[redacted]
Currenta GmbH & Co. OHG
Analytics / Product Compliance
CHEMPARK
Germany

[redacted]
www.currenta.de

Registered office: Leverkusen
Registered:
Local Court of Cologne
HRA 20833

Environment Agency Austria on behalf of the
Federal Ministry for Climate Action, Environment,
Energy, Mobility, Innovation and Technology

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EU PPD Consortium comments on the CLH report and Proposal for Harmonised Classification and Labelling as prepared by the Environment Agency Austria - Species sensitivity and acute toxicity of 6PPD quinone to aquatic species

1.) Introduction

Currenta on behalf of the EU PPD Consortium[1] intends to comment on the draft CLH report as prepared by the Environment Agency Austria on behalf of the Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology.

6PPD quinone is a transformation product of 6PPD and identified as a component of stormwater runoff which is responsible for the acute mortality of *O. kisutch* (coho salmon). *O. kisutch* is a fish species originating from the Northwest pacific in the US. Based on the observed acute effects of 6PPD quinone to *O. kisutch* (Tian et al., 2022), several authors have recently reported adverse effects to other aquatic species. The Austrian Competent Authority has provided a summary of such studies in the draft CLH report. The following table compares the current self-classification of 6PPD and the harmonized classification proposed by the Competent Authority.

Table 1 Comparison between self and harmonized classification

Chemical name	CAS No.	Self-classification	Harmonized classification
N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine (6PPD)	793-24-8	Repr. 1B Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 (M = 10) Aquatic Chronic 1 (M = 10)	Repr. 1B Acute Tox. 4 Skin Sens. 1A Aquatic Acute 1 (M = 10.000) Aquatic Chronic 1 (M = 10)

While in most parts the proposed harmonized classification is following the self-classification (reproductive and acute toxicity, aquatic chronic toxicity) there is a specification of the skin sensitization (Skin Sens. 1 to Skin Sens 1A) and a change in the M-factor from 10 to 10.000 for aquatic acute toxicity. This thousandfold increase of the M-factor is justified with the inclusion of the abovementioned results from a non-guideline 24h study on *O. kisutch* using the test item 6PPD

[1] The EU PPD Consortium consists of the members Flexsys Chemicals Belgium NV, LANXESS Deutschland GmbH and Sennics Europe B.V.

quinone by Tian et al. (2022). The Competent Authority does not discuss the specific sensitivity of salmonids in general and *O. kisutch* in particular. Furthermore, in the draft CLH report there is no discussion of the relevance of *O. kisutch*, which is only indigenous to the north-western pacific coast of the US, but not found in the EU. Finally, considering the number of studies on different species and the variability of the observed (non-)effect, the option of deriving a more realistic hazard estimation by the means of a species sensitivity distribution (SSD) is not considered.

Currenta on behalf of the EU PPD consortium has conducted an extensive literature search and evaluation of studies on 6PPD quinone, including but not limited to those considered in the draft CLH report, which is presented below.

2.) Specific details from each study

An overview of the studies discussed below are given in Table 2.

Tian et al., (2022) assessed the toxic effects of 6PPD quinone to *Oncorhynchus kisutch*. A Klimisch score of 2 was assigned to the study. A 24h LC50 of 0.095 µg/L was estimated.

Lo et al., (2023) investigated the acute toxicity of 6PPD quinone to newly feeding *O. tshawytscha and O. kisutch*. The study was adapted from the Environment and climate change Canada's guidelines (Environment Canada 1990) and a Klimisch score of 2 was assigned. 24-h LC50 of 0.041 µg/L and > 67.307 µg/L were estimated for *O. kisutch* and *O. tshawytscha* which shows that *O. kisutch* is more sensitive by a factor of 1641.

Greer et al., (2023) compared the species sensitivity of 3 Salmonidae fish species (*Oncorhynchus kisutch, Oncorhynchus tshawytscha and Oncorhynchus nerka*) to 6PPD quinone using in vivo and in vitro methods. Both in vivo and in vitro studies revealed the very high sensitivity of *O. kisutch* leading to 24 h LC50 of 0.0804 µg/L and EC50 of 6.1 µg/L, respectively. In contrast, the other 2 species showed very little or no effects in both in vivo (>50 µg/L) and in vitro studies (no effect). Based on these results, *O. kisutch* is more sensitive than the other 2 species with a factor of 621. The relative order of sensitivity (*O. kisutch* > *O. tshawytscha* > *O. nerka*) has been found to be consistent with exposure to undiluted urban stormwater runoff (French et al., 2022).

