



September, 2023

Givaudan Response to CLH report Proposal for Harmonized Classification and Labelling of 3-(4- tert-butylphenyl)propionaldehyde

Chemical names:

3-(4-tert-butylphenyl)propionaldehyde

EC Numbers:

242-016-2 [1]

CAS Numbers:

18127-01-0 [1]

Comment on CLH report Proposal for Harmonized Classification and Labelling of 3-(4-tert-butylphenyl)propionaldehyde

1. Summary

In the CLH report Proposal, 3-(4-tert-butylphenyl)propionaldehyde (Bourgeonal, EC 242-016-2) is grouped for read-across purposes with lysmeral (EC 201-289-8) and *tert*-butylbenzoic acid (tBBA, EC 202-696-3) that already have harmonised classification as Repr. 1B (H360Fd and H360F, respectively) and hence the Dossier Submitter (DS) concludes that Repr. 1B classification should be applied to these compounds with the argument of structural similarity and experimental data demonstrating formation of the metabolite tBBA. However, recent studies on lysmeral and tBBA (the source substances) showed species-specific formation of tBBA-CoA adducts in the male rat, which accumulate in the testes and in livers of male rats being of no or at most questionable relevance to humans. These new data (Hareng et al. 2023) no longer support the current C&L of Lysmeral as Repr. 1B, and hence no Repr. 1B classification of Bourgeonal based on read-across to lysmeral is warranted.

Hareng et al. 2023 shows that tBBA-CoA accumulation upon treatment with p-tBBA was paralleled by effects on late stage spermatogenesis and a dramatic impact on the lipidome in the tissue samples. This change is observed for (i) specific classes of lipids, (ii) individual metabolic markers of lipid molecules and also (iii) in Principle Component Analysis on the complete metabolic profile. A lack of tBBA-CoA accumulation after m-tBBA treatment was paralleled by a lack of effects on late stage spermatogenesis and no large impact on the lipidome in the tissue samples. This new evidence should be taken into account in the classification proposal of Bourgeonal, regardless the classification of lysmeral and tBBA¹. The mechanistic data on mode of action and species comparison to humans leads to a clear pattern of differences that raises significant doubt to the relevancy for a human hazard classification for Bourgeonal.

¹ *Furthermore the proposed classification entry does not conform with Section 1.6.3.3.3 of the ECHA Guidance on CLP for Additivity vs non-additivity of hazards' for mixtures as the need for classification of mixtures based on the sum of the concentrations of individual substances forming the same metabolite is not normally applicable to reproductive hazards under ECHA Guidance on CLP (*Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures*, 2017).

2. Animal Studies on adverse effects on sexual function and fertility of Bourgeonal

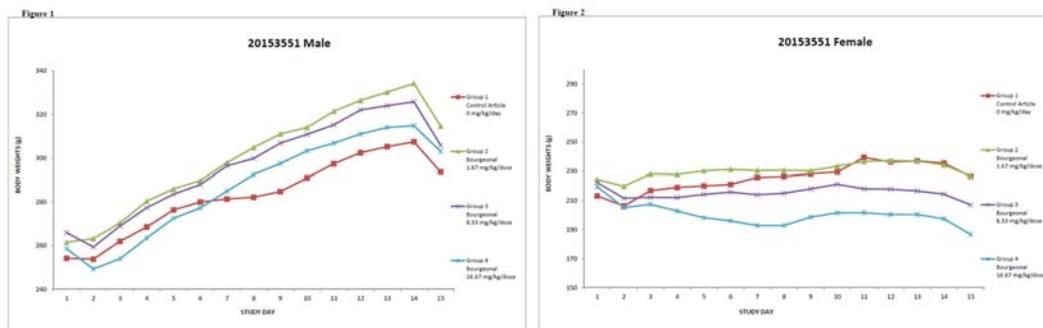
2.1. 14-Day Dose Range-Finding study in Rats

A total of 40 CrI:CD(SD) Sprague Dawley male and female rats (20 rats/sex) were randomly assigned to dose groups, 5 rats/sex/group. Formulations of the test substance, Bourgeonal, or the control substance, Corn Oil, were administered orally by gavage three times (approximately 6 hours apart) daily for 14 consecutive days at 0 (Control), 5, 25, and 50 mg/kg/day. Study parameters included: viability, clinical observations, body weights and body weight changes, food consumption, urinalysis (acid metabolites), macroscopic and microscopic observations, organ weights, and sperm evaluations (motility and concentration) were evaluated.

