Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

metyltetraprole (ISO); 1-[2-({[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxy}methyl)-3-methylphenyl]-4-methyl-1,4-dihydro-5*H*-tetrazol-5-one

EC Number: -

CAS Number: 1472649-01-6

CLH-O-0000007431-80-01/F

Adopted

14 March 2024

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON METYLTETRAPROLE (ISO); 1-[2-({[1-(4-CHLOROPHENYL)-1*H*-PYRAZOL-3-YL]OXY}METHYL)-3-METHYLPHENYL]-4-METHYL-1,4-DIHYDRO-5*H*-TETRAZOL-5-ONE;

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation (09/01/2024-10/03/2023) are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table. Please, note there was later another consultation, and those comments are in another Annex document.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: metyltetraprole (ISO); 1-[2-({[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy}methyl)-3-methylphenyl]-4-methyl-1,4-dihydro-5H-tetrazol-5-one;
EC number: -
CAS number: 1472649-01-6
Dossier submitter: Spain

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.03.2023	France	Sumitomo Chemical Agro Europe S.A.S.	Company-Manufacturer	1

Comment received

It has been proposed that the classification Carc Cat 2 H351 is warranted (CLH report page 286, point 2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria).

The applicant does not agree with this proposed classification and conducted additional histopathological examination on intermediate groups of carcinogenicity studies and statistical analysis to evaluate carcinogenic potential of metyltetraprole more precisely.
Then, a third-party peer review was performed, and the Expert Panel consisting of multiple worldwide expert pathologists concludes that all tumors of ANSES concern are not treatment-related.

The reports of the additional histopathological examinations and of the external peer-review are under finalization and will be available in May 2023:
- Additional histopathology of mouse carcinogenicity study
- Additional histopathology of rat carcinogenicity study
- Statistical analysis of mouse carcinogenicity data
- Statistical analysis of rat carcinogenicity data
- Peer-review of mouse carcinogenicity data
- Peer-review of rat carcinogenicity data
- Expert panel report

In addition, an updated position paper "Metyltetraprole (S-2367): Position Paper on Harmonised C&L (CLH) Proposal for Carcinogenicity Classification" is under preparation and

will be available in May 2023, clearly detailing the weight of evidence demonstrating that there were no treatment related tumours meriting carcinogenicity classification in accordance with the CLP criteria and taking into account the CLP Annex I: 3.6.2.2.6. which lists some important factors which may be taken into consideration, when assessing the overall level of concern for classification.

A summary of the evidences not supporting a treatment-related carcinogenic effect is provided below:
Biological plausibility (paragraph 3.6.2.3.1)
- Lack of statistical significance in the PETO trend and pair-wise tests
- Lack of dose-response relationship
- All tumours were within the HCD range, except for one tumor attributed to high background incidence of the animals used in the study
- None of tumour pathogeneses are supported by the fact that the test substance does not have any hormonal effects and genotoxicity, and any AOPs published
- No higher distribution and accumulation to the sites where tumours were observed than the other tissues, i.e., uterus for malignant schwannoma, and uterus and other sites for histiocytic sarcoma
- The uterus and liver where the malignant schwannoma and/or histiocytic sarcoma were observed, common tissues where they occur spontaneously as well
- Mammary gland tumours were common spontaneous tumours which occurred in association with spontaneous pituitary proliferative lesions in female rat
- Malignant lymphomas were common spontaneous tumours in rats and mice
Comparison with CLP criteria (paragraph 3.6.2.3.2)
(a) Tumor type and background incidence: Some types of tumour were noted, but almost within the HCD range.
(b) Multi-site responses: No clear evidences of multi-site responses.
(c) Progression of lesions to malignancy: No evidences of progression of lesions to malignancy.
(d) Reduced tumor latency: Reduced tumour latency was not observed.
(e) Whether responses are in single or both sexes: No clear evidence that the responses were observed in any sexes.
(f) Whether responses are in a single species or several species: No clear evidence that the responses were observed in any species.
(g) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity: Not structurally similar to substances that have carcinogenic potential.
(h) Routes of exposure: Oral route (dietary administration), which is relevant to consumer dietary risk assessment.
(i) Comparison of ADME between test animals and humans: Suggestive of the similarity between experimental animals and humans.
(j) The possibility of a confounding effect of excessive toxicity at test doses: No evidence of a confounding effect of excessive toxicity.
(k) Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity: No toxicity data supporting particular MOAs for carcinogenicity.
The updated position paper TST-0100 on Historical Control Data is provided in support of this response.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment TST-0100 revised February 2023 (final)_Redacted.pdf
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment TST-0100 revised February 2023 (final).pdf