Hiki et al., (2021) assessed the aquatic toxicity of 6PPD quinone to 2 freshwater fish (*Danio rerio* and *Oryzias latipes*) and 2 crustacean species (*Daphnia magna*, and *Hyalella azteca*). The studies were conducted according to standard guidelines and a Klimisch score of 2 was assigned. 6PPD quinone did not induce acute mortality to the 4 species at its maximum water solubility. The remarkable difference in toxicity values between *O. kisutch* and the tested species is at least a factor of >100 (Hiki et al., 2021).

Brinkmann et al., (2022) assessed the acute toxicity of 6PPD quinone to brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*) and white sturgeon (*Acipenser transmontanus*). Due to high sensitivity in pre-tests, the main test with brook trout was performed for 24 h while for the other species, 96h studies were performed. For brook trout and rainbow trout 24-h LC50 of 0.59 µg/L and 1.96 µg/L (72-96-h LC50 = 1.96 µg/L) were reported,

indicating both species being highly sensitive against 6PPD quinone. For neither Arctic char nor white sturgeon mortalities were observed at the highest test concentrations of 14.2 and 12.7 µg/L after 96h. A Klimisch score of 2 was assigned to the study. Yet, larger/older fish (still sub-adult) than recommended according to various guidelines were used, therefore effects on smaller/younger fish cannot be concluded.

Foldvik et al., (2022) investigated the acute toxicity of 6PPD quinone in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) alevins over a period of 48h. No mortalities were recorded for both fish species at the highest test concentration, therefore no LC50 was reported. A Klimisch score of 2 was assigned to the study. These results indicate that both species, *S. salar* and *S. trutta*, are less sensitive compared to *O. kisutch* (Lo et al. 2023) by a factor of >296.

Varshney et al., (2022) investigated the behavioral responses and cardiotoxicity in zebrafish larvae after exposure to 6PPD and 6PPD quinone. In addition, a fish embryo test (*Danio rerio*) according to OECD 236 was conducted to select the doses for the sublethal toxicity experiments. The 96 h LC50 for 6PPD quinone was found to be 132.92 µg/L. In addition, a 24 h LC50 of 308.67 µg/L was estimated which is a factor of 7512 less sensitive to the 24 h LC50 (0.041 µg/L) reported for *O. kisutch* by Lo et al. (2023).

Di et al., (2022) investigated the enantioselective toxicities of 6PPD and 6PPD quinone to *Oncorhynchus mykiss* and *Gobiocypris rarus* according to OECD 203. The 96 h LC50 values of rac-6PPD-Q, R-6PPD-Q and S-6PPD-Q were determined to be 2.26, 4.31 and 1.66 µg/L for *O. mykiss*, respectively. For *G. rarus*, no acute mortality was observed in rac-6PPD-Q, R-6PPD-Q and S-6PPD-Q test solutions at 500 µg/L. These results also show that these species are less sensitive than *O. kisutch* with a factor >40. Nevertheless, a Klimisch score of 3 was assigned and these results should be taken with caution. The adequacy of the study (reliability and relevance) is in question due to the following reasons: 1) use of enantiomers as test item; 2) lack of data on validity criteria; and 3) exposure concentrations likely above water solubility of ≈67 µg/L reported by Hiki et al., (2021).

Klauschies & Isanta-Navarro, (2022) investigated the individual and mixture effects of 6PPD and salt (NaCl) on the population growth of *Brachionus calyciflorus*, an herbivorous rotifer. In a second step, the effects of 6PPD and 6PPD quinone were also compared. The results show that 6PPD quinone did not affect the population growth of the rotifer at 1000 µg/L. The study was assigned a Klimish score of 3 based on the effects observed in control treatment, exposure concentrations probably above water solubility and lack of chemical analysis to ensure stability over 12 days.

Table 2: Overview of the acute toxicity of 6PPD quinone to aquatic species.