There were no Bourgeonal-related mortalities in females, however, there were two mortalities at 50 mg/kg/day in males. One male was found dead on SD 14. Although there were no clinical signs prior to death or macroscopic findings during necropsy examination the likelihood of Bourgeonal-related toxicity cannot be excluded. The other male was euthanized on SD 1 due to adverse clinical condition. This early death was considered to be unrelated to Bourgeonal because the death was attributed to the jugular blood collection procedure based on the macroscopic findings and timing of death. All other animals survived to scheduled euthanasia on SD 15.

Bourgeonal-related clinical signs were observed in females in the 25 and 50 mg/kg/day dose groups, and included suspected dehydration (based on skin turgor) and a low incidence of hunched posture and thin appearance. There were no Bourgeonal-related clinical observations in males.

In females, statistically significant mean body weight losses ($p \leq 0.01$) of **-15.4 g** and **-33.0 g** were observed at 25 and 50 mg/kg/day, respectively, compared to a mean body weight gain of +13.3 g in controls. Body weight of females on SD15 were 226.4 g (control), 207 g (-8.6% at 25 mg/kg/d), 186.6 g (-17.6% at 50 mg/kg/d) exceeding the maximum tolerated dose. At 50 mg/kg/day, the lower mean body weights were statistically significant ($p \leq 0.05$ or $p \leq 0.01$) on each weighing day between SD 6 and SD 15 compared to the control group. There were no Bourgeonal-related effects on mean body weights or mean body weight gain in males. Figures 1 and 2 summarize the body weigh observed for males and females of the study No. 20153551 (Charles River Study No. 20153551).



Figures 1 and 2. Body weight of males and females during the 14-day dose range finding study. Charles River Study No. 20153551, A 14-Day Oral (Gavage) Dose Range-Finding Study of Bourgeonal in Rats, pages 39 and 40.

Lower mean food consumption was observed in females at 25 mg/kg/day for the intervals of SD 1 to 5, SD 5 to 8, and SD 12 to 14 (84% to 94% of control) and at all tabulated intervals between SD 1 and SD 14 and overall for the interval of SD 1 to 14 at 50 mg/kg/day (**36% to 83% of control**). There were no Bourgeonal-related effects on mean absolute food consumption in males at any dose.

Based on mortality, body weight losses, decrease of food consumption, among other effects, the no-observed-adverse-effect level (NOAEL) for Bourgeonal could not be established in the 14-day DRF study. Therefore, the maximum tolerated dose was determined to be 5 mg/kg/d, based on the above results and taking into account the longer exposure requirements of OECD 422 study (at least 28 days for males and approximately 60 days for females).

2.2. GLP OECD 422 An Oral (Gavage) Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Bourgeonal in Rats

2.2.1 Effects observed in males

No adverse effects were observed in males at any dose. Mean body weights, mean body weight gains, and mean food consumption values were similar across all groups in the males. There were no Bourgeonal-related effects on any mating and fertility parameter at any dose. There were no macroscopic or microscopic observations or alterations in organ weights at any dose. There were no changes in serum T4 concentrations at any dose. In the males, mean serum T4 concentrations were 104%, 86%, and 90% of controls in the 0.5,

1, and 5 mg/kg/dose groups, respectively. The effect observed in T4 was not dose dependent and not associated with any macroscopic or microscopic observations or alteration in thyroid weight.

2.2.2 Effects observed in Females

There were no Bourgeonal-related clinical observations or effects on mean body weights, mean body weight gains, and mean food consumption values during the pre-mating, gestation, or lactation periods in the females at any dose. There were no Bourgeonal-related effects on estrous cycling, mating or fertility, or any natural delivery or litter parameter in the females at any dose. There were no macroscopic or microscopic observations or alterations in organ weights at any dose.

2.2.3. Effects observed in Pups

In the pups, there were no Bourgeonal-related clinical observations or effects on anogenital distance, nipple retention (males), or mean pup body weights at 0.5 and 1 mg/kg/d. The mean pup body weight was reduced at 5 mg/kg/dose group compared to control values on Days 9 and 12 postpartum (88% and 89% of controls) and were considered by the study director unrelated to Bourgeonal, because the reductions were minimal. In addition, there were no Bourgeonal-related macroscopic or microscopic observations in the pups at any dose. There were no Bourgeonal-related changes in serum T4 concentrations in the males or females at any dose. In the male pups, mean serum T4 concentrations were 98%, 82%, and 78% of controls in the 0.5, 1, and 5 mg/kg/dose groups, respectively, on Day 12 postpartum. In the female pups, mean serum T4 concentrations were 82%, 74%, and 74% of controls in the 0.5, 1, and 5 mg/kg/dose groups, respectively, on Day 12 postpartum. There were no Bourgeonal-related microscopic changes in the thyroid or parathyroid glands of the single pup/sex/litter that was microscopically examined from 5 mg/kg/dose group. The differences observed in mean serum T4 concentrations in the females, and considered by the DS as adverse, **did not reflect any other evidence at the tissue level, therefore, were considered unrelated to administration of Bourgeonal.** Moreover, due to the significant differences between rats and humans to regulate thyroid-hormone homeostasis, minor changes of thyroid-hormone concentrations are of little relevance to humans (Bartsch et al 2018, ECHA (2017) Guidance on the Application of the CLP Criteria Annex I: 3.6.2.2.9.)

