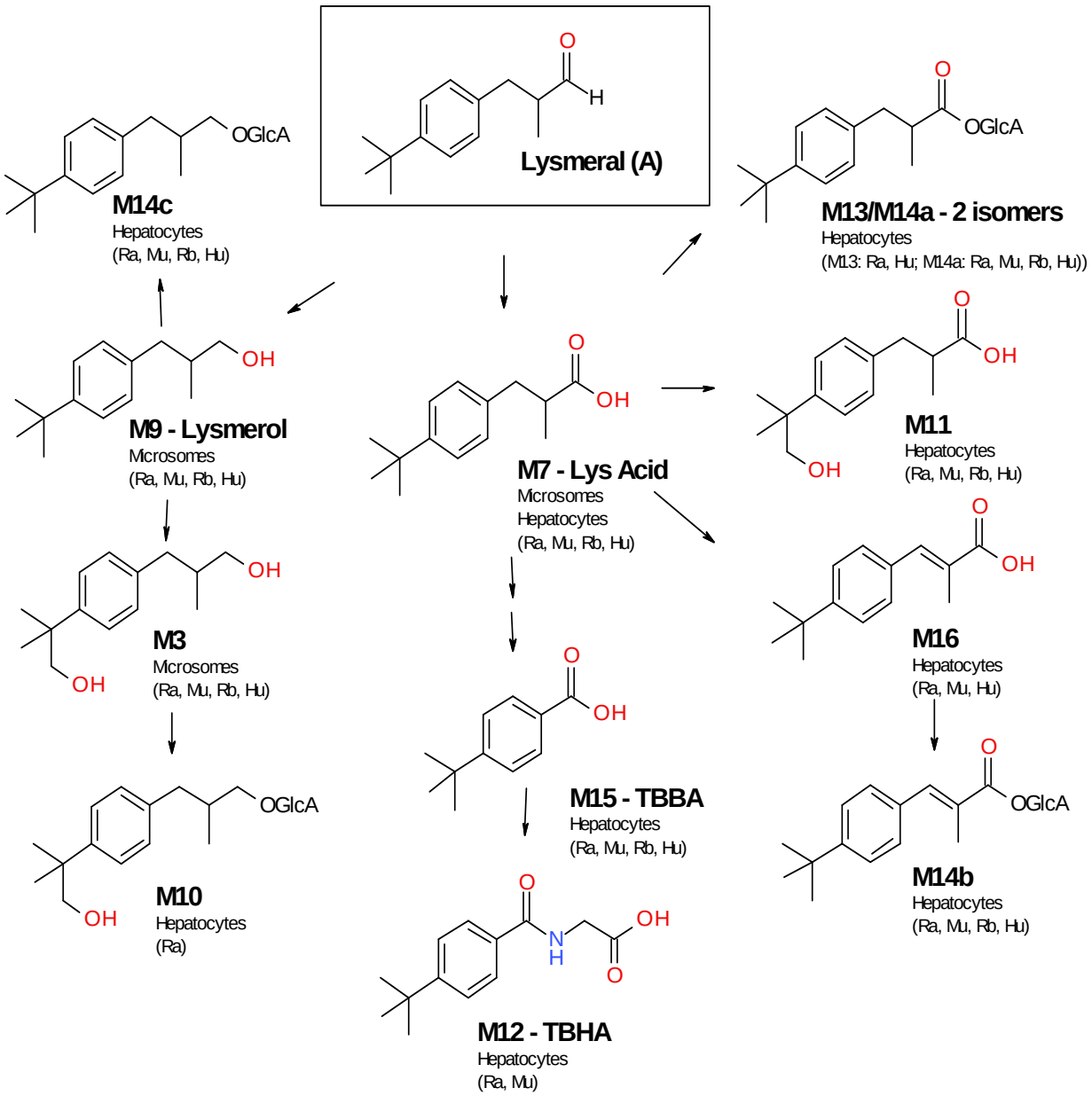
A comparative in vitro metabolism study has been performed in order to study relevant lysmeral specific metabolic pathways (BASF SE 2010). For this purpose liver microsomes and hepatocytes of male Han-Wistar rats, male CD1-mice, male white New Zealand rabbits and male humans were incubated with ^{14}C-lysmeral at nominal substrate concentrations of 10, 50 and 100 µM. Metabolic profiles were detected and quantified by Radio-HPLC after appropriate work up procedures of received incubates. Structure elucidation of formed metabolites was performed from ^{14}C-lysmeral incubates (100 µM) of liver microsomes and hepatocytes of rats and humans by LC/MS-analyses.

On the basis of these findings, metabolic pathways of lysmeral in rodents and non-rodents are depicted in figure 1. In liver microsomes, an oxidation of ^{14}C-lysmeral to its corresponding carboxylic acid (M7-lysmerylic acid) or a reduction to its corresponding alcohol (M9 - lysmerol), being further oxidized at the tert-butyl group to form a hydroxy-metabolite (M3), was observed. In hepatocytes, oxidation to lysmerylic acid was confirmed and its further dehydrogenation (most probably by hydroxylation and dehydration) to (E)-3-(4-tert-Butyl-phenyl)-2-methyl-acrylic acid (M16) was observed. Putative decarboxylation of lysmerylic acid, followed by oxidation to the

propanoic acid derivative and beta-oxidation led to the identified metabolite p-tert-butyl-benzoic acid (TBBA - M15).

This metabolite was conjugated with glycine to form p-tert-butyl-hippuric acid (TBHA - M12) in rodents. In addition to these metabolites, glucuronic acid conjugates of metabolites M3, M7, M9, and M16 were detected. As described below, certain metabolites identified here, i.e. lysmerylic acid, TBBA and TBHA, have also been detected in blood or in urine samples of rodents and non-rodents.

Figure 1: Metabolic pathway of ^{14}C-lysmeral based on the metabolites found in the supernatant of in vitro metabolism studies using liver microsomes and hepatocytes. Ra= rat; Mu=mouse; Rb=rabbit; Hu=human.



After oral administration of radiolabelled [β-^{14}C]-lysmeral in a single dose of 25 and 100 mg/kg to 4 male lbm:RORO (SPF) rats per dose via gavage (Huntingdon Research Centre, 1995) a rapid absorption of the radioactive compound for both doses applied and proportionate plasma maximum concentration (C_{max}) has been observed (table 10). In contrast, AUC was found to increase disproportionate to the dose applied which is interpreted to be indicative for a saturation of the renal clearance of lysmeral acid metabolites.

Table 10: Pharmacokinetic variables of radioactivity in blood plasma after single oral dose administration of [β-14C]- lysmeral to rats. Tmax und Cmax represent the mean of Tmax and Cmax observed for single animals. AUC was estimated up to the last time at which concentrations were above the quantification limit by the linear trapezoidal rule. Testing laboratory specific programs were used for data processing, i.e. "Radioactivity", "Fast Radioactivity" and KIN 5.1.

Dose (mg/kg bw)	C_{max} (µg equivalents/ml)	T_{max} (h)	AUC $_{0-48}$ (µg x h/ml)
25	14.3	3.5 ± 1.9	122
100	52	1.8 ± 0.5	937

After occlusive dermal application of [β-^{14}C]-lysmeral in 70% ethanol to the rat at a single dose of 6.8 mg/kg bw accounting for a topical concentration of 0.2 mg/cm^2 for a maximum of 6 hours (2 animals/time point i.e. 0.5, 1, 3, 6, 12, 24, 48, 72, 120 h sacrifice) good bioavailability and rapid urinary excretion was observed (Huntingdon Research Centre, 1995). In general, the observed maximum plasma concentration was about 20-30 fold lower than after single oral application of 25 mg/kg bw, as seen in the study mentioned above. Up to 120 hours after application of lysmeral, a mean cumulative total of 14.6% of the dose was excreted in urine, 0.8% was recovered in cage washings and 2.0% was excreted via faeces, whereas levels in expired air traps were not detectable. Two animals per time point each were examined for tissue distribution and remaining dose at the application site. 120 hours after application of the test substance, the remaining radioactivity in all tissues investigated – excluding the skin at the application site – amounted to 1.2% of the applied radioactivity. The maximum urinary excretion rate (1.95 µg equivalents/hour) was observed 6 - 12 hours after dermal application of [β-^{14}C]-lysmeral. Dermal radioactivity concentrations were more persistent and declined with a half-life of approx. 152 hours compared to other tissues ranging from 10 - 93 hours (Table 11).

The highest concentration of the absorbed 14C radiolabel was recovered in the liver (Cmax = 15.6 µg/g tissue representing 0.826 % of given dose/g tissue). Overall, a relation of Tmax and the blood perfusion rate of a respective tissue is indicated based on the findings for highly perfused tissues (i.e. lungs, heart) and poorly perfused tissues (i.e. skin, fat).
Although pharmacokinetic variables were not reported for testes in this study, a maximum concentration in this tissue was determined to be 0.008% of the topically applied dose per gram tissue 1 hour post application.

The mean total proportion of dose in excreta and tissues was about 19%, which represents the apparent level of absorption of radioactivity into the systemic circulation.

Table 11. Pharmacokinetic variables of radioactivity in whole blood, plasma and selected tissues after single dermal dose administration of [β-14C]- lysmeral to rats. C_{max} and T_{max} represent the observed values, whereas AUC were estimated up to the last time point at which concentrations were above the quantification limit by the linear trapezoidal rule and extrapolated to infinity (AUC∞). Testing laboratory specific programs were used for data processing, i.e. "Radioactivity", "Fast Radioactivity" and KIN 5.1.

	C_{max} (µg /ml or g tissue)	T_{max} (h)	AUC (µg * h/g)	AUC∞ (µg * h/g)	Terminal rate constant (h)	Terminal half-life (h)
Fat	0.4	24	34.9	-	-	-
Heart	1.3	3	11.7	12.0	0.0644	11
Kidneys	0.6	6	15.6	15.8	0.0655	11
Large Intestines	3.0	12	76.2	86.9	0.0110*	63
Liver	15.6	6	337.5	345.3	0.0205	34
Lungs	0.3	1	7.8	8.6	0.0150*	46
Skin	0.9	24	74.8	183.2	0.0046*	152
Small intestine	2.8	12	75.3	77.9	0.0177*	39
Pancreas	0.5	12	19.4	-	-	-
Stomach	2.5	0.5	16.5	22.6	0.0075*	93
Plasma	0.5	1	6.2	8.9	0.0492*	14
Whole blood	0.4	1	4.6	6.4	0.0521	13

* Period used for the rate constant estimation was < 2-fold above half-life. Therefore, estimated half life and AUC∞ are regarded as approximations.

Blood plasma kinetics of lysmeral and lysmerylic acid in rodents was studied in male Wistar rats (BASF SE 2006A) and male C57BL/6NCrl mice (BASF SE 2006B) after oral application of a single dose of 50 mg/kg bw of each lysmeral and lysmerylic acid by gavage. Blood was taken retroorbitally, 3 days before gavage, directly after the first oral application (i.e. 20 minutes for mice and 10 minutes for rats), as well as 2, 4, 8, and 24 hours after application and blood plasma was analysed for lysmeral and lysmerylic acid by HPLC/MS. After application of lysmeral, no unchanged parent compound was detectable in any plasma sample of both rodent species.

In the male rat, lysmerylic acid was detected in all plasma samples and highest plasma concentration was observed 4 hours after application of lysmeral or directly after application of lysmerylic acid (table 12). In the male mouse, highest plasma concentration of lysmerylic acid was observed directly after application of both lysmeral and lysmerylic acid.

Toxicokinetic parameters for lysmerylic acid in rodents show no species difference, whereas some difference in the toxicokinetics after oral application of lysmeral between rat and mouse can be observed regarding T_{max} and C_{max} (table 12).

Table 12: Plasma kinetics of lysmerylic acid in the male rat and male mouse after application of a single dose of 50 mg/kg bw/day of each lysmeral and lysmerylic acid by gavage (BASF SE 2006A and 2006B). Parameters have been derived using TopFit 2.0 (Heinzel. G. et al.; TopFit 2.0, Pharmacokinetic and Pharmacodynamic data analysis system for the PC; Gustav Fischer Verlag, Stuttgart, Jena, New York; 1993). The AUC was determined according to the linear trapezoidal rule.

Test substance	Species	T_{max}	C_{max} (µg/g)	AUC_{0-24} (µg x h/g)	$T_{1/2}$
Lysmeral	Rat	4 h	8.8	81.4	5.8 h
	Mouse	Directly after application, i.e. 20 minutes.	18.4	85.1	3.3 h
Lysmerylic acid	Rat	Directly after application i.e. 10 minutes.	29.4	89.3	3.6 h
	Mouse	Directly after application, i.e. 20 minutes.	22.1	106.7	4.0 h

Excretion of the expected urinary metabolites of lysmeral, i.e. tert.-butyl benzoic acid (TBBA) and tert.-butyl benzoyl hippuric acid (TBHA) has been compared in the rat, mouse, guinea pig, dog and rhesus monkey (Roche 1985A). Urine was collected for 24 hours after the last bolus oral administration of lysmeral for 5 consecutive days in the 5 species mentioned above. The doses ranged from 45 to 400 mg/kg bw/d differing between species (rat: 50-400 mg/kg bw/d; mouse, guinea pig, and rhesus monkey: 100 mg/kg bw/d; dog: 45 mg/kg bw/d). Urinalysis of tert-butylbenzoic acid (TBBA) and tert-butylhippuric acid (TBHA) was performed by GC/MS. In the control group of all species, no TBBA and TBHA were found. Considering the relation between TBBA and TBHA, the main urinary metabolite in orally treated rats, dogs and rhesus monkeys was found to be TBBA, whereas in the guinea pig and mouse TBHA resulting from glycine conjugation predominates (table 13). Surprisingly in the rat, urinary TBHA level are very low compared to other rodent species in this study, thus glycine conjugation or urinary TBHA excretion might not occur in the same rate as it does in other rodents. The urinary TBBA level in one of the two rhesus monkeys was found to be comparable to rat levels, whereas the other monkey showed 2-3 fold lower TBBA levels than the rat.

Table 13: Urinalysis of tert.-butyl benzoic acid (TBBA) and tert.-butyl benzoyl hippuric acid (TBHA) in five different species after application of comparable lysmeral doses by gavage for five consecutive days (Roche 1985A). Data on urinary metabolites are given in mg metabolite/kg body weight and in % of the dose lysmeral applied.

Species	Gender	No. of animals per dose	Dose (mg/kg bw)	Urinary metabolite			
				TBBA		TBHA	
				mg/kg	% dose*	mg/kg	% dose**
Rat	Male	8	50	4.8	11%	<0.3	
	Male	no data	50	8.1	19%	<0.9	
	Male	8	100	5.9	7%	0.8	1%
	Female	no data	50	6.7	15%	<1.1	
Mouse	Male	5	100	<0.8		14.5	13%
Guinea pig	Male	5	100	<0.03		56	49%
Dog	Male	3	45	1.1	3%	0.6	1%
	Female	3	45	1.4	4%	0.5	1%
Rhesus Monkey	Male	2	100	2 – 10	3 - 11%	0.04 - 0.2	< 0.1%

*% TBBA = TBBA in urine (µM/kg) / applied dose (µM/kg)

**% TBHA = TBHA in urine (µM/kg) / applied dose (µM/kg)

Similarly, species specific differences in the urinary excretion of TBBA have been observed after oral application of p-tert-butylbenzaledhyde (TBB) or p-tert-butyltoluene (TBT).

After 5-day oral administration of 12.5 and 50 mg/kg bw/day p-tert-butylbenzaldehyde (TBB) or 25 and 100 mg/kg bw/day p-tert-butyltoluene (TBT) to rats, p-tert-butylbenzoic acid (TBBA) was identified as metabolite in the urine 24 hours after the last administration, but not the secondary metabolite p-tert-butylhippuric acid (TBHA) was found. Urine levels yielded 17.2 mg TBBA /kg bw after administration of TBT (100 mg/kg bw/d) and 12.7 mg TBBA/ kg bw after administration of TBB (50 mg/kg bw/ day), being approximately 2-3 fold higher compared to TBBA urine levels observed after administration of comparable lysmeral doses to rats. No further metabolites have been investigated and glucuronic acid conjugates could not be identified by the analytical method used (Givaudan 1982B).

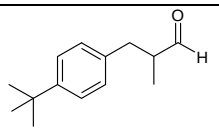
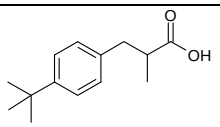
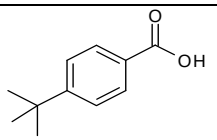
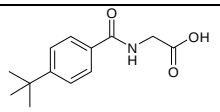
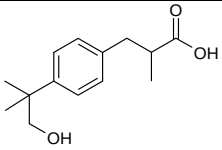
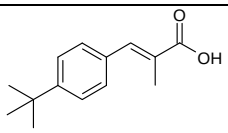
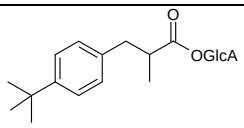
On the occasion of different 5 day oral toxicity studies in mice, guinea pigs and dogs, 100 mg/kg bw/d TBB or TBT was administered for 5 days, urine was collected for 24 h after the last administration and analyzed for TBBA and TBHA by GC analysis.

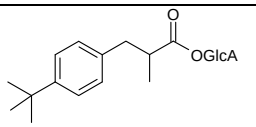
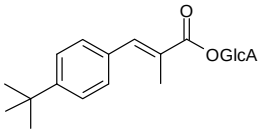
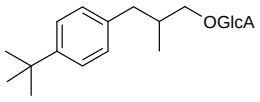
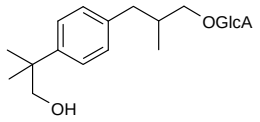
After application of TBB, TBBA was determined as metabolite in urine samples of treated dogs, guinea pigs and at very low levels in the urine of mice. However, TBHA was found to be at higher levels in urine samples of treated mice and guinea pigs compared to TBBA, whereas TBHA levels tended to be lower in the urine of dogs than TBBA levels (Givaudan 1985). A similar pattern was observed after application of TBT. TBBA was determined as metabolite in urine samples of treated dogs and at very low amounts in guinea pigs but not in mice. TBHA was found to be at higher levels in urine samples of treated mice and guinea pigs compared to TBBA, whereas TBHA levels were lower in the urine of dogs than TBBA levels (Givaudan 1985).