Reference	Aquatic species	Guideline	Age	Duration (h)	EC50 / LC50 (µg/L)	Reliability
Tian et al., 2022	*Oncorhynchus kisutch (coho salmon)*	-	Juvenile (1+ years)	24	0.095	2
Lo et al., 2023	*Oncorhynchus kisutch (coho salmon)*	Environment Canada, 1990	Juvenile (~3 weeks)	24	0.041	2
	O. tshawytscha (chinook salmon)	Environment Canada, 1990	Juvenile (~3 weeks)	24	> 67.307	2
Greer et al., 2023	*Oncorhynchus kisutch (coho salmon)*	-	Juvenile (189 days)	24	0.0804	2
	Oncorhynchus tshawytscha (chinook salmon)	-	Juvenile (582 days)	24	>25	2
	Oncorhynchus nerka (sockeye salmon)	-	Juvenile (625 days)	24	>50	2
Brinkmann et al., 2022	*Oncorhynchus mykiss (rainbow trout)*	-	Juvenile (~2 years)	72-96	1	2
	Salvelinus fontinalis (brook trout)	-	Juvenile (~1 year)	24	0.59	2

Reference	Aquatic species	Guideline	Age	Duration (h)	EC50 / LC50 (µg/L)	Reliability
	Salvelinus alpinus (Arctic char)	-	Juvenile (~3 years)	96	>14.2	2
	Acipenser transmontanus (white sturgeon)	-	Juvenile (~4.5 years)	96	>14.2	2
Foldvik et al., 2022	*Salmo Salar (Atlantic salmon)*	-	Alevins	48	>12.16	2
	Salmo trutta (Brown trout	-	Alevins	48	>12.16	2
Hiki et al., 2021	*Danio rerio (Zebrafish)*	OECD 236	Embryo (16 cell stage)	96	>54	2
	Oryzias latipes (Japanese medaka)	OECD 203	Juvenile (41-days-old)	96	>34	2
	Daphnia magna	OECD 202	Neonate (< 24-h-old)	48	>46	2
	Hyalella azteca	Environment and Climate Change Canada (2017).	neonates (3–5-days old)	96	>43	2
Varshney et al., 2022	*Danio rerio (Zebrafish)*	OECD 236	Embryo (16 cell stage)	96	133	2
Di et al., 2022	*Gobiocypris rarus*	OECD 203	Juvenile (2 weeks)	96	>500	3

Reference	Aquatic species	Guideline	Age	Duration (h)	EC50 / LC50 (µg/L)	Reliability
	Oncorhynchus mykiss (rainbow trout)	OECD 203	Juvenile (2 weeks)	96	1.66 – 4.31	3
Klauschies & Isanta-Navarro, 2022	*Brachionus calyciflorus*	-	-	288	>1000	3

3.) Discussion on species sensitivity

Sensitivity to Salmonidae fish family

Based on acute mortality induced by 6PPD quinone, *O. kisutch* is not only significantly more sensitive than other fish and aquatic species but it is also highly sensitive compared to other closely related species in the Salmonidae family (Figure 1 and Figure 2). Among the fish in the Salmonidae family, *S. fontinalis* and *O. mykiss* are the closest in sensitivity to *O. kisutch* by a factor of 14 and 24, respectively. The other species show no adverse effect at maximum exposure concentration (Figure 1). The exceptional high sensitivity of *O. kisutch* in comparison to its close relatives has also been reported by other studies. For example, Greer et al., (2023) could confirm the high acute sensitivity of *O. kisutch* using in vitro cell assays while the cells of other Salmonidae species (O. *tshawytscha, O. nerka and O. mykiss)* showed little or no adverse effects.

Figure 1: Comparison of species sensitivity of O. kisutch and other fish in the Salmonidae family to 6PPD quinone. Bars in the "No Effect" box represent the highest tested concentration and are to be interpreted as LC50 >highest tested concentration as in Table 2.

Sensitivity to other fish and other aquatic species

In comparison to other fish and aquatic species, *O. kisutch* also shows significantly higher sensitivity to 6PPD quinone (Figure 2). The reported toxicity values for aquatic species indicate that only *Danio rerio* showed mortality effects at a concentration which is greater than the water solubility (WS ≈ 67 µg/L). Other tested species showed no effects at maximum exposure concentration (Table 2).

Figure 2: Comparison of species sensitivity of O. kisutch and other aquatic species to 6PPD quinone. Bars in the "No Effect" box represent the highest tested concentration and are to be interpreted as LC50 >highest tested concentration as in Table 2.