There were no Bourgeonal-related effects on mating and fertility in the males or females or any effects on estrous cycling and natural delivery parameters in the females. There were no Bourgeonal-related differences in any preweaning developmental parameter evaluated in the offspring. Furthermore, there were no Bourgeonal-related macroscopic or microscopic findings in the adults or pups or alterations in the organ weights in the adults. Based on these findings, the no-observed-adverse-effect-level (NOAEL) for general toxicity, mating, and fertility for Bourgeonal in males and females was 5 mg/kg/dose. The NOAEL for development of the offspring was also 5 mg/kg/dose. Based on these findings, the no-observed-adverse-effect-level (NOAEL) for general toxicity, mating, and fertility for Bourgeonal in males and females was 5 mg/kg/dose.

3. Conclusion

The DS acknowledges the fact that the studies available for Bourgeonal are considered to be of limited quality or relevance for the purpose of harmonized classification as stand-alone. The available information indicates minimal reduction on mean pup body weight and was considered unrelated to Bourgeonal. Effects on the testes were observed at doses that show effects on body weight gain and food consumption, exceeding the maximum tolerated dose. Recent studies on lysmeral and tBBA showed species-specific formation of tBBA-CoA adducts in the male rat, which accumulate in the testes and in livers of male rats being of no or at most questionable relevance to humans. In light of the new data on lysmeral (Hareng et al. 2023) and limited data on Bourgeonal, a Repr. 1B classification is not justified. Instead, a Repr. 2 classification to recognize remaining uncertainties is proposed.

4. Additional Comment: Clarification on Expiration date of the test substance

On pages 49 and 72 of the CLH proposal, the DS mention a *note* "... the OECD 422 study was conducted in 2019, and according to the study report the expiration date of the lot/batch of the substance used in the study was in October 2011...". In the Charles River Study No. 20128637 (An Oral (Gavage) Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Bourgeonal in Rats, the Certificate of Analysis showing the expiration date of 11 Dec of 2019 can be found on page 206. The material was under perfect conditions for test during the in-life phase of the study. Final report Page 206

Certificate of Analysis

2837903		BOURGEONAL	
Batch Information			
Batch Number	SC00023379		
Manufacturing date	21 Mar 2018		
Best before	11 Dec 2019		
Country of Origin	Spain		
Product Information			
Sensory Profile	Floral, Fresh, Lily-of-the-valley, Green		
Color	colorless to Pale yellow		
Appearance	liquid		
Handling	Protect against light.		
Flashpoint	73 °C (163 °F)		
Storage	Dry, well ventilated, preferably full, hermetically sealed		
Temperature	Ambient / 10-30°C (50-85°F)		
Analysis Methods	Results	Specification Limits	
Appearance	Conform	Conform	
Appearance color against standard			
Sensory evaluation	Conform	Conform	
Sensory Evaluation			
Specific gravity (20/20°C)	0,9605	0,9580 - 0,9640	
Specific Gravity Determination			
Specific gravity (20/4°C)	0,9585	0,9560 - 0,9620	
Specific gravity (25/25°C)	0,9575	0,9550 - 0,9610	
Refractive index (20°C)	1,5108	1,5080 - 1,5120	
Refractive Index Determination			
Refractive index (25°C)	1,5088	1,5060 - 1,5100	
Acid value (KOH)	0,0	<= 3,0 mg/g	
Acid Value with DL53 Titrator			
Conform chromatogram	Conform	Conform	
GC Comparison against Standard			
Sum of the two isomers	97,3	>= 97,0 %	
Purity Determination by GC			



Regional Quality Manager

The information contained herein is, to the best of our knowledge, true and accurate.
All information is valid until revisions are issued.
Version: Final / 5

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Page 1/1

5. References

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- ECHA. (2017). Guidance on the Application of the CLP Criteria. Retrieved from https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5
- Hareng, L., Schuster, P., Haake, V., Walk, T., Herold, M., Laue, H., & Natsch, A. (2023). Towards the mechanism of spermatotoxicity of p-tert-butyl-alpha-methylhydrocinnamic aldehyde: inhibition of late stage ex-vivo spermatogenesis in rat seminiferous tubule cultures by para-tert-butyl- benzoic acid. *Arch Toxicol*, 97(1), 279-294. doi:10.1007/s00204-022-03379-y.