A qualitative and quantitative evaluation of metabolic profiles of different species largely confirmed these findings in an in vitro metabolism study using hepatocytes of male Han-Wistar rats, male CD1-mice, male white New Zealand rabbits and male humans incubated with ^{14}C-lysmeral at nominal substrate concentrations of 10, 50 and 100 µM (BASF SE 2010, see above). The two lower test concentrations chosen (10, 50 µM) reflect plasma levels observed after oral administration of no adverse testicular effect levels of lysmeral whereas 100 µM covers plasma levels obtained after doses exerting testicular toxicity. Cmax for lysmeral metabolites in plasma were 14 µg/ml or approx. 70 µM (assuming the molecular weight for lysmeral) after oral application of 25 mg/kg bw lysmeral (Huntingdon Research Center, 1995). Oral application of 50 mg/kg bw lysmeral yielded a Cmax of 9 µg/ml or approx. 40 µM lysmerylic acid, i.e. the main metabolite (BASF SE 2006A). The Radio-HPLC chromatograms were used to assign ROI values (region of interest = integrated peak area under Radio-HPLC) of each characterized metabolite in order to receive relative amounts of each metabolite in the respective metabolic profile. These ROI values were used for comparison of the incubation concentrations and species tested. As summarized in table 14, lysmeral was metabolized nearly completely in the hepatocytes of all species whereas lysmerylic acid (M7) was quantitatively the main metabolite. The metabolite M16 ((E)-3-(4-tert-Butyl-phenyl)-2-methyl-acrylic acid) was more pronounced in hepatocytes of rats than in hepatocytes of mice or humans (not detected in hepatocytes of rabbits). In line with findings in vivo, species differences in metabolic profiles were seen for M12, representing TBHA, which was more pronounced in mice (4.9 – 27.1 % ROI) than in rats (3.5 – 3.6 % ROI). TBHA was not detectable in incubates of hepatocytes of rabbits and humans.

In rat hepatocytes, an increase of TBBA (M15) levels was found, and the incubation with lower lysmeral concentrations resulted in higher TBBA levels. When compared to other rodent or non-rodent animal species, rats showed the highest amounts of TBBA. Whereas this metabolite contributed to 8.3 – 29.3 % ROI in hepatocytes of rats, its amount was ≤ 0.5 % ROI in mice, ≤ 2.0 % ROI in rabbits. The levels observed in humans were found to be approx. 4 fold lower than in rat hepatocytes for corresponding tested lysmeral concentrations, ranging from 1.9 – 7.5 % ROI. Furthermore, levels of TBBA observed in humans were similar to those found in rabbits at the 50 µM and 100 µM doses (the doses most relevant to plasma levels obtained after doses exerting testicular toxicity). These quantitative differences in TBBA formation between human and rat hepatocytes were apparently less pronounced or absent in the 24 hour urine samples of rats and rhesus monkeys. Since no comparative in vitro data for rhesus monkey hepatocytes are available and only two individual animals have been assessed in the primate study, the inclusion of these data for the overall assessment is questionable. Furthermore, the TBBA levels in the 24 hour urine represent a cumulative amount of the excreted metabolite, whereas the concentrations detected in the supernatant of the hepatocyte cultures are seen as directly proportional to given plasma concentrations in vivo. Since the plasma concentrations (i.e. Cmax) represent a more relevant parameter in respect of the thresholded testicular toxicity observed for lysmeral, the data from the comparative in vitro metabolism study in hepatocytes are considered to better demonstrate species differences in lysmeral metabolism.

Table 14: Summary of detected metabolites in hepatocytes of different species after application of 10, 50 or 100 µM 14C-lysmeral (BASF SE 2010).

			ROI [%=rel. peak area] after incubation with 10, 50, 100 µM Lysmeral			
Metabolite(s)	**Retention time [min]**	**Structure[1)]**	**Rat**	**Mouse**	**Rabbit**	**Human**
Lysmeral	35.5-35.7		-	-	0.8 0.8 0.3	-
Lysmerylic acid M7	31.5-31.8		27.2 60.1 64.6	62.8 74.2 82.8	84.8 92.4 95	74.4 82.9 88.1
TBBA M15	28.7-29.0		29.3 12.7 8.3	- 0.5 -	1.3 2 1.3	7.5 3.1 1.9
TBHA M12	24.4-24.6		3.6 - 3.5	27.1 8.7 4.9	-	-
Hydroxy-lysmerlyic acid M11	23.6-24.0		8.5 7.5 8.1	4.4 6.1 4.9	13.1 3.4 2.4	8.8 6.8 5.5
(E)-3-(4-tert-Butyl-phenyl)-2-methyl-acrylic acid M16	32.4-32.5		24.9 15.5 10.7	5.8 9.7 7.4	-	3.4 2.7 1.4
Glucuronide M13	24.5-24.8		- 1.3 -	-	-	1.2 1.3 -

			ROI [%=rel. peak area] after incubation with 10, 50, 100 µM Lysmeral			
Metabolite(s)	**Retention time [min]**	**Structure[1)]**	**Rat**	**Mouse**	**Rabbit**	**Human**
Glucuronide M14	25.9-26.4		4.6 2.8 4.8	- 0.7 -	- 1.4 1	4.7 2.7 3.1
						
						
Glucuronide M10	20.3-20.5		1.8 - -	-	-	-

[1)]: structure elucidation was performed from the incubation in rat and human hepatocytes (100 µM)

- not detectable

4.1.2 Human information

See Chapter 4.1.1.

4.1.3 Summary and discussion on toxicokinetics

Quantitative data on the toxicokinetics of lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) are available from rat, mouse, rabbit, guinea pig, dog and rhesus monkey and humans. Based on its physico-chemical properties, lysmeral is considered to have a high bioavailability via the oral route and a limited bioavailability via the inhalation route. After acute and repeated oral and dermal administration of lysmeral to experimental animals and humans there is clear evidence of systemic absorption. However, in humans only very limited percutaneous absorption of lysmeral is observed especially when compared to the rat. Distribution predominately to the liver and rapid urinary excretion has been observed in rats after dermal administration and can be assumed for the oral route as well. A detailed in vivo study on the metabolism of lysmeral is not available.

Comparative assessment of the urinary metabolites in different laboratory animal species reveal species specific differences in the urinary excretion of p-tert-butylbenzoic acid (TBBA) and p-tert-butyl-hippuric acid (TBHA). Furthermore, these data substantiate, that TBBA is formed as common metabolite after administration of lysmeral, p-tert-butyltoluene (TBT) or p-tert-butylbenzaldehyde (TBB) and their potency for testes toxicity correlates with systemically formed urinary TBBA levels (see chapter 4.11).

On the basis of a qualitative and quantitative evaluation of metabolic profiles for different species in an in vitro metabolism study, a predominant formation of TBBA levels in rat hepatocytes was found when compared to other rodent, non-rodent animal or human hepatocytes. The TBBA levels observed in the model using human hepatocytes were found to be approx. 4 fold lower compared to rat hepatocytes at corresponding incubation concentrations, which reflect plasma levels obtained after oral administration of lysmeral doses below and above the lowest adverse testicular effect level. Furthermore, the TBBA levels formed in human hepatocytes after incubation of lysmeral concentrations related to adverse testicular effect doses were comparable to TBBA levels found in the rabbit, a species not sensitive to testicular toxicity.

Overall, species specific differences in the formation of metabolites have been clearly identified both in vitro and in vivo between responder (e.g. rat) and non-responder species (e.g. mouse, rabbit) with respect to reproductive toxicity. The species specific organ toxicity after repeated oral application of lysmeral can be attributed to the toxic metabolite TBBA. In vitro studies show significantly lower production of TBBA in humans than in rats, with human TBBA production similar to that observed in rabbits at toxicologically relevant doses.

4.2 Acute toxicity

Not evaluated in this report.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this report.

4.4 Irritation

Not evaluated in this report.

4.5 Corrosivity

Not evaluated in this report.

4.6 Sensitisation

Not evaluated in this report.

4.7 Repeated dose toxicity

4.7.1 Non-human information

For the purpose of this report, evaluation of available data on repeated dose toxicity is mainly focused on adverse effects on the male reproductive organs. However, other adverse effects are discussed in the context of reproductive toxicity. Studies designed for the assessment of fertility and developmental toxicity are discussed in chapter 4.11. Short-term studies in the rat with an application period of 1 to 14 days have been performed for the oral and dermal route. In addition, short-term oral testing for 5 days has been performed in the mouse, guinea pig, and rhesus monkey. Long-term studies are available for the rat and the dog for the oral route.

General adverse effects, such as decreases in body weights and food consumption and/or clinical signs of toxicity were observed after oral administration of lysmeral (2-(4-tert-butylbenzyl) propionaldehyde). In studies applying more detailed clinical chemistry and histopathological examinations, adverse effects on the liver became evident. At the dose levels showing general and liver toxicity, adverse testicular effects of lysmeral after oral administration were found as well. Several of the present repeated doses studies mainly focused on testicular toxicity but did not provide full information on liver toxicity after oral administration of lysmeral. However, when assessed, no testicular toxicity in the absence of other toxicological findings was observed.

Considering the repeated short and long term oral administration studies in rats, a clear effect level for testicular toxicity can be set at 50 mg/kg bw/day. The effect levels observed did not differ between short term (≤ 14 day) and subchronic (90 day) application periods. Adverse testicular findings were observed already after a single oral administration, suggesting acute effects on male reproductive organs. In contrast, dermal administration in rats led to testicular toxicity only at an excessive dose level (above the limit dose), whereas at the limit dose of 1000 mg/kg body weight, no adverse testicular effects were observed. In dogs, general adverse effects, together with liver and testicular toxicity was observed after oral administration at higher dose levels, i.e. 200 mg/kg bw/day. Thus, concerning testicular toxicity after orally administered lysmeral, dogs were found to be less sensitive of than rats.

No testicular toxicity was observed in the mouse, guinea pig, rabbit and primates, substantiating species specificity for the testicular toxicity observed after oral administration of lysmeral. For a summary of available studies with focus on adverse effects of male reproductive organs, see table 16 in chapter 4.11.

4.7.1.1 Repeated dose toxicity: oral

Rodent studies.

In a 90 day rat oral toxicity study (following OECD 408 with a few deviations), six test groups each consisting of 14 animals per sex were dosed with lysmeral (analytical purity 97.8%) via gavage with 2, 5, 25, and 50 mg/kg body weight/day (five days a week) (Givaudan 1986A). For the high dose group a satellite group of 14 animals per sex was included for a post-treatment period of 4 weeks. As an adverse clinical sign, alopecia was observed in the females of the high dose group. Organ specific toxicity included the liver, as seen by elevated absolute (24% - 45% and 57% - 69%

increase in males and females respectively) and relative (21% - 45% and 59% - 75% increase in males and females respectively) liver weights starting at 25 mg/kg bw/day. A histopathologic correlate (hepatic lipid droplet content) at 50 mg/kg was observed in both genders. Furthermore, a significant decrease in plasma cholinesterase activity ranging from 30% to 70% of respective controls and lower plasma cholesterol levels ranging from 40% to 70% of respective controls at 25 and 50 mg/kg bw/day in both genders was detected.

A slight but significant increase in aspartate aminotransferase activity was observed in males of the high dose group, whereas other liver enzymes such as alanine aminotransferase activities were not influenced. Effects on clinical chemistry were reversible in the recovery group. In addition, in female rats treated with 25 and 50 mg/kg bw/day, elevated absolute (16% - 30% increase) and relative (18% - 36% increase) weights of adrenal glands and hypertrophy of the zona fasciculata were observed. Findings in both, adrenals and liver were shown to be reversible in the recovery group.

In parallel, test substance related testicular toxicity such as spermatoceles in the epididymides and testicular atrophy was observed at 50 mg/kg bw/day (Table **15**). 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.

Table 15: Histological findings in testes and epididymides in a 90 day rat oral toxicity study (Givaudan 1986A).

Dose group (mg/kg bw/d)	Histological findings (percentage of organs affected)
0	2/14 animals: • Disturbed spermatogenesis (11%) • Sertoli cell-only tubules (11%) • Increased surface density of Leydig cells (11%) • Decreased density of spermatozoa in epididymides* (11%) • Nucleated cells in epididymides* (11%) • Increased number if unusual clear cells in epididymides* (11%)
2	No pathologic findings in the testes and epididymides
5	1/14 animals: • Disturbed spermatogenesis (7%) • Sertoli cell-only tubules (7%) • Increased surface density of Leydig cells (7%)
25	1/14 animals: • Unilateral disturbance of spermatogenesis (4%) • Sertoli cell-only tubules (4%) • Decreased density of spermatozoa in epididymides* (4%) • Increased number if unusual clear cells in epididymides * (4%)
50	14/14 animals: • Disturbed spermiogenesis (21%) • Disturbed spermatogenesis, (29%) • Sertoli cell-only tubules (29%) • Increased surface density of Leydig cells (21%) • Decreased density of spermatozoa in epididymides* (80%) • Nucleated cells in epididymides* (100%) • Spermatoceles in the epididymides* (67%)
50 Recovery group (4 weeks)	14/14 animals: • Disturbed spermatogenesis (27%) • Sertoli cell-only tubules (27%) • Increased surface density of Leydig cells (8%) • Decreased density of spermatozoa in epididymides* (33%) • Nucleated cells in epididymides* (67%) • Spermatoceles in the epididymides* (79%)

* Only evaluated in epididymides without spermatocele(s)

Oral administration of lysmeral (analytical purity 99.1%) and lysmerylic acid to rats (50 mg/kg bw/day by gavage) for 1, 2, 3, 4 or 14 days was performed in a study with main focus on male reproductive organs (BASF SE 2006A). This study aimed for the comparison of lysmeral and lysmerylic acid in terms of potency, time dependency of adverse testicular and spermatotoxic effects and species specificity based on an analogous study performed in mice ((BASF SE 2006B). In rats, slight to severe testicular atrophy with an incidence of 2/5 animals for lysmeral and 3/5 animals for lysmerylic acid after a single application, and in all animals after longer application periods was observed (BASF SE 2006A). Generally, testicular effects were described as diffuse tubular testicular degeneration, fine vacuolar change of pachytene spermatocytes up to apoptotic cell death. Furthermore, sperm parameter examined i.e. sperm motility, spermatid count in testes, cauda epididymal sperm count, and sperm morphology were affected solely after an application period of 14 days, as seen for two test substances. Although not statistically significant, body weight gains were found to be decreased by 25% and 20% below controls after application of lysmeral and lysmerylic acid for 14 days.

For comparison, oral administration of lysmeral and lysmerylic acid via gavage in mice (50 mg/kg bw/day) for 1, 2, 3, 4 or 14 days led to a reduction in the ratio of normal to abnormal sperm in animals exposed only for 3 and 4 days (BASF SE 2006B). However, other treatment periods did not influence this parameter and other sperm parameters, i.e. sperm motility, spermatid count in testes or cauda epididymidis. Single administration (1 day) of lysmerylic acid led to a significant reduction in the ratio of normal to abnormal sperm and reduced total sperm numbers in cauda epididymidis in the group exposed for 2 days was observed. All other examined sperm parameters were not influenced for all treatment periods. For both substances, macroscopic and microscopic evaluation of the testes revealed no pathological changes in all groups observed. A statistically non-significant decrease in body weight gains (33% below controls) was found after application of lysmerylic acid after 14 days. Since the changes observed in single sperm parameters were inconsistent, did not follow a kinetic and were not verified histologically in testes, a substance related origin is unlikely. In line, a further study supports the absence of testicular toxicity in mice, as presented further below.

As supportive evidence, several oral gavage studies for 5 consecutive days in rats at doses ranging from 25 to 400 mg/kg bw/day confirmed clinical signs of toxicity, body weight loss and macroscopic changes in the liver starting from 50 mg/kg bw/day lysmeral. At same dose levels, changes in seminiferous epithelium with degenerated/reduced numbers of germ cells, were found, whereas decreased testes and kidney weights and decreased sizes of prostate & seminal vesicles became evident at higher dose levels (Givaudan 1986B, Givaudan 1991A, Newberne 1990A).

However, oral administration of 100 mg/kg bw/day lysmeral for five consecutive days in male mice or guinea pigs showed neither any general adverse systemic effects nor adverse effects on the male reproductive organs (Newberne 1990 B-C). In these studies, lysmeral was administered by gavage to 5 SPF albino male mice or SPF Himalayan spotted male guinea pigs. Body weights, signs of toxicity and mortality was monitored. Survivors were sacrificed and necropsied, the testes were weighed and examined by histopathology.

Non-rodent studies.

In a pilot study, lysmeral (analytical purity 95%) was administered to two male beagle dogs by oral administration via gelatine capsules in subsequently increasing doses (47 -564 mg/kg bw/day) for 9 weeks (Givaudan 1990A). As general adverse effects, occasional vomiting in both animals, diarrhoea in one animal and body weight reduction together with an increase in clinico-chemical parameters (glutamate dehydrogenase, alanine aminotransferase) was found. 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, i.e. administration of 4.4, 22.3 or 44.6 mg/kg bw/day lysmeral (analytical purity 97.6%) to each 3 male/female dogs (Givaudan 1990B) or 200 mg/kg bw/day to 3 female dogs for 90 days in gelatine capsules (Givaudan 1990C). In the former study occasional diarrhoea at 22.3 or 44.6 mg/kg bw/day and vomiting at the high dose group was observed but no other alterations and no findings from the latter study were attributable to treatment. In male animals, no alterations on reproductive organs were observed.

Based on the indications of adverse testicular effects observed in the two dogs of the pilot study (Givaudan 1990A), a testicular toxicity screening study in beagle dogs at comparable dose levels was performed for further confirmation. This study intended to clarify, whether testicular toxicity after oral administration of lysmeral occurs in a non-rodent species. In this study, lysmeral (analytical purity 99.1%) was administered to groups of 4 purebred male Beagle dogs via gelatine capsules at dose levels of 0, 40, 200 and 1000/500 mg/kg bw/day for 2 weeks (reduction of dose levels in the high dose group due to vomitus and diarrhoea) (BASF SE 2008A). Besides clinical/hematological examinations, urinalyses and a gross-pathological assessment, specific histopathological investigation on reproductive organs and liver was performed.

Systemic effects, such as a retardation in body weight gains and body weight loss in distinct animals together with decreases in food efficiency were observed in combination with vomitus and soft faeces/diarrhea in all animals of the mid and high dose group. Furthermore, significant absolute and relative liver weight increases between 30-40% above control values, and centrilobular hypertrophy of hepatocytes were observed in the mid and high dose groups.

Distinct clinical parameters were altered, i.e. prolongation in activated partial thromboplastin time, increases in serum magnesium, potassium and inorganic phosphate levels in mid/high dose animals and decreased glucose levels in high dose animals. Decreases in aspartate aminotransferase and alanine aminotransferase were found in mid/high dose animals.

A massive diffuse degeneration of seminiferous tubules combined with a hyperplasia of Leydig cells in the testes and an aspermia and epithelial vacuolation in the epididymides was found in one dog of the mid dose, which showed also a decrease in relative testis weights. Furthermore, a reduced size of testes and epididymides was observed in this animal. A second animal in the mid dose group showed a slight, one-sided and focal degeneration of seminiferous tubules, which was observed in historical control data as well and might therefore be considered as spontaneous in nature. In contrast, no such adverse testicular effects were observed in animals of the low and high dose group. Decreases in prostate sizes were observed in low dose and high dose group animals. However, due to the lack of histopathological findings and the absence of a dose response relationship, these effects are not considered to be substance related.

To further clarify the findings of the study above, a follow-up study, involving a higher animal number per dose group and additional andrological/ spermatological examinations prior and during the test substance administration period was performed. Lysmeral was administered to groups of 10 male purebred Beagle dogs via gelatine capsules at concentrations of 0 and 200 mg/kg body weight/day for 2 weeks (BASF SE 2008B).