4.) Conclusion

The differential sensitivity of *O. kisutch* in comparison to other aquatic species is unlikely explained by experimental factors but could be reasonably driven by inherent susceptibility and specific mechanisms of action displayed in *O. kisutch* (Hiki et al., 2021). Therefore, *O. kisutch* does not appear to be representative for the acute mortality of 6PPD quinone to aquatic species and may not be used as the main species for classification due to over-estimation of effects and unrealistic hazard assessment. According to ECHA guidance R7b, justification should be given for using a species. The OECD 203 TG gives examples of important criteria for selecting a species such as; ready availability throughout the year, ease of maintenance, convenience for testing and any relevant economic, biological or ecological factors. *O. kisutch* is: 1) not indigenous to EU environment and may not be readily available to be utilized for testing at EU labs; 2) not easily accessible and cultivated in the EU environment; and 3) not representative of other species due to its extremely high sensitivity. Furthermore, sufficient toxicity data exist for other relevant and standard species which are more representative and could be readily used for classification. In addition, no urban runoff mortality

events have been noted in the EU to our knowledge. The EU PPD Consortium wants to point out that the CLP regulation is supposed to focus on the hazard towards the EU specifically.
In conclusion, the PPD Consortium asks to reconsider the proposed M-factor for aquatic acute toxicity considering EU relevant and representative species and the possibility to realistically assess the hazard of 6PPD and its transformation products via SSD.

Senior Expert

Product-Compliance

References

Brinkmann, M., Montgomery, D., Selinger, S., Miller, J. G., Stock, E., Alcaraz, A. J., Challis, J. K., Weber, L., Janz, D., & Hecker, M. (2022). Acute toxicity of the tire rubber-derived chemical 6ppd-quinone to four fishes of commercial, cultural, and ecological importance. Environmental Science & Technology Letters, 9(4), 333-338.

Di, S., Liu, Z., Zhao, H., Li, Y., Qi, P., Wang, Z., Xu, H., Jin, Y., & Wang, X. (2022). Chiral perspective evaluations: Enantioselective hydrolysis of 6PPD and 6PPD-quinone in water and enantioselective toxicity to Gobiocypris rarus and Oncorhynchus mykiss. Environment international, 166, 107374.

Foldvik, A., Kryuchkov, F., Sandodden, R., & Uhlig, S. (2022). Acute Toxicity Testing of the Tire Rubber–Derived Chemical 6PPD-quinone on Atlantic Salmon (Salmo salar) and Brown Trout (Salmo trutta). Environmental toxicology and chemistry.

French, B. F., Baldwin, D. H., Cameron, J., Prat, J., King, K., Davis, J. W., ... & Scholz, N. L. (2022). Urban roadway runoff is lethal to juvenile coho, steelhead, and chinook salmonids, but not congeneric sockeye. Environmental Science & Technology Letters, 9(9), 733-738.

Greer, J. B., Dalsky, E. M., Lane, R. F., & Hansen, J. D. (2023). Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products. Environmental Science & Technology Letters.

Hiki, K., Asahina, K., Kato, K., Yamagishi, T., Omagari, R., Iwasaki, Y., Watanabe, H., & Yamamoto, H. (2021). Acute toxicity of a tire rubber-derived chemical, 6PPD quinone, to freshwater fish and crustacean species. Environmental Science & Technology Letters, 8(9), 779-784.

Lo, B. P., Marlatt, V. L., Liao, X., Reger, S., Gallilee, C., & Brown, T. M. (2023). Acute toxicity of 6PPD-quinone to early life stage juvenile Chinook (Oncorhynchus tshawytscha) and coho (Oncorhynchus kisutch) salmon. Environmental toxicology and chemistry.

Tian, Z., Gonzalez, M., Rideout, C. A., Zhao, H. N., Hu, X., Wetzel, J., Mudrock, E., James, C. A., McIntyre, J. K., & Kolodziej, E. P. (2022). 6PPD-quinone: Revised toxicity assessment and quantification with a commercial standard. Environmental Science & Technology Letters, 9(2), 140-146.

Varshney, S., Gora, A. H., Siriyappagouder, P., Kiron, V., & Olsvik, P. A. (2022). Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae. Journal of Hazardous Materials, 424, 127623.