Mean body weight loss (-0.2 kg compared to 0.1 kg in controls after day 14) due to mainly 2 of 10 animals with a massive body weight loss were observed, together with a slightly reduced food consumption (up to 25% below controls) starting from day 3 onwards. In line, a negative value for food efficiency was found. Vomitus in 7 of 10 animals and diarrhoea in 4 of 10 animals was

observed as further parameters for systemic toxicity. Significant increases in absolute and relative liver weights (14% and 17% above controls, respectively) with centrilobular hypertrophy of hepatocytes became evident in the dosed animals.

Furthermore, distinct clinical parameters were statistically significantly altered (values refer to respective mean control levels after day 14), i.e. increases in alanine aminotransferase by 80% and aspartate aminotransferase activities by 310%, prolongation in activated partial thromboplastin time by 10%, decrease in serum triglyceride levels by 35% compared to control. Decreases in red blood cell counts and haemoglobin by 5%, and hematocrit values by 10% together with a decrease in reticulocyte counts by 60% indicate an anemic situation after test substance application. Increases in serum urea by 45%, creatinine by 25%, calcium by 5% and magnesium levels by 20% indicate adverse effects on the kidneys, however, no kidney weight changes were observed.

Decreases in absolute and relative testes weights by approx. 25% along with a slight to severe degeneration of seminiferous tubules in 9 of 10 animals were observed. Unilateral decrease in testicular length or width was found in 6 of 10 animals.

Furthermore, effects on spermatological parameters, i.e. decrease of progressively motile spermatozoa and/or morphological alterations were found in 9 of 10 dogs after treatment when compared to the values of the respective animals before treatment.

Morphological sperm alterations consisted mainly of mid-piece anomalies (cytoplasmatic droplets) and less frequently in sperm neck anomalies (paraxial tail attachment, cytoplasmatic droplets). Prostate weights were slightly decreased and respective minimal to moderate multifocal atrophies were found in 3 of 10 animals.

A screening study on the male reproductive function in rabbits was performed in order to clarify, whether testicular toxicity or spermatotoxic effcts after oral administration of lysmeral occurs in a further non-rodent species.

In this screening study, rabbits were treated via gavage for 15 days at doses of 30, 100 and 300 mg/kg bw/day lysmeral (analytical purity 99.1%; BASF SE 2008C). No test substance related findings on clinical observations, body weights and food consumption were observed in all dosing groups. Neither testes nor cauda epididymis weights were affected. A moderate diffuse degeneration of the seminiferous tubules combined with a moderate oligospermia and a moderate mixed inflammation in the epididymides was observed in 1/5 animals of the low dose group. The inflammation found in the epididymides is assumed to be causative for the degenerative changes in the testis. In the mid dose group, a reduced testes and epididymides size with severe diffuse degeneration of seminiferous tubules in the examined left testis and a severe atrophy plus aspermia in the left epididymides was observed in 1/5 animals. However, sperm evaluation did not reveal any treatment related effect in this or any other treated animal. Based on the absence of a dose response relationship, the isolated occurrence in one single animal and the absence of adverse effects on spermatological parameters in the respective animal, a treatment related origin of the observed findings seem unlikely.

In a study on primates, using a limited number of animals, oral administration of 100 mg/kg bw/day lysmeral for 5 days did not lead to any general adverse effects or testicular toxicity (Newberne 1990; Givaudan 1984G). In this study using 2 male rhesus monkeys (Macaca mulatta), clinical signs of toxicity and mortality was monitored and animals were weighed at test day 1 and 6. Animals were sacrificed by perfusion with glutaraldehyde and subjected to a complete necropsy. All organs and tissues were examined grossly and testes and epididymides were examined by histopathology. No incompatibility reactions were seen in clinical observations during the in-life period and body weights were not significantly affected. In histological examinations, only small

foci in one epididymis of one animal and small hollow spaces in the epithelium of one epididymis of the other animal was observed. The testes of both animals were found to be free of lesions. Overall, general and testicular toxicity was not observed under the conditions of this study in primates.

4.7.1.2 Repeated dose toxicity: inhalation

No data available

4.7.1.3 Repeated dose toxicity: dermal

Dermal administration of lysmeral (analytical purity 99.1%) to rats 6 hours per day for 5 days (250, 500 1000, 2000 mg/kg bw/day) caused very slight decrease in body weights by 2% and marked testicular atrophy at the high dose only (Givaudan 1991A). Seminiferous tubules with disorganization of the epithelial structure, decrease of the number of germ cells, increase of the number of degenerating germ cells (inclusive giant cells) were observed in combination with immature/degenerating germ cells in epididymides and the occurrence of spermatocele. No clinical signs and substance related necropsy findings were observed. No further observations were performed in this study to assess adverse effects other than testicular toxicity.

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

No data available

4.7.1.7 Summary and discussion of repeated dose toxicity

For a detailed discussion of repeated dose studies and studies designed for the assessment of fertility, see Chapter 4.11.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not evaluated in this report.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this report.

4.10 Carcinogenicity

Not evaluated in this report.

4.11 Toxicity for reproduction

Table 16: Summary table of relevant reproductive toxicity studies and repeated dose toxicity studies focused on adverse effects on the male reproductive organs.

Method			Results		Remarks	Reference
Species	**Study period**	**Dosage** [mg/kg bw/day]	**$LOAEL_{testes/sperm}$** [mg/kg bw/day]	**$NOAEL_{testes/sperm}$** [mg/kg bw/day]		
Rat	5 days	Dermal. 250, 500, 1000, 2000; daily for 6 hours	2000 Testicular toxicity (with additional systemic toxicity)	1000		Givaudan 1991A
Rat	1, 2, 3, 4, 14 days	p.o. 50; daily 5/ time point investigated	n.d. Testicular toxicity on day 1 and following, spermatotoxic effects (with additional systemic toxicity on day 14)	n.d.		BASF SE 2006A
Rat	5 days	p.o. 25, 50, 100, 200, 400; daily 8/dose group	50 Testicular toxicity (with additional systemic toxicity)	25		Givaudan 1986B
Rat	5 days	p.o. 25, 50, 100; daily 5/dose group	50 Testicular toxicity (with additional systemic toxicity)	25		Givaudan 1991A
Rat	5 days	p.o. 50, 100, 200, 400; daily 8/dose group	100 Testicular toxicity (with additional systemic toxicity)	50		Newberne 1990A
Rat	90 days	p.o. 2, 5, 25, 50; 5 days/week 14/dose group	50 Testicular toxicity (with additional systemic toxicity)	25		Givaudan 1986A
Rat	12 weeks	Feed; 400, 800, 1700, 3400 ppm; daily	62.6 (1700 ppm) Testicular toxicity, spermatotoxic effects (with additional systemic toxicity)	28.7 (800 ppm)	Info given refers to male animals	BASF SE 2006C
Dog	14 days	Gelatine capsule, 40, 200, 1000/500; daily 4/dose group	200 Testicular toxicity in 1 animal of mid dose group. (with additional systemic toxicity)	40		BASF SE 2008A
Dog	14 days	Gelatine capsule, 200, daily 10/dose group	n.d Testicular toxicity, spermatotoxic effects	n.d.		BASF SE 2008B

			(with additional systemic toxicity)			
Dog	9 weeks	Gelatine capsule, 47 - 564 2 animals	n.d. Testicular toxicity (with additional systemic toxicity)	n.d.		Givaudan 1990A
Dog	90 Dog days	Gelatine capsule, 4.4, 22.3, 44.6; daily 6/dose group	> 44.6	44.6		Givaudan 1990B
Mouse	1, 2, 3, 4, 14 days	p.o. 50; daily 5/ time point investigated	--	50		BASF SE 2006B
Mouse	5 days	p.o. 100; daily 5/dose group	--	100		Newberne 1990B
Guinea pig	5 days	p.o. 100; daily 5/dose group	--	100		Newberne 1990C
Rhesus monkey	5 days	feed; 100; daily 2/dose group	--	100		Newberne 1990; Givaudan 1984G
Rabbit	15 days	p.o. 30, 100, 300; daily 5/dose group	--	300		BASF SE 2008C

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Concerning fertility, a one-generation range finding study and a variety of repeated dose studies on male rats are available; some of them were designed for the assessment of male reproductive organ toxicity. Furthermore, repeated dose studies on dogs, mice, guinea pigs, rabbits and primates are available for the assessment of reproductive toxicity of lysmeral (2-(4-tert-butylbenzyl) propionaldehyde; see Chapter 4.7).

Evidence for testicular toxicity of lysmeral after bolus oral application via gavage is available from existing repeated dose studies in rats, however, data on continuous oral application of lysmeral is lacking. Concerning the properties of lysmeral as fragrance material, palatability has been assessed in a preliminary feeding test in rats (BASF 2002). Lysmeral (analytical purity 99.1%) has been administered for 14 days to 3 Wistar rats per sex and dose at a dietary concentration of 0, 100 and 1000 ppm. Food consumption and body weights were monitored and animals were examined for signs of toxicity and mortality. Clinical examinations and palpations have been performed.

Mean test substance intake ranged from 10.1-10.6 mg lysmeral/ kg bw/ day in the low dose group and 97-105.3 mg lysmeral/ kg bw/ day in the high dose group. Mean food consumption was slightly decreased in males of the high dose group (-6% versus controls at day 7 and 14) and in females of

the high dose group (-12% and -3% versus controls after day 7 and day 14 respectively) without gaining statistical significance.

Food efficiency has been decreased significantly in high dose males after day 7 and in high dose females at both observation time points.

In male animals, mean body weights were slightly decreased in the high dose group (-5% and -7% compared to controls after day 7 and day 14 respectively) and body weight gains were slightly decreased in the low dose group and more severe in the high dose group (-2% and -20% versus controls, respectively) at both observation time points. In female animals, slightly decreased body weights were found in the low dose group (-3% versus controls) and a statistically significant decrease has been observed (-10% versus controls) in the high dose group after 14 days. Body weight gains were slightly decreased in the low dose females (-8% and -14%) and significantly decreased in the high dose females (-39% and -42%) after day 7 and day 14 respectively.

No substance related clinical signs of toxicity were observed and no deaths occurred during the observation period.

For further assessment of lysmeral induced effects after continuous oral application via feed, the subsequent study, i.e. a one-generation range finding study for a two-generation study, has been performed using microencapsulated lysmeral in order to exclude stability issues and palatability induced effects when applied over a longer time period.

Such a one-generation range finding study has been performed, in order to:

- verify the relevance of the adverse testicular effects observed after bolus application via gavage compared to a continuous application via feed
- close the gap between the observed adverse changes in testes and sperm and infertility
- to assess the no effect levels concerning fertility for a sound risk assessment

In this range finding study, the test substance was administered to groups of 10 male and 10 female young Wistar rats (F0 parental generation) via the diet (30.7% lysmeral, microencapsulated) (BASF SE 2006C). In the 400, 800, 1700 and 3400 ppm group, the uptake of lysmeral via the diet was accounted to 14.5, 28.7, 62.6 and 119.7 mg/kg bw/day, respectively. Due to dose adjustment during gestation and lactation, the dams received 200, 400, 850 and 1700 ppm of the test substance in feed, resulting in an uptake of 12.9/10 and 25.8/18.3 mg/kg bw/day lysmeral for the two low dose groups, respectively (no assessment of the two high dose groups was performed due to the absence of offspring). About 6 weeks after the beginning of treatment, F0 animals were mated to produce a litter (F1). The female F0 animals were allowed to deliver and rear their F1 pups until weaning (postnatal day 21). The study was terminated with the sacrifice of the F1 weanlings and F0 adult animals.

Male F0 animals showed dose dependent reduced body weights and body weight gains (5-30% and 10-40% below control, respectively) and food consumption was 15% below controls in the high dose group. Increases in relative liver weights (10-20% above control) starting at the 800 ppm dose group and decreases in relative kidney weights (15% below control) were found in the high dose group. Significant changes in clinical chemistry such as increased levels of plasma alanine aminotransferase by 20-45%, alkaline phosphatase by 30-55% and a 4 to 5 fold increase of the glutamate dehydrogenase above mean controls were observed starting at 1700 ppm. Mean gamma glutamyltransferase was increased two fold in the high dose group only compared to controls.

Testicular toxicity and spermatotoxicity, i.e. effects on sperm parameters, decreases in relative testes (30-45% below control) and cauda epididymis (30-40% below control) weights, diffuse testes degeneration and aspermia of the epididymis, were observed at the 1700 ppm and 3400 ppm groups. In the high dose group, weights of additional organs were decreased (i.e. seminal vesicle (10%) and prostate (20%) below control) and hyperplasia of Leydig cells was observed.

Maternal toxicity was manifested by decreases in body weights and body weight gain (5-10% and 10-30% below control) during/after premating in the 800 ppm group and at higher doses. In the groups delivering offspring, i.e. the two low dose groups, mean maternal body weights and body weight gain were approx. 10% below control in the 800 ppm group during gestation and lactation and food consumption during lactation was 20% below controls. Furthermore, significant changes in clinical chemistry were seen in all dose groups observed, i.e. a 2-8 fold increase in gamma glutamyltransferase and decreases in serum cholinesterase by 50-65% compared to controls. From 800 ppm onward, glutamate dehydrogenase was found to be increased by 5-75% as well. However, no significant changes in mean relative liver or kidney weights were observed.

In the 1700 ppm group only 1 of 8 mated females became pregnant and a relationship to the adverse effects observed in male reproductive organs is indicated.

No viable offspring has been derived from animals treated with 1700 ppm and 3400 ppm microencapsulated lysmeral. In the 1700 ppm group, the only pregnant female had only 1 implant which was resorbed. In contrast, only a slight and non-significant increase in mean implantation losses have been observed for the two lower dose groups; i.e. 16% and 11% mean losses per litter in dose groups 400 ppm and 800 ppm versus 5% in controls, respectively (see table **17**). No corpora lutea have been determined in this study

A slight decrease in the mean number of delivered pups per dam (7.9 in dose group 800 ppm versus 9.4 and 8.7 in controls and dose group 400 ppm) was recorded. However, no effects on gestation and the live birth indices became evident, due to the absence of any stillborn in the lysmeral treated dose groups with offspring.

Pup survival was minimally decreased for postnatal day 0 to 4 (94% in the 800 ppm dose group versus 99% in the 400 ppm dose group and controls), and no pup mortality was observed between postnatal day 4 and 21 in all dose groups with offspring. Overall the respective viability and lactation index was not considered to be affected by treatment.

For the 400 and 800 ppm dose groups, a significant reduction in birth weights (19% and 22% below controls, respectively) and pup weight at weaning (17% and 21% below controls, respectively) has been recorded for male and female pups. Accordingly the pup body weight gain was decreased in the 400 and 800 ppm dose groups (16% and 21% below controls, respectively).

Table 17: Overview on reproductive parameters from the one-generation range finding study with Wistar rats (BASF SE 2006C).

Dose group [ppm]	Fertility index (male)[a]	Fertility index (female)[b]	Mating index (male/female)[c]	Mean implantation sites	Mean Postimplantation loss[d]	Mean pups delivered	Number of litters
0	100 %	100 %	100 %	9.9	5.1±9.27%	9.4±3.95	10
400	100 %	100 %	100 %	8.5	16.2±30.3%	8.7±1.41	9
800	100 %	100 %	100 %	8.8	11.1±10.16%	7.9±2.23	10
1700	10 %	13 %	80 %	1	100 ±0%	0	0
3400	0 %	0 %	50 %	0	-	0	0

[a]Male fertility index = Number of males proving their fertility / Number of males placed with females * 100

[b]Female fertility index = Number of females pregnant / Number of females mated * 100

[c]Mating index = Number of animals mated or with confirmed matings / number of males placed with females * 100

[d]Postimplantation loss = number of implantations – number of pups delivered / number of implantations

To facilitate comparison with testicular toxicity observed in repeated dose studies a summary of the various studies previously discussed in chapter 4.7 is provided in table 16 focussing on (no-)effect dose levels for testicular toxicity.

In addition, an in vitro study on chorionic gonadotrophin stimulated testosterone secretion of primary rat Leydig cells after treatment with lysmeral did not indicate any specific inhibition at the level of testosterone secretion. It was therefore assumed, that the atrophic changes of the testes in vivo might not be attributed to a specific and direct action of lysmeral on the main function of Leydig-cells which is the secretion of testosterone (Roche 1994).

Testicular toxicity induced by p-tert-butylbenzoic acid (TBBA).

Clear evidence of adverse testicular and spermatotoxic effects, being identical in quality to lysmeral induced testicular toxicity, have been observed in repeated dose studies, testicular toxicity screening studies and fertility studies with p-tert-butylbenzoic acid (TBBA). For comparison, a summary is provided in table 18 focussing on (no-)effect dose levels for testicular toxicity. Further study details are given in Annex 2.

Table 18: Summary of relevant studies for p-tert-butylbenzoic acid (TBBA) with focus on adverse effects of the male reproductive organs

Species	Test substance	Study period	Dosage	LOAEL/$C_{testes/sperm}$	NOAEL/$C_{testes/sperm}$	Reference
Rat	TBBA	Single treatment	p.o. 500, 630, 800, 1000, 2000 mg/kg bw	500 mg/kg bw Testicular toxicity	<500 mg/kg bw	Hunter 1965
Rat	TBBA	Single treatment	p.o. 700, 720 mg/kg bw	700 mg/kg bw Testicular toxicity	<700 mg/kg bw	Hazleton 1986
Rat	TBBA	Single treatment	inhalation 495, 668, 958, 1802 mg/m^3; 4 hour whole body	495 mg/m^3 Testicular toxicity	<495 mg/m^3	Lu 1987
Rat	TBBA	5 days	p.o. 12.5, 25, 50, 100 mg/kg bw/d;	25 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	12.5 mg/kg bw/d	Givaudan 1982D
Rat	TBBA	90 days	p.o. 100, 316, 1000, 3160, 10000 ppm in feed	100 ppm (6-8 mg/kg bw/d) Testicular toxicity (with additional systemic toxicity)	<100 ppm (6-8 mg/kg bw/d)	Hunter 1965
Rat	TBBA	70 days	p.o. 20, 100, 500 ppm in feed	100 ppm (7.9 mg/kg bw/d) Testicular toxicity/impaired fertility	20 ppm (1.6 mg/kg bw/d)	Hoechst 1987
Rat	TBBA	28 days	dermal 7.5, 15, 30, 60 mg/kg bw/d	60 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	30 mg/kg bw/d	Shell 1975
Rat	TBBA	90 days	dermal 17.5, 35, 70, 140 mg/kg bw/d	70 mg/kg bw/d Testicular toxicity, spermatotoxic effects (with additional systemic toxicity)	35 mg/kg bw/d	Cagen 1989, Lu 1987

Rat	TBBA	7 days	inhalation 12.5, 106, 525 mg/m^3; 6 hours/day, whole body	12.5 mg/m^3 Testicular toxicity, spermatotoxic effects (with additional systemic toxicity)	<12.5 mg/m^3	Shell, 1982
Rat	TBBA	28 days	inhalation 1.5, 4.7, 15.7 mg/m^3; 6 hours/day, snout-only	No testicular toxicity observed.	15.7 mg/m^3	HRC 1995

Testicular toxicity induced by p-tert-butyl-benzaldehyde (TBB) and p-tert-butyltoluene (TBT).

As described in Chapter 4.1, oral administration of two other substances, namely p-tert-butyl-benzaldehyde (TBB) and p-tert-butyltoluene (TBT), resulted in the formation of systemic TBBA. Therefore, both compounds and lysmeral share TBBA as a common metabolite. As outlined below, oral TBB and TBT administration to rats resulted in adverse testicular effects, being identical to the testicular findings observed for TBBA and lysmeral. In line with findings for lysmeral, the rat represents the most sensitive species and evidences for testicular effects in dogs exist, whereas other species, i.e. mouse or guinea pigs, show low susceptibility for testicular toxicity.

For comparison, a summary is provided in table 19 focussing on (no-)effect dose levels for testicular toxicity. Further study details are given in Annex 3.

Table 19: Summary of relevant studies for p-tert-butylbenzaldehyde (TBB) and p-tert-butyltoluene (TBT) with focus on adverse effects of the male reproductive organs.

Species	Test substance	Study period	Dosage	LOAEL/$C_{testes/sperm}$	NOAEL/$C_{testes/sperm}$	Reference
Rat	TBB	5 days	p.o. 6.5, 12.5, 25, 50 mg/kg bw/d;	25 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	12.5 mg/kg bw/d	Givaudan 1981
Rat	TBB	5 days	p.o. 100 mg/kg bw/d	100 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	n.d.	EPA (TSCAT) 1982
Mouse	TBB	5 days	p.o. 100 mg/kg bw/d	No evident testicular toxicity observed	100 mg/kg bw/d	Givaudan 1984A

Guinea pig	TBB	5 days	p.o. 100 mg/kg bw/d	No evident testicular toxicity observed	100 mg/kg bw/d	Givaudan 1984B
Dog	TBB	5 days	p.o in gelatine capsule. 100 mg/kg bw/d 2 animals	100 mg/kg bw/d Testicular toxicity in 1/2 animals.	n.d.	Givaudan 1984C
Rat	TBT	5 days	p.o. 12.5, 25, 50, 100 mg/kg bw/d;	50 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	25 mg/kg bw/d	Givaudan 1982C
Rat	TBT	5 days	p.o. 200 mg/kg bw/d	200 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	n.d.	EPA (TSCAT) 1982
Mouse	TBT	5 days	p.o. 100 mg/kg bw/d	No evident testicular toxicity observed	100 mg/kg bw/d	Givaudan 1984D
Guinea pig	TBT	5 days	p.o. 100 mg/kg bw/d	Slight testicular toxicity observed in single animals	n.d.	Givaudan 1984E
Dog	TBT	5 days	p.o in gelatine capsule. 100 mg/kg bw/d 2 animals	100 mg/kg bw/d Slight testicular toxicity observed in 1/2 animals.	n.d.	Givaudan 1984F
Rat	TBT	28 days	p.o. 1.5, 5, 15, 50 mg/kg bw/d;	50 mg/kg bw/d Testicular toxicity (with additional systemic toxicity)	15 mg/kg bw/d	Furuhashi 2007A
Rat	TBT	50-52 days (males) 41-45 days (females)	p.o. 1.5, 5, 15, 50 mg/kg bw/d;	15 mg/kg bw/d Testicular toxicity, spermatotoxic effects (with additional systemic toxicity)	5 mg/kg bw/d	Furuhashi 2007B

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

Table 20: Summary table of relevant developmental toxicity studies

Method	Results	Remarks	Reference
• Prenatal Developmental Toxicity Study (OECD Guideline 414, GLP) • rat (Wistar) • oral: gavage • 0, 5, 15, 45 mg/kg bw/d (nominal dose) • 0, 4.1, 12.7, 40.7 mg/kg bw/d (actually ingested)	• NOAEL (maternal toxicity): 5 (4.1) mg/kg bw/d • NOAEL (prenatal developmental toxicity): 5 (4.1) mg/kg bw/d		• BASF SE (2004)

4.11.2.1 Non-human information

For the assessment of developmental toxicity of lysmeral (2-(4-tert-butylbenzyl) propionaldehyde), a study in Wistar rats was performed in accordance with the OECD test guideline No. 414 in line with the OECD Principles of Good Laboratory Practice (BASF SE 2004). Lysmeral (analytical purity 98.1%) was administered via gavage at nominal doses of 5, 15 and 45 mg/kg bw/day from day 6 through day 20 post coitum (p .c.). The effective dose levels amounted to 4.1; 12.7 and 40.7 mg/kg body weight/day.

Clear signs of maternal toxicity were observed starting at the mid dose level. The high dose animals showed transient salivation. Slight but statistically significant reduction of mean food consumption (18% below controls) was observed in the high dose group on day 6-8 p.c. By study termination, food consumption was comparable to control animals (see table 11). Although no evident decrease in food consumption was detectable in mid dose animals, mean maternal weight gains significantly decreased on day 6-8 p.c of about 56 % below control which recovered during the study period.

In high dose animals, a statistically significant mean body weight loss was observed on day 6-8 p.c and the mean body weight gain over the entire treatment phase was found to be about 25% below controls. Furthermore, a statistically significant reduction of mean body weights on day 13 - 20 p.c. (about 7% below controls at study termination) was found. In line, the corrected body weight gain was statistically significantly lower (about 32% below control), representing a direct, substance-related sign of maternal toxicity.

Concerning clinical chemistry, increases in mean alanine aminotransferase levels (20-30% above control) and decreases in serum cholinesterase levels (20-45% below control) were found, starting from the mid dose. In the high dose group, mean glutamate dehydrogenase levels were found to be 79% above controls.

Increases in absolute and relative liver weights (10% and 10-20% above controls, respectively) were found at all dose levels, however, due to the lack of changes in respective clinical parameters,

only the liver weight changes in the mid and high dose group were considered as adverse. In high dose animals, reduced mean uterus weights (20% below controls) were observed.

Gestational parameters such as number of corpora lutea, implantation sites and preimplantation loss were not influenced by the test substance at any dose level (see table **21**). However, mean postimplantation losses (mainly early resorptions) were found to be increased significantly in the high dose group. In animals receiving 45 mg/kg bw per day, mean resorptions accounted to 15.1% per dam compared to 4.4%; 4.7% or 4.9% resorptions at 0, 5 or 15 mg/kg body weight/day, being outside the historical control range. Subsequently, a decrease in the mean number of fetuses and live fetuses per dam became evident in the high dose group, i.e. 7.4, when compared to controls or lower dose groups (8.1; 8.2 and 8.8 at 0; 5 and 15 mg/kg body weight/day). These high dose findings were slightly below the historical controls of the mean number of fetuses per dam. Sex distribution and placental weights were not influenced by the test substance. No dead fetuses, abortions or premature births have been observed in control and all dose groups of this study.

Table 21: Overview on reproductive parameters from the developmental toxicity study in Wistar rats (BASF SE 2004). * = p ≤ 0.05, mg/kg bw/d (mg/kg body weight/day); HCD (Historical control data).

Dose group [mg/kg bw/d]	Conception rate[a]	Pregnant at terminal sacrifice	Mean Corpora lutea	Mean implantation sites	Mean Preimplantation loss[b]	Mean Postimplantation loss[c]	mean number of fetuses / life fetuses
0	92%	92%	9.2±1.41	8.5±1.5	7.6±12.33%	4.4±7.35%	8.1±1.5
5	88%	88%	9.0±1.25	8.6±1.4	4.6±7.42%	4.7±7.59%	8.2±1.18
15	92%	92%	9.9±1.29	9.3±1.22	5.5±7.17%	4.9±10.56%	8.8±1.37
45	92%	92%	9.3±1.36	8.8±1.68	5.9±9.36%	15.1±20.25%*	7.4±2.15
HCD	92%	92%	9.2-11.3 (range per study)	8.1-10.2 (range per study)	3.5-12.2% (range per study)	3.4-11.3% (range per study)	7.6-9.8 (range per study)

[a]Conception rate = number of pregnant animals / number of fertilized animals * 100

[b]Preimplantation loss = number of corpora lutea - number of implantations / number of corpora lutea * 100

[c]Postimplantation loss = number of implantations - number of live fetuses / number of implantations * 100

Sporadic malformations were observed in 3 out of 170 or 1.8% of all high dose group fetuses. Three out of 23 or 13% of the litters were affected in this dose group. Findings were reported as anasarca with a small spleen, polydactyly due to a supernumerary phalanx and cervical hemivertebra. The mean percentages of affected fetuses per litter with total malformations amounted to 0; 0; 0 and 2.4% at 0; 5; 15; or 45 mg/kg bw/day. These findings are not regarded as sufficient evidence for a selective teratogenic effect of lysmeral, since the observed malformations lacked a consistent pattern and were not found in any other dose group. Furthermore, they occurred in very few of the large number of examined fetuses and its low incidence is to be found within the respective control range of the given testing laboratory; i.e. affected fetuses (0-2.7%), affected litters (0-25%) and affected fetuses/litter (0-2.79%).

External variations were not observed and soft tissue variations (dilated renal pelvis, ureters and/or cerebral ventricles) occurred in a dose independent manner in all test groups including control animals. The mean percentages of affected fetuses per litter with total soft tissue variations amounted to 7.8%, 7.7%, 3.6% and 5.3%, in controls, low, mid and high dose animals, respectively.

Skeletal variations were seen in all tested dose groups and litters including controls. Every litter was affected, resulting in 100% litter incidence for all groups assessed. Although within the historical control range and lacking a relation to dosing, a statistically significant increase in mean percentages of affected fetuses per litter were found in mid and high dose animals (99.1% and 98.3% in mid and high dose groups versus 89.1%, 92.%, in controls and the low dose group, respectively). The fetal incidence of skeletal variations in total was increased accordingly.

As shown in table 22, these skeletal variations mainly represent delays and minor disturbances in ossification of the skull, sternebrae and pubic girdle. Non or incompletely ossified structures were statistically significantly increased in the mid and/or high dose group compared to the concurrent control, and incidences of single findings were above the study related and/or current historical control range, i.e. incomplete ossification of supraoccipital, sternebra, pubis or unossified sternebra. As described in detail for skeletal structural variations further down, these findings coincide with decreased mean fetal body weights. These were dose dependently lowered in the mid (10% below controls) and high dose groups (20% below controls). Approximately 85 % of the fetuses in the high dose group and 50 % in the mid dose group showed body weights below one standard deviation of the control group mean body weight (< 3.3 g). Decreases in mean maternal body weight gains or even body weight losses in combination with decreased food consumption occurred at these dose levels as well.

Although significantly increased, structural skeletal variations such as supernumerary (14^{th}) ribs were found in control and dosed animals at high incidences within historical control ranges, whereas incidences for a supernumerary thoracic vertebra (14^{th}) or a misshapen sacral vertebra (1^{st} sacral arch; right or left side) were increased in the high dose group fetuses above historical control ranges.

One fetus of one litter in the control group showed a supernumerary thoracic vertebra which places the study specific control group parameters into the lower part of the updated current historical control range. High dose group incidences are well above the historical control range whereas mid dose group values are within the updated current historical data range. Both dose groups contained single litters with multiple fetuses showing a supernumerary thoracic vertebra. Findings from these litters are the main driver for the increased fetal incidences and affected fetuses/ litter given in table 22. Out of 23 litters, a single mid dose litter with 4 affected fetuses and 2 mid dose litters with 2 affected fetuses each were observed. Similarly, a single litter contained 4 affected fetuses and 3 litters contained 2 affected fetuses each in the high dose group. For each of these litters, a decreased mean litter weight has been observed, i.e. ≤3.3 g in the mid dose group and ≤3.0 g in the high dose group. The respective dams showed a decreased body weight gain in the mid dose and a body weight loss in the high dose group on day 6-8 p.c. Furthermore, increases in absolute and/or relative liver weights and changes in clinical chemistry were noted. Three additional litters contained 1 fetus with such a variation in the mid and high dose group, being within the range of incidence per litter also observed in the control group. One additional vertebra in the thoracolumbar region is generally considered to be a variation in the rat and occurs quite frequently in the rabbit (Solecki 2001).

A misshapen sacral vertebra was found in 1 fetus of 2 control group litters each (fetal incidence of 2.1% and litter incidence of 8.7%). Incidences in the high dose group exceeded the historical

control data. Two of 23 litters contained 2 fetuses with the named variation. In addition, 7 litters contained 1 affected fetus each, leading to an increase in the litter incidence. For all these litters, mean fetal body weights were decreased (≤3.0 g/litter) and respective dams experienced a body weight loss and decreased food consumption on d6-8 p.c., changes in clinicochemical parameters and increases in absolute and/or relative liver weights. Furthermore, the observed structural changes in the morphology of the sacral vertebra were minor and provide, together with the high control animal incidence, the rationale for classifying them as variations.

As described above, the observed skeletal variations are well correlated to the statistically significantly decreased mean fetal body weights and maternal adverse effects in the respective dose groups. Such a delay in fetal body weight development and subsequent increase in skeletal variations is considered to be caused by the evident maternal toxicity.

Significantly increased mean percentages of affected fetuses per litter with incomplete ossification of parietal (29.8% versus 11.7% in controls) or interparietal (36.0% versus 20.7% in controls) with unchanged cartilage was observed only in the low dose group and considered to be spontaneous in nature, since no dose dependency was observed. Discoloration of fetal livers was evident in some mid and high dose animals with a mean percentage of affected fetuses per litter of 1.7% and 15.5%, respectively, being in line with the liver changes of the respective dams.

Based on these findings, the NOAEL is set at 4.1 mg/kg bw/day for maternal and prenatal developmental toxicity.

Table 22: Maternal systemic toxicity based on body weight/ changes and food consumption. Effects on mean fetal body weights on a litter basis and occurrence of statistically significantly increased fetal skeletal variations (expressed as mean percentage of affected fetuses/litter). All statistically significant differences, which showed a dose-response relationship and/or were outside historical control ranges (at date of study) were marked in bold types. * = p ≤ 0.05; ** = p ≤ 0.01; mg/kg bw/d (mg/kg body weight/day);.

Finding		0 [mg/kg bw/d]	5 [mg/kg bw/d]	15 [mg/kg bw/d]	45 [mg/kg bw/d]	HCD report[1] Mean % (range)	HCD 2012[2] Mean % (range)
Mean maternal food consumption (d6-8 p.c.)	grams/ animal/ day	15.4	15.0	14.7	12.7**	-	-
Mean maternal body weight change (d6-8 p.c.)	grams (± SD)	5.0	4.2	2.2**	- 5.5**	-	-
Mean maternal body weight change (d6-20 p.c.)	grams (± SD)	75.3	75.7	77.0	56.8**	-	-
Mean maternal body weights (d20 p.c.)	grams (± SD)	264.4	265.2	268.1	247.2*	-	-
Mean net body weight change from day 6	grams (± SD)	30.0	30.6	30.6	20.5**	-	-
Mean fetal weights	grams (± SD) (on a litter basis)	3.6	3.5	3.3**	2.9**	3.5 (2.8-4.2)	3.6 (2.6-5.5)
Misshapen sacral vertebra	Fetal incidence %	2.1	0	2.7	**12**	0.7 (0-3.5)	1.8 (0-7.1)
	Litter incidence %	8.7	0	13	**39***	3.4 (0-16.7)	7.6 (0-28)
	Affected Fetuses/litter	1.7	0	3	**11.9****	0.8 (0-3.8)	1.9 (0-7.5)
Supernumerary thoracic vertebra	Fetal incidence %	1	2.1	**10**	**14**	1.9 (0-4.7)	2.9 (0-10.1)
	Litter incidence %	4.3	9.1	**26***	**30***	7.3 (0-16.7)	10.7 (0-30)
	Affected Fetuses/litter	1.1	2.7	**9.8***	**13.6****	1.9 (0-5.3)	3.0 (0-11.5)
Supernumerary rib (14th), cartilage present	Fetal incidence %	1	2.1	**10**	8.7	4 (0-8.7)	6.2 (0-19.2)
	Litter incidence %	4.3	9.1	26*	22	13.4 (0-29.2)	20 (0-62.5)
	Affected Fetuses/litter	1.1	2.3	9.8*	8.4*	4.1 (0-10.2)	6.2 (0-18.3)
Supernumerary rib (14th), cartilage not present	Fetal incidence %	41	31	51	39	41.9 (31.3-58.1)	48.8 (31.2-72)
	Litter incidence %	78	64	91	65	81.2 (65.2-95)	85.7 (65.2-100)
	Affected Fetuses/litter	38.5	30.7	51.1*	36.7	41.7 (32.4-58.8)	48.6 (32.4-73.1)
Incomplete ossification of supraoccipital,	Fetal incidence %	13	19	17	**22**	14 (5.8-21.4)	17.9 (5.8-46.2)

unchanged cartilage	Litter incidence %	39	55	43	**61**	41.5 (22.2-60)	48.1 (22.2-92)
	Affected Fetuses/litter	12.8	20.1	17.2	**24.6***	14 (6.2-21.2)	17.8 (6.2-45.1)
Supraoccipital hole(s)	Fetal incidence %	23	17	28	42	31.3 (8-59.3)	29.2 (4-59.3)
	Litter incidence %	52	50	70	87*	70.7 (33.3-100)	67.4 (19-100)
	Affected Fetuses/litter	23.4	17.3	28.4	39.8*	31.7 (8.6-60.5)	29.4 (3.6-60.5)
Incomplete ossification of skull, unchanged cartilage	Fetal incidence %	0	4.3	2.7	7.6	5.4 (0-10.8)	5.5 (0-15.2)
	Litter incidence %	0	14	13	26*	17.3 (0-30.4)	18.1 (0-44.0)
	Affected Fetuses/litter	0	5.1*	2.8*	6.7**	5.4 (0-11)	5.4 (0-15.8)
Unossified sternebra, unchanged cartilage	Fetal incidence %	3.1	8.5	13	**46**	10.6 (3.4-35.7)	7.9 (0.9-35.7)
	Litter incidence %	13	32	26	**83****	32.2 (13-70.8)	24.9 (4.2-70.8)
	Affected Fetuses/litter	3.3	9.4	12.8	**49.9****	11.1 (3.4-39)	8.0 (0.8-39)
Incomplete ossification of sternebra, unchanged cartilage	Fetal incidence %	47	61	**76**	**77**	56 (38.5-73.7)	64.3 (38.5-87.3)
	Litter incidence %	78	91	100*	96	88.5 (70-100)	91.6 (70-100)
	Affected Fetuses/litter	46.1	60.1	**76.7****	**76.6****	55.9 (38.4-74.6)	63.6 (38.4-87.2)
Bipartite ossification of sternebra, unchanged cartilage	Fetal incidence %	0	0	2.7	0	0.8 (0-4.9)	0.4 (0-4.9)
	Litter incidence %	0	0	13	0	2.9 (0-13)	1.6 (0-13)
	Affected Fetuses/litter	0	0	2.8*	0	0.8 (0-4.6)	0.4 (0-4.6)
Incomplete ossification of sacral arch, cartilage present	Fetal incidence %	1	2.1	0	12	12.5 (0-46.2)	6.9 (0-46.2)
	Litter incidence %	4.3	9.1	0	39**	31 (0-87)	18.8 (0-87)
	Affected Fetuses/litter	1.1	2.4	0	16.2**	12.2 (0-46.1)	6.8 (0-46.1)
Incomplete ossification of pubis, cartilage present	Fetal incidence %	0	0	**1.8**	**5.4**	0.4 (0-1.2)	0.3 (0-1.9)
	Litter incidence %	0	0	**8.7**	**22***	2 (0-5.6)	1.6 (0-8.3)
	Affected Fetuses/litter	0	0	**2**	**8****	0.4 (0-1.2)	0.4 (0-2.1)
Incomplete ossification of interparietal, unchanged cartilage	Fetal incidence %	21	**36**	21	11	21.6 (13.6-33.3)	23.4 (12.7-36.1)
	Litter incidence %	52	**82***	65	35	58.5 (37.5-81.8)	60.4 (37.5-82.6)
	Affected Fetuses/litter	20.7	**36***	20.9	9.8	21.5 (12.5-33.3)	23.3 (12.4-35.3)
Incomplete ossification of parietal, unchanged cartilage	Fetal incidence %	11	**30**	14	14	15.2 (3.2-29.9)	15.0 (3.2-29.9)
	Litter incidence %	39	68*	52	48	43.4 (12.5-68.2)	43.7 (12.5-68.2)
	Affected Fetuses/litter	11.7	**29.8****	13.6	13.4	15.4 (3.1-27.6)	15.3 (3.1-27.6)
Total fetal skeletal	Fetal	91	91	99	98	94.6	96.6

variations	incidence %					(88-99.2)	(88-100)
	Litter incidence %	100	100	100	100	100 (100-100)	100 (100-100)
	Affected Fetuses/litter	89.1	92	99.1**	98.3*	94.7 (87-99.2)	96.6 (87-100)

[1] HCD report: historical control data available at the study finalisation date.

[2] HCD 2012: updated historical control databasis, i.e. including data available up to 2012.

Further information on developmental toxicity can be deduced from the respective endpoints of the one-generation rangefinding study. In this study, the test substance was administered to groups of 10 male and 10 female young Wistar rats (F0 parental generation) via the diet (30.7% lysmeral, microencapsulated) (BASF SE 2006C). In the 400, 800, 1700 and 3400 ppm group, the uptake of lysmeral via the diet was accounted to 14.5, 28.7, 62.6 and 119.7 mg/kg bw/day, respectively. Due to dose adjustment during gestation and lactation, the dams received 200, 400, 850 and 1700 ppm of the test substance in feed, resulting in an uptake of 12.9/10 and 25.8/18.3 mg/kg bw/day lysmeral for the two low dose groups, respectively (for further details see Chapter 4.11.1.1.).

No viable offspring has been derived from animals treated with 1700 ppm and 3400 ppm microencapsulated lysmeral. In the 1700 ppm group, the only pregnant female had only 1 implant which was resorbed. In contrast, only a slight and non-significant increase in mean implantation losses have been observed for the two lower dose groups; i.e. 16% and 11% mean losses per litter in dose groups 400 ppm and 800 ppm versus 5% in controls, respectively (see table **17**).

A slight decrease in the mean number of delivered pups per dam (7.9 in dose group 800 ppm versus 9.4 and 8.7 in controls and dose group 400 ppm) was recorded. However, no effects on gestation and the live birth indices became evident, due to the absence of any stillborn in the lysmeral treated dose groups with offspring.

Pup survival was minimally decreased for postnatal day 0 to 4 (94% in the 800 ppm dose group versus 99% in the 400 ppm dose group and controls), and no pup mortality was observed between postnatal day 4 and 21 in all dose groups with offspring. Overall the respective viability and lactation index was not considered to be affected by treatment.

No effects in sex ratios have been observed and pup necropsy revealed only sporadic and non-dose related findings, including post mortem autolysis, situs inversus, hemorrhagic thymus, dilated renal pelvis and a small kidney. The overall pup incidence for these observations was 6.4%, 2.6%, and 1.3% in controls and dose groups 400 ppm and 800 ppm respectively.

For the 400 and 800 ppm dose groups, a significant reduction in birth weights (19% and 22% below controls, respectively) and pup weight at weaning (17% and 21% below controls, respectively) has been recorded for male and female pups. Accordingly the pup body weight gain was decreased in the 400 and 800 ppm dose groups (16% and 21% below controls, respectively).

Taken together, significant developmental toxicity has been observed in the one-generation rangefinding study only in terms of decreased pup weights and pup weight gains. These dose levels were associated with changes in clinical chemistry of parental females, i.e increased gammaGT and GLDH activities and decreased activity of serum cholinesterases. In the 800 ppm dose group, impaired maternal body weight development and food consumption was observed during premating phase, gestation and lactation (for further details see Chapter 4.11.1.1.).

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

No data available.

4.11.4 Summary and discussion of reproductive toxicity

Summary of Fertility

Repeated dose studies in male rats, partly with focus on male reproductive organs, provide evidence for adverse effects on male reproductive organs in rats after oral lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) administration. These effects were observed concomitantly with signs of general toxicity and adverse effects on the liver. Testicular toxicity was observed at dose levels of 50 mg/kg bw/day and above, and the NOAEL for these effects after oral administration is set at 25 mg/kg bw/day. This effect level was found to be independent from treatment duration. Adverse testicular findings were observed even after a single oral administration. These data support the conclusion for a clear dose threshold for the induction of testicular toxicity in rats independent of dose duration.

Accordingly, impairment of male fertility combined with signs of general toxicity and changes in clinical parameters of the liver was observed in the one-generation range finding study in the rat after oral administration of lysmeral. Due to the obvious testicular and spermatotoxic effects of lysmeral, the relation between the observed lack of pregnancies, lack of delivered offspring and impairment of male fertility is clearly indicated. These findings were obtained at comparable dose levels also used in repeated dose studies. In contrast, dermal administration on rats led to no testicular toxicity except for dose levels above the limit dose. In dogs, general adverse effects together with liver and testicular toxicity were observed after oral administration, however, adverse testes effects occurred at higher dose levels than in the rat. Considering the findings from the available studies in dogs, a NOAEL for testicular toxicity is set at 44.6 mg/kg bw/day. No testicular toxicity was observed in the mouse, guinea pig, rabbit and primates.

Identical adverse testicular effects and species specificity has been observed after oral administration of p-tert-benzaldehyde (TBB) and p-tert-butyltoluene (TBT); (see Annex 3). The rat has been found to be the most sensitive species for TBB and TBT induced testicular toxicity. In analogy to lysmeral, systemic formation of p-tert-butylbenzoic acid (TBBA) has been observed after oral administration of TBB and TBT, being approximately 2-3 fold higher compared to lysmeral treated animals on the basis of urinary TBBA levels (for details see Chapter 4.1). Therefore TBB, TBT and lysmeral all share the same metabolite, namely TBBA. The oral, dermal or inhalation administration of the identified metabolite TBBA to the rat results in the same testicular findings as observed for lysmeral, TBB or TBT (see Annex 2). Comparing the available lowest adverse effect levels for testicular toxicity in the rat, the lowest potency can be assigned to lysmeral with a $LOAEL_{testicular\ toxicity}$ of 50 mg/kg bw/day after oral application. TBT and TBB were found to be more potent with $LOAELs_{testicular\ toxicity}$ of 15 and 25 mg/kg bw/day, respectively. The magnitude of difference in testicular toxicity potency between lysmeral and TBB/TBT are comparable to the differences observed in the urinary TBBA levels formed. The highest potency can be assigned to TBBA with a $LOAEL_{testicular\ toxicity}$ of 8 mg/kg bw/day.

Taken together, the comparable pattern of testicular effects, the species dependencies and the observed differences in potencies substantiate, that the formation of systemic TBBA represents the mode of action for lysmeral and TBB/TBT induced testicular toxicity.

Summary of Developmental Toxicity

Developmental toxicity of lysmeral has been assessed by oral (gavage) administration of lysmeral to pregnant rats in a developmental toxicity study according to OECD test guideline No. 414.

High dose dams (41 mg/kg bw/d) showed clinical signs (transient salivation), transient reduction of mean food consumption and body weight loss on day 6-8 p.c. Mean body weight gain was decreased over the entire treatment phase resulting in lower mean body weights on day 13 - 20 p.c. and net body weight gain compared to controls. Increased levels of alanine aminotransferase and glutamate dehydrogenase, decreases serum cholinesterase levels and organ weight changes (increased liver weights, reduced uterus weights) were noted.

In mid dose dams (13 mg/kg bw/d) body weight gains were transiently decreased on day 6-8 p.c. Furthermore, alanine aminotransferase levels were increased, serum cholinesterase levels were decreased and increased liver weights were found.

These findings reflect a lysmeral induced general systemic and liver toxicity for high dose and less pronounced for mid dose dams.

The number of mainly early resorptions was increased due to postimplantation losses in the high dose group whereas gestational parameters were not significantly influenced in lower dose groups (5, 15 mg/kg bw/d). Subsequently, the number of fetuses and live fetuses per dam was found to be slightly below the respective historical control range in the high dose group.
Concomitantly, prenatal developmental toxicity in terms of reduced fetal body weights was observed in the mid and high dose groups. These findings coincided with significant maternal toxicity at the same dose levels.

Sporadic malformations were observed, which lacked a consistent pattern, occurred in very few of the large number of examined fetuses and there incidences were found within the respective historical control ranges. External variations were not observed and soft tissue variations occurred in a dose independent manner in all test groups including control animals.

In contrast, an overall incidence of skeletal variations was statistically significantly increased in mid and high dose animals. These variations represented mainly delays and minor disturbances in ossification processes of the skull, sternebrae and pubic girdle. Supernumerary (14^{th}) ribs were found in control and dosed animals at high incidences, and structural variations like a supernumerary thoracic vertebra (14^{th}) or a misshapen sacral vertebra (1^{st} sacral arch) were found to be increased evidently in the high dose group fetuses. The observed skeletal variations are well correlated to statistically significantly decreases in mean fetal body weights and evident maternal toxicity in the respective dose groups. Clustering of incidences for a supernumerary or misshapen vertebra in single litters was observed, and a maternal predisposition which affects the respective offspring in situations of maternal stress conditions could be hypothesized here.

Supernumary ribs and delays of ossification in rodent offspring are among the common endpoints related to chemical exposure stress (ECETOC, 2004). Delays in ossification are by definition

transitory, occur in conjunction with decreased fetal weights and represent an indicator for adverse effects on fetal maturation rather than a teratogenic potential (Daston, 2007).

Overall, the increased numbers of fetuses with common skeletal variations are considered an embryo-/fetotoxic effect due to fetal growth retardations, representing a manifestation of a non-specific stress on the dams and not a teratogenic effect of lysmeral. Increased early resorptions and the subsequent decrease in number of fetuses are further manifestations of the non–specific maternal stress induced by lysmeral administration.

The findings of the one-generation rangefinder study are largely consistent with the effects observed in the present key teratogenicity study. Slight, non-significant and dose independent increases in postimplantation losses was found in both dose groups having offspring. A slight reduction in the number of delivered pups could be observed at the highest dose group having offspring. Furthermore, a significant reduction in birth weights, pup weights at weaning and pup weight gain has been seen in both dose groups when compared to controls. These findings coincided with adverse systemic effects to the dams.
No effects on the gestation and live birth indices were observed due to the absence of any stillborn in the dosed animals. Viability and lactation indices were not significantly affected, no changes in sex ratios have been observed and no test substance related findings in pup necropsy have been found.

Taken together, developmental toxicity has been observed at doses leading to evident maternal toxicity and is considered to be a secondary non-specific consequence of general systemic toxicity in the dams. Therefore, these findings do not warrant a classification with respect to developmental toxicity.

Discussion

Adverse effects of lysmeral on the male reproductive system have been observed in various oral repeated dose toxicity studies in rats and were confirmed in a feeding one-generation range-finding study, whereas no evidence for testicular toxicity was observed in the mouse and guinea pig. Considering non-rodent species, the dog has been shown to be susceptible towards testicular toxicity as well, however at higher dose levels than the rat. Testicular toxicity in rats and dogs after oral lysmeral application was only observed at dose levels showing also general signs of toxicity in these animals. In studies with more detailed observations, the liver was found to be the main affected organ upon treatment with doses also inducing testicular toxicity. In contrast, short-term oral exposure to rabbits did not indicate a potential of lysmeral to induce testicular toxicity. Furthermore in rhesus monkeys, no indication of testicular toxicity, at doses causing testicular toxicity in the rats, was observed. Therefore, the rat appears to be the most susceptible species, and it appears, that a single oral exposure to lysmeral above a clearly defined threshold dose seems sufficient to cause testicular toxicity.

Based on the clear evidences from animal studies, it is considered appropriate to classify lysmeral for reproductive toxicity, i.e. adverse effects on fertility. In determining the respective hazard category, the assessment of the relevance of the hazard to humans is to be considered.

The adverse effects on male reproductive organs are considered to underlie species specific mechanisms and the present data indicate, that primates are considerably less or even not susceptible towards the testicular toxicity observed in the dog and more effectively in the rat. In accordance, quantitative differences in the formation of metabolites such as TBBA exist and the

urinary excretion of glycine conjugated TBBA differs between the rat and the other rodent species investigated, i.e. mouse and guinea pig.

On the basis of a qualitative and quantitative evaluation of metabolic profiles for different species in an in vitro metabolism study, a predominant formation of TBBA levels in rat hepatocytes was found when compared to other rodent, non-rodent animal or human hepatocytes. The TBBA levels observed in the model using human hepatocytes were found to be approx. 4 fold lower compared to rat hepatocytes at corresponding incubation concentrations (10, 50 100 µM), which reflect plasma levels obtained after oral administration of lysmeral doses below and above the lowest adverse testicular effect level. Furthermore, the TBBA levels formed in human hepatocytes after incubation of lysmeral concentrations related to adverse testicular effect doses (50 and 100 µM) were comparable to TBBA levels found in the rabbit, a species not sensitive to testicular toxicity.

Testicular effects and species dependencies identical to lysmeral have been observed after application of p-tert-benzaldehyde (TBB) and p-tert-butyltoluene (TBT). Para-tert-butylbenzoic acid (TBBA) is formed as metabolite after administration of TBT, TBB or lysmeral. Therefore TBB, TBT and lysmeral all share the same metabolite, namely TBBA. TBBA application in rats revealed the highest testicular toxicity potency based on effect levels and is included in Annex VI of the CLP regulation with a classification as Repr. Cat2 R60 and Repr. 1B. In line, TBB and TBT showed lower potencies in exerting comparable testes effects. Lysmeral showed the lowest potency in testes toxicity when compared to TBB, TBT and especially to TBBA. Testes toxicity potencies correlated with systemically formed urinary TBBA levels. Overall, these findings substantiate, that the formation of systemic TBBA represents the mode of action for lysmeral and TBB/TBT induced testicular toxicity.

The human odour threshold for racemic lysmeral is set at 1-2 ppb (0.01 mg/m3 for R-lysmeral, >2.5 mg/m3 for L-lysmeral; van Gemert, 2003). Due to its properties, a concentration 1000 fold above the human odour threshold would usually be perceived as rather unpleasant. Therefore a prolonged human uptake of lysmeral doses inducing systemic toxicity, i.e. testes toxicity or spermatotoxic effects, is highly unlikely. Perceivable lysmeral concentrations would result in a calculated internal dose of 0.007 mg/kg lysmeral (considering a daily respiration volume of 20 m^3 and an average body weight of 60 kg for the general population). Given an allometric scaling factor of 4, the perceivable odour threshold would result in human doses approximately 3 orders of magnitude below non-effective rat dose levels concerning testicular toxicity.

The adverse testicular effects in the rat and dog were observed after administration of lysmeral via the oral route. Besides test substance stability issues, palatability was a major obstacle due to the unpleasant smell of concentrated lysmeral in order to attain study relevant doses. Therefore, administration needed to be performed via gavage or encapsulation, representing an unrealistic and non-relevant form of application with respect of realistic use patterns.

The dermal route is the most relevant route to humans in both an occupational and consumer setting (exposure via inhalation may occur but to a much lower extent). Compared to oral studies, dermal administration of lysmeral in rats led to testicular toxicity only at an excessive dose level, clearly above the limit dose, whereas at 1000 mg/kg body weight, no adverse testicular effects were observed. Furthermore, compared to rats, very limited percutaneous absorption of the test substance in humans was observed. Taking into account conservative assumptions, the potential lysmeral uptake of workers is of low magnitude. Based on the high margin in relation to the oral dosing studies in the rat, it is unlikely that a worker would reach relevant systemic levels of lysmeral or respective metabolites under normal working conditions or following an accidental acute exposure (for detailed information, see Annex 1). Due to much lower use concentrations in formulated

consumer products, a lysmeral uptake by the consumer would in general be much lower than for the worker.

Overall, studies on species variability provide clear evidence that higher order mammalian species including humans are less or not susceptible than rats due to the observed lack of testicular toxicity and differences in metabolic profiles. Furthermore, a low odour threshold and unpleasant perception at toxicologically relevant but unrealistic concentrations are intrinsic properties of lysmeral. Based on the fact that testicular toxicity in susceptible species has a clear threshold, it is highly unlikely that lysmeral levels taken up by humans would lead to the formation of relevant systemic levels of TBBA. Taken together, the relevance of the species specific testes toxicity observed in test animals for humans is doubtful.

4.11.5 Comparison with criteria

Lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) has been found to induce testicular toxicity and spermatotoxicity when administered orally to rats and at higher dose levels to dogs. Infertility in rats due adverse effects of orally administered lysmeral on the male reproductive system has been confirmed in a feeding one-generation range-finding study. Based on clear evidences from experimental animals, it is considered appropriate to classify lysmeral for reproductive toxicity, i.e. adverse effects on fertility.

The CLP regulation criteria for classification in Repr. 1B (fertility) are as follows:

“The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.”

Besides the reference to clear evidences from experimental animals, the CLP regulation further states in its criteria for classification in Repr. 1B (fertility):

“However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.”

In determining the appropriate hazard category, the assessment of the relevance of the given hazard to humans needs to be taken into account.

- Species specificity for lysmeral induced testicular toxicity has been observed. Adverse effects of lysmeral on the male reproductive system at a clearly defined threshold dose have been found in rats whereas no evidence for testicular toxicity was observed in the mouse and guinea pig. Considering non-rodent species, the dog has been shown to be susceptible towards lysmeral induced testicular toxicity. In contrast, short-term oral exposure to rabbits did not indicate a potential of lysmeral to induce testicular toxicity. Furthermore in rhesus monkeys, no indication of testicular toxicity, at doses causing testicular toxicity in the rats, was observed.
- Based on the accordance in the testicular toxicity profile of lysmeral, p-tert-benzaldehyde (TBB), p-tert-butyltoluene (TBT) and the shared metabolite para-tert-butylbenzoic acid

(TBBA), the formation of the systemic TBBA intermediate is the causative agent responsible for the mode of action for lysmeral induced testicular toxicity.

- Species specificity for lysmeral induced testicular toxicity is reflected by species dependent differences in the conversion of lysmeral to TBBA in hepatocytes. TBBA formation in human hepatocytes is of low magnitude compared to rats and is comparable to levels found in the rabbit at toxicologically relevant doses, a species not sensitive to lysmeral induced testicular toxicity.
- Due to its low odour threshold and the perception as rather unpleasant at toxicological relevant but unrealistic concentrations (1000 fold above the respective odour threshold), a prolonged human uptake of lysmeral resulting in doses as in animals is highly unlikely.
- Because of the properties of lysmeral as fragrance material leading to palatability issues, substance administration needed to be performed via gavage or encapsulation. This represents a non-relevant form of application with respect of realistic use patterns. Studies via the relevant route to humans (i.e. dermal) in rats showed no testicular toxicity up to the limit dose. On the basis of comprehensive conservative assumptions, a potential human lysmeral uptake would be of low magnitude when compared to doses leading to rat testicular toxicity

A clear evidence for a species specificity and, if at all, a lower human susceptibility concerning lysmeral induced testicular toxicity raises doubt about the relevance of the effect for humans. Furthermore, evident reproductive toxicity has been observed after substance administration via gavage or encapsulation, representing a non-relevant form of application with respect of realistic use patterns. A low odour threshold and unpleasant perception at toxicologically relevant but unrealistic concentrations are intrinsic properties of lysmeral, making a human lysmeral uptake above the clearly defined threshold dose for lysmeral induced testicular toxicity in rats highly unlikely.

As outlined in the CLP criteria listed above, a classification as substance to be suspected of damaging fertility, i.e. Repr. 2 (H361f; regulation 1272/2008) and Repr. Cat3 R62 (67/548/EEC) is appropriate**.**

Concerning developmental toxicity, the CLP regulation states as a basis of classification:

"…Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

Developmental toxicity has been observed at doses leading to evident maternal toxicity and is considered to be a secondary non-specific consequence of general systemic toxicity in the dams. Therefore, based on the present data, no classification concerning developmental toxicity is warranted.

4.11.6 Conclusions on classification and labelling

Lysmeral (2-(4-tert-butylbenzyl) propionaldehyde) has been identified to induce testicular toxicity when administered orally to rats and at higher dose levels to dogs. Infertility in rats due adverse

effects of orally administered lysmeral on the male reproductive system has been confirmed in a feeding one-generation range-finding study. Based on clear evidences from animal studies, it is considered appropriate to classify lysmeral for reproductive toxicity, i.e. adverse effects on fertility. In determining the respective hazard category, the assessment of the relevance of the given hazard to humans needs to be taken into account.

Species specificity for lysmeral induced testicular toxicity has been observed. Adverse effects of lysmeral on the male reproductive system at a clearly defined threshold dose have been found in rats but not in the mouse and guinea pig. Considering non-rodent species, the dog has been shown to be susceptible towards lysmeral induced testicular toxicity. In contrast, short-term oral exposure to rabbits did not indicate a potential of lysmeral to induce testicular toxicity. Furthermore in rhesus monkeys, no indication of testicular toxicity, at doses causing testicular toxicity in the rats, was observed

Based on the accordance in the testicular toxicity profile of lysmeral, p-tert-benzaldehyde (TBB), p-tert-butyltoluene (TBT) and the shared metabolite para-tert-butylbenzoic acid (TBBA), the formation of the systemic TBBA intermediate is the causative agent responsible for the mode of action for lysmeral induced testicular toxicity. On the basis of the effective doses determined, lysmeral possesses evidently a much lower potency for testicular toxicity than TBBA.

Species specificity for lysmeral induced testicular toxicity is reflected by species dependent differences in the conversion of lysmeral to TBBA in hepatocytes. TBBA formation in human hepatocytes is of low magnitude compared to rats. In fact, TBBA formation in humans is comparable to levels produced in species that did not demonstrate testicular toxicity (i.e. rabbits) at biologically relevant doses. Therefore, clear evidences for a lower susceptibility of humans regarding lysmeral induced testicular toxicity further support the absence of its human relevance.

Due to its low odour threshold and the perception as rather unpleasant at toxicological relevant but unrealistic concentrations (1000 fold above the respective odour threshold), a prolonged human uptake with lysmeral doses resulting in such effective internal doses is not to be expected. Therefore, the occurrence of lysmeral induced testicular toxicity in humans is extremely unlikely.

Because of the properties of lysmeral as fragrance material leading to palatability issues, substance administration needed to be performed via gavage or encapsulation. This represents a non-relevant form of application with respect of realistic use patterns. Studies via the relevant route to humans (i.e. dermal) in rats showed no testicular toxicity up to the limit dose (1000mg/kg bw/day). On the basis of comprehensive conservative assumptions, a potential human lysmeral uptake would be of low magnitude when compared to lysmeral doses leading to rat testicular toxicity. This estimation further supports, that it is unlikely that a human would reach relevant systemic levels of lysmeral specific metabolites.

Given the intrinsic properties of lysmeral (i.e. low odour threshold and unpleasant perception at toxicologically relevant but unrealistic concentrations), which leads to the conclusion that a hazardous situation for humans would be unrealistic, the relevance of the observed testicular effects for humans is doubtful. Furthermore, clear evidence for a species specificity and, if at all, a lower human susceptibility concerning lysmeral induced testicular toxicity question a relevance for humans.

Therefore, a classification as substance to be suspected of damaging fertility, i.e. Repr. Cat3 R62 (67/548/EEC) or Repr. 2 (H361f; regulation 1272/2008), is warranted**.**

Developmental toxicity has been observed at doses leading to evident maternal toxicity and is considered to be a secondary non-specific consequence of general systemic toxicity in the dams.

Therefore, based on the present data, no classification concerning developmental toxicity is warranted.

4.12 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

This substance has been registered according to the requirements of the REACH legislation. In addition, the substance is currently under evaluation in the framework of the Community Rolling Action Plan (CoRAP). The evaluation started in the year 2012 and is still ongoing. The listing was based on concerns regarding human health due to CMR properties wide dispersive use and consumer use.

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8 ANNEXES

ANNEX 1: HUMAN LYSMERAL UPTAKE AND REPRODUCTIVE TOXICITY HAZARD ASSESSMENT.

Summary

Lysmeral has been identified to induce testicular toxicity when administered orally to rats and dogs, but not to mice, guinea-pigs, rabbits and monkeys. Orally administered lysmeral in rats resulted in the formation of systemic p-tert-butyl benzoic acid (TBBA). TBBA has been found to induce identical testicular toxicity in rats at significantly lower doses compared to lysmeral.

A dermal study in rats showed no testicular toxicity at up to the limit dose (1000 mg/kg bw/day).

It is considered appropriate to classify lysmeral for reproductive toxicity, i.e. adverse effects on fertility. In determining the appropriate hazard category for lysmeral, an assessment of the relevance of the hazard to humans has been made. This conservative assessment is meant to clarify the identification of human relevance and includes the estimation of lysmeral doses taken up by workers and the general population and comparison to the effective level of the most sensitive species via the oral route.

Taking conservative assumptions into account, the estimated lysmeral doses in an occupational setting are of low magnitude. Due to the much lower levels of lysmeral in formulated consumer products, lysmeral doses taken up by the consumer would be in general significantly lower than for the worker.

The potential for effects from the estimated worker lysmeral uptake under normal working conditions or following an accidental acute event would result in systemic doses of lysmeral or respective metabolites such as TBBA, far below levels that have been shown to induce testicular toxicity in the rat. Further, from the observed species variability in metabolism of lysmeral showing that human relevance is questionable and considering the significantly lower doses of lysmeral likely in the general population, the induction of testicular toxicity is highly unrealistic.

Introduction and approach

Lysmeral is used as fragrance in a wide number of industries. It shows an intensive, radiant, floral odor with a typical lily-of-the-valley note. As a component of fragrance mixtures the main uses include cosmetic/personal care products and washing/cleaning products. Lysmeral is further included as a fragrance substance in air care products, biocidal products, coatings and paints, fillers/plasters, ink/toners, polishes/wax blends and scented articles (clothes, eraser, toys, paper articles, CD). On the basis of animal data, showing testicular toxicity of lysmeral, an assessment of human doses resulting from the current uses of lysmeral is compared in this chapter with doses from respective animal studies in support of an appropriate hazard classification.

Exposures of lysmeral to the general public are minimal, since the substance is only used in trace amounts in final products, is poorly absorbed dermally in humans (6% of a dermally applied dose) and has a low volatility (vapor pressure = 0.25 Pa). Thus, lysmeral doses taken up by a consumer would be expected to be very low and significantly lower than by workers involved in manufacturing, compounding, formulating and in industrial/professional uses in final products, i.e. cleaning.

These assessments for potential relevant routes of exposure (i.e. dermal and inhalation), have been developed using conservative assumptions in first tier or higher tier exposure estimation tools (ECETOC TRA, Stoffenmanager, RiskofDerm). Overall, this assessment determines the potential occupational exposure to lysmeral during manufacturing, compounding, formulating and industrial/professional uses in final products.

Exposure assessment

For the assessment of the workplace, models, i.e. ECETOC TRA, Stoffenmanager 4.0 or RISKOFDERM 2.1 have been used. This assessment is supported by workplace measurements covering several process categories.

Exposures in the workplace

It is generally assumed that oral exposure to industrial chemicals in the workplace can be discounted and therefore, it is considered unlikely that any oral uptake with liquid lysmeral will occur during manufacture, compounding, formulation and industrial/professional uses in final products. Ingestion is usually controlled by straightforward good hygiene practices such as segregating working and eating facilities and adequate washing prior to eating. Overall, a putative oral uptake has not been included in a combined worker exposure estimation.

For the assessment of the uptake via the dermal route, a low dermal absorption of 6% of the applied dose is assumed based on a value of 2% from a study in human subjects and a safety factor of 3 due to limited number of test subjects and the detected variability between individual test subjects (Huntingdon Research Centre Ltd 1994; for further details see Chapter 4.1.). External dermal exposure has been calculated by the first tier exposure estimation tool ECETOC TRA or the higher tier exposure estimation tool RISKOFDERM 2.1 (see table 23), and an internal dermal exposure has been calculated assuming a 6% dermal absorption of the external dermal dose. For all process categories, the use of suitable gloves as personal and product protective equipment has been included in the exposure assessment resulting in an additional reduction by a factor of 10 of the external dermal dose.
Inhalation of lysmeral is considered to be of low relevance, since lysmeral has a low vapor pressure (0.25 Pa) and the major industrial scenarios do not include the formation of aerosols. Inhalation has been estimated using the first tier estimation tool ECETOC TRA or the higher tier estimation tool Stoffenmanager 4.0 (see table 23). The given external inhalation exposure estimate has been calculated into an internal inhalation estimate assuming a 100% absorption via inhalation, a mean worker respiratory volume of 10 m^3 and a mean body weight of 70 kg. For confirmation of the calculated estimates, occupational measurements by personal air sampling are available, covering the relevant process categories in the manufacture exposure scenario (table 22). These data support, that the calculations made are based on conservative assumptions, leading to an overestimation compared to the realistic levels not exceeding 1 µg/m^3. Furthermore in a model setup, a stationary measurement of the air concentration 5 cm above the liquid surface of lysmeral resulted in a concentration of 0.18 mg/m^3 at room temperature. Sampling has been performed for 150 minutes above a dish containing 20 ml lysmeral.

This setup represents a worst case scenario compared to realistic scenarios and further confirms the conservatism in the calculation for certain process categories, where relevant uptake via inhalation might occur (e.g. mixing or industrial/professional spray applications of final products). Such model setup can also be used for the assessment of lysmeral uptake following an accident in the workplace such as spilling. The concentrations of lysmeral in air would be considered not to exceed 0.18 mg/m^3 as determined in the setup, i.e. air concentration 5 cm above the liquid surface. Considering a respiration volume of 10 m^3 during a shift and a mean body weight of 70 kg, a daily internal dose would result in 0.026 mg/kg bw for workers not wearing respiratory protection. When comparing with the no adverse effect level for testicular toxicity in the rat as most sensitive species including an allometric scaling factor of 4, the margin of safety would be approx. 250 for such an accidental situation.

Table 23: BASF SE Workplace measurements. Personal air sampling according to EN 481 und 482 have been performed. For the determination of lysmeral concentrations in air, defined air volume have been collected into a cartridge containing 2,4-dinitrophenylhydrazine. Adsorbed lysmeral has been eluted with acetonitrile and quantified by liquid chromatography via comparison with a calibrator solution.

Detection limit (mg/m3)*	Measurement duration (minutes)	Description of Task
< 0.001	250	Activities in laboratories
< 0.00098	240	Activities in laboratories
< 0.00098	240	Transfer of substance into drums
< 0.00098	240	Transfer of substance into drums
< 0.00098	240	Transfer of substance into drums
< 0.001	500	Drum filling and control activities in production facility
< 0.001	500	Sampling and control activities in production facility
< 0.001	500	Sampling and control activities in production facility
< 0.00098	240	Activities in production facility - not further specified
< 0.00098	240	Activities in production facility - not further specified
< 0.001	250	Activities in production facility - not further specified
< 0.001	250	Activities in production facility - not further specified
< 0.001	250	Activities in production facility - not further specified

*All measurement results have been below the detection limit given in the table

As presented in Table 23, the external dermal dose in a worker specific exposure scenario does not exceed 0.7 mg/kg bw day on the basis of the conservative assumptions of ECETOC TRA, leading to a potential internal dose of 0.04 mg/kg bw/day. Furthermore, the calculated mean inhalation exposure estimates during an 8 hour shift do not exceed 0.22 mg/m^3 resulting in an internal dose of 0.03 mg/kg bw/day.

Overall, the comparison of the combined internal exposure estimate (dermal and inhalation) of all relevant process categories for lysmeral and the no adverse effect level for testicular toxicity in the most sensitive species, i.e. rats, revealed a margin above 100. In considering the high margins in relation to the rat as most sensitive species for lysmeral induced testicular toxicity, uptake of lysmeral in the workplace will not have an impact on the fertility of male workers.

Table 23: Combined exposure assessment and risk characterization of all relevant process categories in the workplace.

Exposure scenario	Process category PROC[1]	Exposure estimate long-term inhalation external [mg/m³]	Exposure estimate long-term inhalation internal [mg/kg bw/d][2]	Exposure estimate long-term systemic dermal external [mg/kg bw/d]	Exposure estimate long-term systemic dermal internal [mg/kg bw/d][3]	Exposure estimate long-term systemic combined internal [mg/kg bw/d]	NOAEL [mg/kg bw/d][4]	Margin of safety[5]
Manufacture	2	0.0097	0.0014	0.1371	0.0082	0.0096	25	650
	8b (vessel)	0.0201	0.0029	0.6857	0.0411	0.0440	25	142
	8b (drums)	0.0091	0.0013	0.6857	0.0411	0.0424	25	147
	15	0.0201	0.0029	0.0343	0.0021	0.0049	25	1267
Compounding	1	0.0170	0.0024	0.0343	0.0021	0.0045	25	1392
	2	0.1703	0.0243	0.1371	0.0082	0.0325	25	192
	3	0.0220	0.0031	0.0343	0.0021	0.0052	25	1201
	5 (automated)	0.0539	0.0077	0.00077	0.0000	0.0078	25	806
	5 (manual)	0.0539	0.0077	0.00077	0.0000	0.0078	25	806
	8b	0.0660	0.0094	0.6857	0.0411	0.0506	25	124
	15	0.1703	0.0243	0.0069	0.0004	0.0247	25	253
Formulation	1	0.0004	0.0001	0.0009	0.0001	0.0001	25	55678
	2	0.0426	0.0061	0.0034	0.0002	0.0063	25	994
	3	0.1277	0.0182	0.0009	0.0001	0.0183	25	342
	5	0.0539	0.0077	0.0343	0.0021	0.0098	25	640
	8b	0.0228	0.0033	0.0686	0.0041	0.0074	25	848
	9	0.0228	0.0033	0.0686	0.0041	0.0074	25	848
	14	0.2128	0.0304	0.0017	0.0001	0.0305	25	205
	15	0.0228	0.0033	0.0034	0.0002	0.0035	25	1806
Cleaning industrial	5	0.0102	0.0015	0.0005	0.00003	0.0015	25	4188
	7	0.1705	0.0244	0.0017	0.00010	0.0245	25	256
	8a	0.0068	0.0010	0.0005	0.00003	0.0010	25	6214
	8b	0.0034	0.0005	0.0003	0.00002	0.0005	25	12428
	10	0.0204	0.0029	0.0011	0.00007	0.0030	25	2094
	13	0.0068	0.0010	0.0005	0.00003	0.0010	25	6214
Cleaning professional	8a	0.0170	0.0024	0.0005	0.00003	0.0025	25	2536
	8b	0.0068	0.0010	0.0003	0.00002	0.0010	25	6318
	10	0.0511	0.0073	0.0011	0.00007	0.0074	25	849
	11	0.0385	0.0055	0.0043	0.00026	0.0058	25	1086
	19	0.0170	0.0024	0.0057	0.00034	0.0028	25	2255

[1]process categories (PROCs) are defined according to REACH Guidance on information requirements and chemical safety assessment Chapter R.12: Use descriptor system.

[2]calculated as follows: Exposure estimate inhalation external x respiration volume (10 m3) / body weight (70 kg)

[3]calculated as follows: Exposure estimate dermal external x dermal penetration human (6% of applied dose)

[4]NOAEL for testicular toxicity in rats

[5] NOAEL / systemic combined internal exposure estimate x allometric scaling (4)

Exposures via consumer products

Lysmeral is found in a broad array of consumer products as a component of fragrance mixtures. However, lysmeral has not been used as flavor ingredient. The main sources of lysmeral stem from cosmetic/personal care products and washing/cleaning products. Lysmeral is further included in air care products, biocidal products, coatings and paints, fillers/plasters, ink/toners, polishes/wax blends and scented articles (clothes, eraser, toys, paper articles, CD). As described above, estimated worker doses of lysmeral are of low magnitude. Due to the much lower levels of lysmeral in formulated consumer products, an uptake of lysmeral by a consumer would be in general significantly lower than for the worker. Lysmeral is used in trace amounts in final product up to 0.04 % in washing/cleaning products and up to 0.6 % in personal care products. Highest use levels for lysmeral were determined for air care products (up to 7%), which contain high concentrations of fragrance mixes. Despite the obvious intention of incorporating fragrances into consumer products for their olfactory properties, inhalation appears to represent a minor route of systemic exposure to fragrances, even when highly exaggerated airborne levels and rather unlikely exposure scenarios are used (Cadby 2002). Furthermore lysmeral has a low volatility (vapor pressure = 0.25 Pa), making exposure via the inhalation route less likely. Since lysmeral is not used as flavor agent nor is included in oral care products, only indirect oral exposure (e.g. via dishwashing residues) is to be expected. Therefore, the major route of systemic exposure is almost certainly by deposition on the surface of the skin.
A broad variety of consumer products contain lysmeral as an integral part of fragrance mixtures included in these products. However, the final concentrations in these products are usually in the range or below the concentrations found in the main product/article categories described above. Since an uptake of lysmeral by a consumer is expected to be significantly lower than by a worker involved in manufacturing, compounding, formulating and in industrial/professional uses in final products, i.e. cleaning, the exposure of the general population to products containing lysmeral will not have an impact on male fertility.

Conclusion

In conclusion, based on the high margins of safety in relation to oral dosing studies in the rat as most sensitive for lysmeral induced testicular toxicity, it is unlikely that the uptake of lysmeral would lead to the formation of relevant systemic levels of metabolites, such as p-tert-butylbenzoic acid, in the human, meaning the likelihood of a concern would be very low.

ANNEX 2: OVERVIEW ON STUDIES ADRESSING TESTICULAR TOXICITY INDUCED BY P-TERT-BUTYLBENZOIC ACID (TBBA).

Evidences for testicular toxicity after oral administration of p-tert-butylbenzoic acid were found in an acute oral toxicity study in albino Carworth Farm rats (Hunter 1965). Groups of 4 animals/sex were administered with doses of 500, 630, 800, 1000 and 2000 mg/kg bw. When male survivors were examined 18 or 24 days after treatment, testicular atrophy was observed in 4/5 males exposed to a single dose of 500 mg/kg bw. Testes were shrunken, their parenchyma was pinkish and felt like "bags of jelly". These organs weighted 50- 60% of the normal weight. This bilateral atrophy was due to a degeneration of the generative cells in the seminiferous tubules. In contrast, ovaries of the surviving females were of normal appearance and presented no evidence of abnormal oogenesis at microscopy. Cohabitation of surviving male animals with untreated female animals did not result in pregnancies, indicating disturbed male fertility. An oral LD50 of 735 mg/kg bw was determined in this study.

Single oral dose by gavage with 700 mg/kg bw of p-tert-butylbenzoic acid to 10 male Sprague-Dawley albino resulted in a high mortality rate (7/10 rats) within 24 hours (Hazleton 1986). After administration of 720 mg/kg bw 2/10 rats died within 2 hours. In both groups the only treatment-related macroscopic and microscopic observation was small testes observed in one animal and hypospermatogenesis of the testes in all treated animals. Even though the cells appeared normal, these animals had fewer spermatogenic cells in the seminiferous tubules than controls. Mean absolute and relative testes weights were significantly lower in treated animals.

Single whole body exposure for 4 hours to respirable dust of p-tert-butylbenzoic acid (0, 495, 668, 958 or 1802 mg/m^3) in 6 Fischer 344 rats/sex resulted in reduced testes weights (50% compared to controls) in all p-tert-butylbenzoic acid exposed animals (Lu 1987). Reduction in spermatid counts, tubular degeneration, reduction of spermatogonia, multinucleated giant cells in the tubuli seminiferi and vacuolization in the plasma of Sertoli cells was observed. The LC50 determined in this study was >1802 mg/m^3.

In a testicular toxicity screening test using Fü albino SPF rats (8 males/ dose group) were orally treated with 12.5, 25, 50 and 100 mg p-tert-butylbenzoic acid /kg bw/day for 5 consecutive days, whereas 4 control males received the vehicle only (Givaudan 1982D). Mortality, general symptoms, body weights were recorded and all rats were autopsied. The testes of all rats were examined microscopically. One animal in the 100 mg/kg bw/ day dose group died by unknown causes. No signs of intoxication were observed, but a dose-related loss of body weights were observed from 25 mg/kg bw/ day onward. As necropsy findings, marmoration of the liver, delineation of hepatic lobules, pale livers and kidneys were observed in some animals of all dose groups. Increases in mean liver weights by 9% compared to the control group were found in the high dose group. Mean testes weights decreased by 15% compared to controls at the top dose.

Treatment related histological changes in the seminiferous epithelium were found. Cross-sectioned seminiferous tubules of controls and rats treated with 12.5 mg/kg bw/day showed normal patterns with orderly germ cell arrangement. At higher dose levels, degeneration of germ cells especially of spermatids and spermatocytes were observed and number of spermatozoa were reduced. Sporadic giant cells were found. These changes showed a dose related increase in incidence and severity.

A $NOAEL_{general\ toxicity}$ and a $NOAEL_{testicular\ toxicity}$ of 12.5 mg/kg bw/ day can be set for this study.

In an oral 90 day study, p-tert-butylbenzoic acid was administered to albino Carworth Farm rats (10 animals/sex/group) via the diet containing doses of 0, 100, 316, 1000, 3160 and 10000 ppm (0, 6, 21, and 75 mg/kg bw/d for males and 0, 8, 27, 89 mg/kg bw/d for females for doses up to 1000 ppm; two top doses not calculated) (Hunter 1965). In this study food consumption and body weights were recorded and urinalysis, hematology, clinical chemistry, gross and microscopic examinations were performed. Dietary levels of 3160 and 10000 ppm resulted in high percentages of premature deaths or animals to be killed in extremis, whereas no deaths were observed in the three lower exposure groups. Hind limb paralysis was reported for one male and one female exposed to diet concentration of 3160 ppm and one female at 1000 ppm. Final body weights were significantly decreased in males at diet concentrations from 316 ppm onward and in female rats from 1000 ppm onward. The feed consumption was reduced by 50-70% compared to controls in the 3160 and 10000 ppm dose group. In hematology, only changes with minor pathological relevance, i.e. decreased erythrocyte counts and changes in the differential blood counts were observed in the two high dose groups. Reduced total protein levels in male rats receiving 100 to 1000 ppm and dose-dependent increases in urea concentrations in males and female rats from 1000 ppm onward were found. In the urinalysis, increased urine volume and reduced urine osmolarity was found in treated rats from 3160 ppm onwards and protein concentrations were elevated in animals of the 10000 ppm dose group.

Increased relative liver and kidneys weights were observed in all dose groups and animals of the two top doses revealed congested and speckled livers and hydronephrosis, hydroureter, ureteral obstructions, hematuria in the urinary tract at necropsy. Findings in liver and kidney were confirmed microscopically. Sinusoidal congestion and fatty degeneration of centrilobular hepatocytes were found and intra-luminal cell debris, necrosis of the tubular epithelium, papillary necrosis were reported as the causes of the obstructive urinary tract lesions. Renal tubular necrosis and papillary necrosis was evident in all treated animals, showing dose dependent increases in incidences.

Evident testicular toxicity was observed by decreased testes to body ratios in all treated males. Testes weights were statistically significantly ($p<0.05$) reduced in the 1000 ppm (1.21 g) and 316 ppm (2.67 g) exposure groups compared to controls (3.45 g). Bilateral atrophy of the testes was found in all treated animals. The testes atrophy was related to degenerated epithelium of seminiferous tubules.

The authors indicate that atrophy of the testis was found even in the lowest dosage group of 100 ppm. Overall, a NOAEL on male reproductive organ toxicity and general toxicity could not be determined.

A $LOAEL_{general\ toxicity}$ and a $LOAEL_{testicular\ toxicity}$ of 100 ppm has been derived, referring to 6 or 8 mg p-tert-butylbenzoic acid/kg bw/day.

In a fertility study with male Wistar rats, ten animals per dose group were fed diets containing 0, 20, 100, or 500 ppm p-tert-butylbenzoic acid continuously for a period of 70 days before starting with mating trials (Hoechst, 1987). On the basis of the recorded food consumption a mean daily intake of 1.6 (20 ppm), 7.9 (100 ppm) and 41 (500 ppm) mg p-tert-butylbenzoic acid/kg bw/d was calculated.

During exposure, the animals were checked regularly for general condition, behavior, and body weight and food consumption. Each male was then mated to two non-exposed virgin females for a period of one week (first mating trial) and the females were checked daily for cyclicity and sperm. Proof of fertility was taken from successful impregnation of at least one of the two females. Males that had not been fertile during the first trial were kept for another 70 days without dietary test

substance exposure and then were again mated to virgin females for a period of one week (second mating trial - recovery group). Length of gestation, numbers of live and dead born, sex, weight and any externally visible anomalies of the newborns, which were finally sacrificed, have been assessed. Males were terminated at delivery of their impregnated dams or at the end of the mating trials and macroscopically investigated. Organ weights were taken of brain, heart, liver, spleen, kidney, testes and epididymides. Testes, epididymides, prostate and seminal vesicles were subjected to histopathological investigation. Females were terminated either one day after delivery or 25 days after the last mating trial and macroscopically investigated and numbers of implantation sites counted.

Reversible reduction in body weight gains was observed in treated animals at the 500 ppm dose level only, resulting in 14% lower body weights than controls at the end of the treatment period. Parental organ weights for brain, heart, liver, spleen and kidneys of the treated groups did not differ from those of the controls.

No treatment-related effects were observed for duration of the gestational period and on parturition and no differences in the numbers of live born per litter, sex ratio and mean body weights of the newborns between the controls and the treatment groups were found. No externally visible anomalies in newborns were recorded.

No pregnancies were produced during the first mating interval from males exposed to dietary levels of 500 ppm. Three males inseminated one female each; however, no pregnancies resulted, whereas from the other 7 males no sperm was detected in vaginal smears of their female partners. In contrast, all males of former 500 ppm group impregnated one or both females during the second mating trial 70 days after the end of the treatment period, i.e. the recovery group. One male of the 100 ppm group was not successful in impregnating but sired one of its females. All other males in this dose group, at lower dose or in the control group proved to be fertile by impregnating one or two females.

In males of the 500 ppm group mean absolute testes weights were reduced (2.76 g) in comparison to that of the controls (3.14g) after the recovery period, whereas testes weights at lower dose groups did not differ from controls. Exposure to 500 ppm p-tert-butylbenzoic acid resulted in minor lesions of the germinative epithelium which were confined to few tubules only, but no histopathological changes were found in prostate, seminal vesicles, epididymides and its sperm. No histopathological differences were observed in lower dose groups when compared to controls.

Overall, a $NOAEL_{testicular\ toxicity}$ of 20 ppm (1.6 mg/kg bw/d) and a $NOAEL_{general\ toxicity}$ of 100 ppm (7.9 mg/kg bw/d) can be derived from the study based on the finding of infertility/inability to impregnate at dietary dosages of 100 ppm and reduced body weight gain at 500 ppm.

Evidences for testicular toxicity via dermal application of p-tert-butylbenzoic acid have been found in a subacute and subchronic dermal toxicity study.

In a 28 day dermal study, Carworth Farm E strain rats (8 animals/sex and dose) received 0, 7.5, 15, 30 and 60 mg/kg bw/d 4-tert-butylbenzoic acid (0.2 mg/kg of 3.75, 7.5, 15 or 30% w/v solutions of p-tert-butylbenzoic acid in DMSO) topically on shaved skin (Shell 1975). Sixteen animals per sex served as control being exposed to solvent only. Body weights were recorded daily. Four animals/group were necropsied at the end of the study and the liver, kidneys and the gonads were examined histologically.

No mortality or clinical signs were observed, but body weight gains were reduced in animals exposed to 30 and 60 mg/kg bw/d. Significantly lower final body weights of males of these dose groups were observed compared to controls. Dose-related significant increases in absolute and relative liver weights were seen in female rats of all dose groups and in male rats exposed to 15 mg/kg bw/d and above. Increased relative kidney weights were observed in two top doses of female rats. No other relevant and test substance related adverse effect was observed in the liver and the kidneys of all treated animals.

Testicular toxicity has been observed by a decrease in relative and absolute testes weights in rats receiving 60 mg/kg bw/d. Histopathology of the testes revealed a degeneration of germinal epithelium in these animals. Approximately half of the tubuli seminiferi were affected. Reduced mitotic activity in spermatogonia led to germinal epithelium degeneration and reduction of spermatozoa. Multinucleated spermatocytes were found in the affected tubuli.

A $NOAEL_{testicular\ toxicity}$ of 30 mg/kg bw/d can be derived from this dermal study based on the findings of testes weight reduction and effects on the germinative epithelium. A $NOAEL_{general\ toxicity}$ can be set at 7.5 mg/kg bw/d for males and A $LOAEL_{general\ toxicity}$ can be set at 7.5 mg/kg bw/d for females based on the liver effects.

Fischer 344 rats (20 animals/sex and dose) were treated topically (once a day /five days a week) on skin clipped free of hair with 1.0 ml/kg of p-tert-butylbenzoic acid and diethanolamine salt prepared in deionized water (simulating cutting fluid) for either 7 weeks (7 animals/sex/group) or 13 weeks (13 animals/sex/group) (Cagen 1989; Lu 1987). Treatment resulted in daily exposures of 0, 17.5, 35, 70 and 140 mg/ kg bw/ d p-tert-butylbenzoic acid. Study examinations included observation of clinical signs, body weights, food consumption, clinical chemistry, hematology and urinalysis. Furthermore macroscopic findings, organ weights of lungs with trachea, larynx, liver, kidneys, brain, heart, uterus, and spleen have been investigated. Histopathology of various organs/tissues (including those weighed and sciatic nerve and spinal cord) has been performed. Absolute and relative testis weights, sperm head count and LDH-X enzyme assays as a measure for surviving spermatocytes and spermatids were performed.

No exposure related deaths or any clinical signs of toxicity were observed up to the top dose. Significantly lower body weights and body weight gains were observed for males and females exposed to 140 mg/kg bw and for females exposed to 70 mg/kg bw.

A significant increase in urine volume occurred during week 13 at 70 and 140 mg/kg bw/d in males and at 140 mg/kg bw/d in females. Cholesterol concentrations were reduced in all dose groups, and the levels of BUN and phosphorus were increased in males/females at 70 and 140 mg/kg bw/d.

Dose-related significant increases in relative and absolute hepatic and renal weights were seen in all dose groups. In line, in the 70 and 140 mg/kg bw/d dose groups cytoplasmic vacuolation in the liver; pallor, dilatation, degeneration and regeneration of distal convoluted tubular epithelium, tubular casts, interstitial nephritis and papillary necrosis of the kidneys has been observed microscopically. Liver cell vacuolation was also evident in 17.5 and 35 mg/kg bw/d dose groups (females). This lesion was characterized as multifocal to diffuse perilobular to panlobular, lipidpositive vacuolation of hepatocytes. Accompanying aberrations in clinical chemistry values suggested altered hepatic and renal function. In general, a contribution of DEA to the observed effects in this study, especially on the liver metabolism cannot be ruled out.

Concerning testicular toxicity and spermatotoxicity, absolute and relative testes weights, sperm head count and LDH-X enzyme activities were significantly reduced compared to controls after dermal exposures of 70 and 140 mg/kg bw for 7 and 13 weeks.

Furthermore, exposure related microscopic lesions occurred in rat of the 70 and 140 mg/kg bw exposure groups. Lesions were characterized by moderate to severe diffuse seminiferous tubular degeneration. Most affected tubules were reported to contain spermatogonia, primary and secondary spermatocytes, early spermatids and Sertoli cells but were devoid of late spermatids. Occasionally, few seminiferous tubules in the same testis contained only Sertoli cells and a few spermatogonia. Testicular giant cells were reported to be quite numerous in the degenerative tubules and occurred in even greater numbers within epididymal tubular lumina.

Overall, a $NOAEL_{testicular\ toxicity}$ of 35 mg/kg bw/d can be derived from the study based on the findings of testes weight reduction, hypospermia and degeneration of the germinative epithelium, whereas a NOAEL for other adverse systemic effects, i.e. on the liver, could not be established, resulting in a dermal LOAEL of 17.5 mg/kg bw/day.

In an subacute inhalation toxicity study, groups of Fischer 344 rats (8 animals per sex and concentration) were exposed (whole body) to concentrations of 12.5, 106, or 525 mg/m^3 for 6 hours/day on 4 consecutive days, followed by 3-4 days without test substance treatment and subsequent 3 additional exposure days (Shell, 1982). Mean particle diameters ranged from 3.6 to 4.3 µm (MMAD). Mortality and clinical signs, body weights, clinical chemistry and hematology were assessed. Rats were terminated on the day after the last exposure. Organ weights for liver, kidneys, lungs, heart, spleen and brain, gross necropsy were assessed and histopathology on spinal cord, nasal passages, trachea, larynx, lungs, liver, kidneys and all lesions was performed. Furthermore, testes weights, sperm counts, and testicular histology were investigated.

Unscheduled deaths occurred at concentrations of 106 and 525 mg/m^3. Animals of the mid and high concentration groups showed concentration-dependent significant loss of body weights during the study period. Abnormal neurobehaviour has been observed, i.e. fore and hind limb neuropathy, hunched posture, tremors, convulsions, gait abnormalities, prolapsed penis, hypoactivity and abnormal respiration) in the mid and high dose groups. High concentration females had a mild but significantly decreased haemoglobin concentrations and hematocrit and increases in mean white blood cell counts were found in mid and high concentration groups. Clinical chemistry parameters were affected such as a reduced activity of alkaline phosphatase in all treated females.

Macroscopic findings (perineal and abdominal urine staining, dehydration, white powder on the haircoat, small red thymus, bright red lungs, pinpoint red gastric foci, enlarged tan livers, reduced digesta and body fat stores) were primarily noted in mid and high concentration groups.

Absolute and relative organ weights of the liver and kidneys were increased in all animals of the 106 and 525 mg/m³ groups, and females of the 12.5 mg/m³ group showed also increased absolute and relative liver weights.

In histopathology, treatment-related lesions were seen in the kidneys (bilateral multifocal cytoplasmic eosinophilia of cortical tubular cells, intracytoplasmatic vacuolation) of all treated animals. In mid and high concentration group animals, livers showed vacuolar degeneration in peripherilobular/periportal or panlobular hepatocytes (negative for lipid staining) and increased rate of mitotic cells. The thymus in animals of the mid and high concentration groups showed lymphocytic necrosis and atrophy in the cortical region plus medullary congestion and haemorrhage. In the 106 and 525 mg/m³ concentration groups, spinal cords showed severe focal or regional poliomyelopathy. Lesions of the central nervous system were reported for animals, which demonstrated clinical signs of paraplegia.

Testicular toxicity and spermatotoxicity was observed in terms of small soft testes and focal epididymal lesions in animals of the mid and high concentration groups. Absolute and relative weights of the testis were reduced for males of the mid and high concentration groups, which revealed to be lethal. Testicular sperm counts (left testis of all surviving rats) decreased concentration dependent and a reduction by 21% was still observed in animals of the low concentration group (12.5 mg/m³). From histopathological analysis no apparent effects on spermatogenesis were observed in the low concentration group, however, multifocal to diffuse degeneration of the germinal epithelium has been found in the testes of higher concentration groups. Severe tubular changes consisting of absence of late spermatids, reduction in spermatogenic cell types, giant cell bodies, cellular debris, and atrophy of epididymides were observed.

Overall, a $LOAEC_{testicular\ toxicity}$ and a $LOAEC_{general\ toxicity}$ of 12.5 mg/m³ can be derived from this study based on the findings of a reduction of testes sperm numbers and effects on liver and kidney.

Testicular toxicity could not be confirmed in a 28-day inhalation study with a specific design to evaluate neurotoxicity (HRC 1995). Sprague Dawley rats (8 animals per sex and concentration) were exposed to a particulate atmosphere containing 1.5, 4.7 or 15.7 mg/m³ p-tert-butyl benzoic acid (MMAD values of 3.2 - 3.9 µm) by snout-only exposure for 6 hours a day, 5 days a week (Monday to Friday) for 4 weeks.

Standard clinical observations/body weights and neurobehaviour was examined within a functional observational battery (FOB), organ weight analysis, macroscopic and microscopic examination of the adrenals, heart, kidneys, liver, lungs, spleen, testes with epididymides, and gross abnormalities was performed and specific neurohistopathological examinations have been conducted in this study.

No test substance related clinical signs, bodyweight changes, effects on food consumption, macroscopic or microscopic changes were observed. Minor differences in female absolute liver weights, i.e. an increase by 9% compared to controls were observed.

Behavioral observations revealed a slight increase in the incidence of body tremor, that further increased during the observation time of 4 weeks at 15.7 mg/m3 and a significant decrease in activity counts with tendency towards decreased rearing counts was found.

The number of males with decreased arousal and urinating/defecating while in the arena was increased. Also, facial staining and hair loss occurred with slightly increased frequency in high concentration males. No similar findings were noted among treated females.

Microscopic examinations of the organs examined including the testis and epididymides revealed no lesions, which were attributable to treatment with p-tert-butylbenzoic acid.

Putative spermatotoxic effects associated with occupational exposure to p-tert- butylbenzoic acid were investigated in a cohort of 90 male volunteers working in a p-tert-butylbenzoic acid producing facility (Whorton 1981). The control group consisted of 103 volunteers who did not work in the facility and who had not been exposed to any known testicular toxin. Exposures were indexed (with a weighted relative exposure point system) and an exposure index for each person was calculated based on the relative exposure point values and the amount of time in a given job. Medical evaluations were based on self-administered questionnaire for marital and reproductive history and smoking history, administered questionnaire for work history and genitourinary medical history, a brief physical examination of the male genitalia, venous blood sample for haematology and clinical chemistry and two semen samples that were analyzed for volume, sperm count and sperm morphology. Because of the large range of sperm counts found in healthy men who have not been exposed to chemicals that could influence sperm production, the distribution of sperm counts in a group of subjects was taken as a criterion of possible testicular damage in order to improve the evaluation.

Of the 90 participants 33 had undergone a vasectomy and did not contribute to semen analysis. A total of only 51 of the 90 participants provided at least one semen sample. Thirty-nine men provided a total of two semen samples. Exposure indices were similar between both groups, the semen sample providing men as well as the non semen providing participants. Analysis of the sperm count data of the 51 individuals of the study group yielded a median sperm count of 72 million sperm/ml semen, while that of the control group was 78 million sperm/ml. Eight individuals in the study group (15.7 %) had sperm counts of less than 20 million sperm/ml (e.g. in the sub-fertile range), compared to 7 subjects in the control group (6.8%). The authors calculated that this difference was not significant and concluded that p-tert-butylbenzoic acid, at the exposures experienced at that plant, had no clinically detectable effect on testicular function of the workers. Also, there were no indications that p-tert-butylbenzoic acid caused infertility in men who took part in this study. No adverse effects on liver and kidney function or on blood composition were observed. The levels of the hormones studied were in the normal range in the semen providing and the other participants.

To obtain a better statistical analysis, the control group was increased in size by including 232 men who had served as controls in other similar studies. Of the group of non-exposed men (n= 335), 25 (7.5%) had sperm counts less than 20 million/ml. It is reported, that depending on the process used for statistical analysis, the slight difference between the study subjects and the non-exposed group might or might not have been significant. Closer analysis of the urological-clinical data for the men with oligospermia in the study group of the plant revealed that a multitude of other potential factors, such as orchitis after mumps, testicular hernias and sclerosis of the penis could have been responsible for the reduced sperm density. The urological-clinical data for the control group could not be evaluated to further improve the statistical analysis.

The small size of the study group together with the manifold urological findings makes the toxicological significance of the difference from the control group questionable.

ANNEX 3: OVERVIEW ON STUDIES ADRESSING TESTICULAR TOXICITY INDUCED BY P-TERT-BUTYL-BENZALDEHYDE (TBB) AND P-TERT-BUTYLTOLUENE (TBT).

In SFP albino rats, a 5-day oral exposure to p-tert-butylbenzaldehyde (TBB) caused marked testicular damage. Eight male rats per dose group received 0, 6.5, 12.5, 25 or 50 mg/kg bw/d of the test material via gavage once daily for 5 consecutive days; 4 additional control animals received the respective vehicle in the same manner (Givaudan 1981). Body weights, clinical signs were assessed and gross necropsy was performed. Liver, kidneys and testes were weighed. The testes of all rats were microscopically examined.

No mortality was observed. Three rats treated with 12.5 mg/kg bw/d showed slight aggressiveness on test days 3 and 4. From days 3 to 6, a slight loss of hair was seen in one animal of the 50 mg/kg bw/d group. The test material did not affect body weights of animals treated with 6.5 and 12.5 mg/kg bw/d. Rats treated with 25 mg/kg bw/d initially showed a slight weight loss and returned to normal at the end of the treatment. The animals of the highest dose group showed a severe weight loss throughout the study. During the dissection, a marbled liver was recorded in 2 rats treated with 50 mg/kg bw/d. In one rat treated with 25 mg/kg bw/d a small dell was seen in the right kidney.

Testes weights of rats treated with 50 mg/kg bw/d were significantly lower than those recorded for the controls. Histopathological changes of the testes were circumscribed in the seminiferous epithelium. Interstitial cells and Sertoli cells were unaffected. Disorganization of the epithelial structure, degeneration of cells and reduction of the spermatozoa were observed. One testis of a control rat showed about 80 % convoluted tubules with a normal epithelium, and about 20 % convoluted tubules with a normal epithelium, but with degenerated cells or detritus in the lumen. The same ratio occurred in the animals of the 6.5 and in the 12.5 mg/kg bw/d dose group. An alteration of this ratio was seen from the 25 mg/kg bw/d group on. In addition, severe injuries were observed in the seminiferous epithelia of the testes of one animal treated with 25 mg/kg bw/d. Moderate to severe injuries were discovered in the seminiferous epithelia of all rats treated with 50 mg/kg bw/d.
The NOAEL derived for testicular toxicity in rat was 12.5 mg/kg bw/d TBB, based on the histological changes and a slight decrease of the testicular weights. Systemic toxicity in terms of body weight changes were found at doses also exerting testicular toxicity.

A similar test protocol has been applied in a subacute testicular toxicity screening study using p-tert-butyltoluene (TBT). Eight male SFP albino rats per dose group received 12.5, 25, 50, 100 mg/kg bw/d via gavage once daily for 5 consecutive days; 4 additional control animals received the respective vehicle in the same manner (Givaudan 1982C).
No mortality was observed and clinical signs (loss of hair, shaggy fur, hunched posture, lethargy and diarrhea) occurred in the dose group 50 and 100 mg/kg bw/d. A slight and transient body weight loss was observed at 25 mg/kg bw/d, and a more severe body weight loss (reaching the initial weight by the end of the study period) was observed at 50 mg/kg bw. A marked progressive body weight loss was found in rats treated with 100 mg/kg bw/d. Necropsy revealed a delineation of hepatic lobules and pale livers at the 50 and 100 mg/kg bw/d dose groups.
Testes weights were decreased by 23% in rats of the 100 mg/kg bw/d dose group compared to controls. In line, histopathological examinations revealed severe cell-deformations in the germinal epithelium in rats treated with 50 and 100 mg/kg bw/d.

Controls, 12.5 and 25 mg/kg bw/d dose groups showed approximately 85 % convoluted tubules with a normal epithelium, and about 15 % convoluted tubules with a normal epithelium, but with degenerated cells or detritus in the lumen. An alteration of this ratio was seen at 50 mg/kg bw/d group and above. Furthermore the incidence of tubuli with severe destruction of the epithelium which mainly consisted of spermatogonial and Sertoli cells dose dependently increased at 50 and 100 mg/kg bw/d, i.e. 20% and 86% respectively.
The NOAEL derived for testicular toxicity in rat was 25 mg/kg bw/d TBT, based on the histological changes and a slight decrease of the testicular weights. Systemic toxicity in terms of body weight changes were found at doses also exerting testicular toxicity.

In further testicular toxicity screening studies with male SFP albino rats (7 males) and mice (6 males), Himalayan guinea pigs (5 males) and Beagle dogs (2 males), 100 mg/kg bw/d TBB was administered orally (gavage or via gelatin capsules) once daily for five consecutive days (EPA (TSCAT) 1982, Givaudan 1984A; Givaudan 1984B; Givaudan 1984C). In these experiments, body weights and clinical signs were monitored. Rats, mice, guinea pigs were inspected by gross necropsy. Testes were weighed in rats, mice, guinea pigs and histopathological examination of the testes was performed in all species examined.

In rats, no mortality occurred throughout the study and all animals appeared normal. During the first two days an initial body weight loss was observed, but these rats showed subsequent weight gain at the end of the treatment period. However, final mean body weights in treated animals were still below controls. One of the 7 tested animals had an agenesia of the left kidney and testis. Testes weights of the treated animals were decreased compared to controls whereas weight of liver and kidney showed no relevant difference. Histological examination revealed signs for acute hepatitis and acute interstitial nephritis occurred in animals of treated and control groups. Since these histological findings are commonly seen according to the authors, a parasitic infestation is considered to be the cause rather than compound related effects.
In the testes the treated animals showed injuries in the seminiferous epithelium. Five treated animals showed minimal to moderate degeneration of spermatids and spermatocytes. One treated animal showed a minimal reduction of spermatozoa and all treated animals showed minimal to moderate appearance of multinucleate giant cells. Sertoli cells and Leydig cells were unaffected. In contrast, these findings were not observed in control animals.

In mice, no treatment-related findings on body weights and clinical signs of toxicity were observed. At necropsy, a turbid pericardiopleural region with deposits was seen in 2 treated mice, but has been considered to be unrelated to treatment with the test substance. Testes weights of the treated animals showed no effect when compared to control animals. Histological examination revealed a slight damage of the germinal epithelium in testes of 1 control and 4 treated animals. A marginally higher incidence of seminiferous tubules showing, many degenerated cells in the epithelium and disorganization of the epithelial structure (0% versus 0.2%) or severe destruction of the epithelium (0.2% versus 1.2%) were observed for control and test substance treated animals respectively.

In guinea pigs, no treatment-related clinical findings, changes on body weights or necropsy findings were observed. The mean testes weights of the control group and of the treated group were not significantly altered. In histology, a slight damage of the germinal epithelium was seen in 2 control animals and in 1 treated animal.
Furthermore, marginal incidences of seminiferous tubules showing severe destruction of the epithelium (0.2% and 0.1%) were observed for control and test substance treated animals respectively.

In dogs, application of TBB resulted in a slight body weight loss up to day 6 (Dog 1, treated: 12.2 kg on day 1, 11.6 kg on day 6; dog 2, treated: 11.1 kg on day 1, 10.0 kg on day 6). There were about 60 cross sectioned seminiferous tubules with nearly total depopulation of germinal epithelium in both testes of one dog. In these seminiferous tubules, early stages of spermatogenesis and Sertoli cells were preserved only. With the exception of the occurrence of multinucleated giant cells - a background finding seen also in the control animal- , no abnormalities were discovered in the testes of the other treated dog. No changes were seen in epididymides of both dogs. The slight damage of germinal epithelium of one dog was considered to be related to treatment.

Analogous testicular toxicity screening studies have been performed for TBT using male SFP albino rats (7 males) and mice (6 males), Himalayan guinea pigs (5 males) and Beagle dogs (2 males). A single dose (200 mg/kg bw/d in rats and 100 mg/kg bw/d in other animals) TBB was administered orally (gavage or via gelatin capsules) once daily for five consecutive days (EPA (TSCAT) 1982, Givaudan 1984D; Givaudan 1984E; Givaudan 1984F;).

In rats, no mortalities but lethargy and shaggy fur was recorded. Slight body weight loss during the first 3 days was observed with a tendency to return to normal body weights at the end of treatment. Necropsy findings revealed inflammation in the liver in one treated animal. Testes weights decreased compared to controls. Histopathology revealed changes of the seminiferous epithelium in terms of degeneration of spermatocytes and spermatids, reduction of spermatozoa and appearance of giant cells, whereas Sertoli and Leydig cells were unaffected.

In mice, no treatment-related clinical signs and findings on body weights and in necropsy were observed. Testes weights of the treated animals were slightly increased compared to control animals. Histological examination revealed a slight damage of germinal epithelium in testes of 1 control and 3 treated animals. A marginally higher incidence of seminiferous tubules showing, many degenerated cells in the epithelium and disorganization of the epithelial structure (0% versus 0.2%) or severe destruction of the epithelium (0.2% versus 0.7%) were observed for control and test substance treated animals respectively.

In guinea pigs, no mortality, treatment-related clinical findings, changes in body weights or necropsy findings were observed. The mean testes weights of the control group and of the treated group were not significantly altered. In histology, a slight damage of germinal epithelium was seen in 2 control animals and in 1 treated animal and a moderate damage was seen in one TBT treated animal. Slightly increased incidences of seminiferous tubules showing many degenerated cells in the epithelium and disorganization of the epithelial structure (1.8% vs. 0%) or severe destruction of the epithelium (2.7% vs. 0.2%) were observed for TBT treated versus control animals respectively. However, the severity of these findings were much lower when compared the testicular effects observed in rats under the same testing conditions.

In dogs, no mortality, symptoms of incompatibility or effects on body weights were observed after application of TBT. A small quantity of seminiferous tubules with nearly total depopulation of germinal epithelium in both testes of one dog was observed, i.e. approx 20 in testis 1 and 10 in testis 2. These tubules showed early stages of spermatogenesis and Sertoli cells only. With the exception of the occurrence of multinucleated giant cells - a background finding seen also in the control animal- , no abnormalities were discovered in the testes of the other treated dog. No changes were seen in epididymides of both dogs. The observed slight damage of germinal epithelium of one dog cannot be clearly attributed to treatment.

TBT induced testicular toxicity in rats was confirmed in a subacute toxicity study equivalent to OECD TG 407. TBT was administered to 12 Sprague-Dawley rats/sex/dose by gavage at dose levels of 0, 1.5, 5, 15, 50 mg/kg bw/day for 28 days (Furuhashi 2007A). Satellite groups were allowed a 14-day recovery.

No deaths occurred and no treatment-related clinical signs of toxicity or changes in body weights were observed. Lower food consumption was noted in the 15 mg/kg males and in both sexes given 50 mg/kg. Higher water intake was noted in the males at 15 mg/kg and in both sexes at 50 mg/kg. Clinical chemistry parameters such as decreases in total protein, albumin, cholesterol, triglycerides and ions and increases in AST, A/G ratio, gamma-GTP, bilirubin, urea nitrogen and creatinine were affected starting from dose group 5 mg/kg bw/d for males and 15 mg/kg bw/d for females. Hematology parameters, i.e. shortened APTT, prolonged PT and decreased fibrinogen concentration were affected starting from dose group 5 mg/kg bw/d for males and 50 mg/kg bw/d for females. Urinalysis revealed a higher urinary volume, decreased urinary specific gravity, and decreased protein levels from dose group 15 mg/kg bw/d for males and 50 mg/kg bw/d for females. Organ weight measurement showed increased absolute/ relative liver weights starting from dose group 15 mg/kg bw/d onward. Increased relative kidney weights, increased relative adrenals weights and decreased absolute ovary weights were found in the high dose females. No gross pathological findings were reported. In histopathology a hypertrophy of periportal hepatocytes in high dose males and females has been observed.

Testicular toxicity was evident in terms of decreased absolute and relative testis and absolute epididymis weights in males of the 50 mg/kg bw/d dose group. In histopathology, atrophy of seminiferous tubules, hyperplasia of Leydig cells and a decrease in sperm count in the lumen of the ductus epididymis was found in the high dose males. At the termination of the recovery period, atrophy of seminiferous tubules in the testes and decrease in sperm in the epididymides were still evident in the males which had received 50 mg/kg bw/d.

Taken together a $NOAEL_{general\ toxicity}$ can be set at 1.5 mg/kg bw/d (males) and 5 mg/kg bw/d (females) and a $NOAEL_{testicular\ toxicity}$ can be set at 15 mg/kg bw/ day for this study.

In a reproduction / developmental toxicity screening study by the same authors in accordance with OECD TG 421, TBT was administered to 12 Sprague-Dawley rats/sex/dose by gavage at dose levels of 0, 1.5, 5, 15, 50 mg/kg bw/day.

Mortality was observed at 50 mg/kg bw/d (1 male and 6 females) and at 15 mg/kg bw/d (1 female). Hypothermia, decrease in locomotor activity, soiled fur, reddish urine, hypothermia, adoption of a prone position, a staggering gait, piloerection, lacrimation, bradypnea, diarrhea, and muscle relaxation were noted starting from 15 mg/kg bw/d. Body weights were decreased in males from 15 mg/kg bw/d onward and in females from 5 mg/kg bw onward. Transient decreases in food consumption was noted in males at 50 mg/kg bw/d and in females at 5 mg/kg bw/d during the lactation period.

No changes attributable to administration of the test substance were noted in the estrous frequency, copulation index, or number of days before copulation. No substance related changes were noted at 1.5, 5, or 15 mg/kg bw/d in terms of numbers of corpora lutea, implantation sites, implantation rate or gestation index.

Concerning the offspring, number of pups on day 0 of lactation was zero at 50 mg/kg bw/d, since no females conceived. In the 15 mg/kg bw/d dose group, lower numbers of pups, number of pups on day 0 of lactation, the delivery index, birth index, live birth index, number of pups on day 4 of lactation, and the viability index on day 4 of lactation was observed. The number of stillbirths tended to be higher in this dose group. No external abnormalities attributable to the compound administration were noted. Lowered pup body weights in both sexes on Days 0 and 4 of lactation were found at 5 and 15 mg/kg bw/d.

Concerning testicular toxicity and spermatotoxicity, decreased absolute testis and epididymis weights at 15 mg/kg bw/d were observed. The relative testis and epididymis weights were lowered at 50 mg/kg bw/d. Sperm examinations revealed a decreased motility ratio, path velocity, straight line velocity, curvilinear velocity, viability, survivability, number of sperm, number of sperm/g of the left caudae epididymidis and an elevation of the proportion of abnormal sperm at 15 and 50 mg/kg bw/d. A higher value for beat cross frequency was noted at 15 mg/kg bw/d. At necropsy, atrophy of the testes and epididymides was noted in males at 15 and 50 mg/kg bw/d. Histopathological examinations confirmed an atrophy of seminiferous tubules, hyperplasia of Leydig cells and decrease in sperm in the epididymides at 15 and 50 mg/kg. Furthermore, the fertility index was significantly decreased at 15 and 50 mg/kg bw, and no animal became pregnant in the high dose group, substantiating the adverse effects of TBT on male fertility.

Taken together a $NOAEL_{general\ toxicity}$ can be set at 5 mg/kg bw/d and a female specific $NOEL_{general\ toxicity}$ can be set at 1.5 mg/kg bw/d. A $NOAEL_{testicular\ toxicity}$ can be set at 5 mg/kg bw/ day and a $NOAEL_{developmetal\ toxicity}$ can be set at 1.5 mg/kg bw/ day for this study.