Dossier Submitter's Response
The DS acknowledged the conduct of additional histopathological examination on low and intermediate dose groups of the rat and mouse carcinogenicity studies, as well as the conduct of further statistical analysis (please note that appropriate trend tests and pairwise comparisons are expected to be conducted). These data should be provided to ECHA as soon as available. The updated position paper TST-0100 on historical control data (HCD) was analysed by the DS. One additional study, dated 2018 and conducted by dietary administration, was included in the HCD already available at the time of the CLH/DAR submission. Therefore, updated tables summarising neoplastic lesions observed with metyltetraptole along with updated HCD can be found below. Two additional sets of HCD are proposed compared to the HCD presented in the CLH/DAR: - **HCD from 2010-2016, diet**: already presented in the CLH/DAR - **HCD from 2010-2018, diet:** includes the new study from 2018 available in the updated position paper on HCD - **HCD from 2014-2018, diet:** includes the new study from 2018 available in the updated position paper on HCD and excludes the HCD from the years 2010-2013. Indeed, HCD should be centered as closely as possible to the date of the study within a 5-year period). Both experimental parts of the carcinogenicity studies being conducted in 2015-2017, the DS considers that HCD from years 2014 to 2018 are the most relevant. As can be seen from the tables below, no significant differences are seen in the three sets of HCD, except that no incidence at all of malignant lymphomas and malignant uterine schwannomas were reported in female rats in HCD from 2014 to 2018. The DS would like to reiterate that the proposal of the applicant to consider a longer period of time (i.e. a period of 11 years from 2008 to 2018 in the updated paper on HCD) is not agreed upon since the provided linear regression between incidence of neoplasms and years of the studies is not considered appropriate. As reported by the DS in the DAR Vol 3CA B6 (in Annex of the CLH/DAR Vol 1): *"Exploring relationship between incidence and year of study does not provide biologically relevant metrics by means of linear regression. In terms of methodology variable Year should be treated as categorical variable, which is not the case. Even if so had been done by applicant, interpretation of this regression would have been questionable and would have not provided any useful information whatsoever since the linear regression is definitely not the methodology of choice to assess variability of incidence across years of studies. More useful information would have been provided if methodologies and recommendations from EFSA Journal 2018;16(1):5122 and EFSA Journal 2018;16(1):5123 had been followed. As such, rationale and analysis from applicant in TST-0100 is considered unacceptable"* In addition, to perform the regression analysis, the applicant considered all the studies in the HCD, regardless the administration route. It is reminded that the DS does not consider appropriate to use historical control data from other routes/methods of administration. Indeed, the method of administration (e.g. feeding versus gavage) could result in different conditions of stress in the animals that could have an impact on the background incidences of some type of tumours. This is for example illustrated by the differences of incidences of malignant schwannomas in the uterus of rats for the period 2014-2018: 0 incidence in 4 studies conducted by dietary administration *versus* mean of 1.4% (range 0 – 3.6%) in 9 studies conducted by gavage. **Updated tables of the CLH/DAR Vol 1 (updates highlighted in yellow):**

Malignant lymphomas in rats:

Organ/Tissue	Finding	Dose level (ppm)							
		Male				Female			
		0	2000	6000	20000	0	2000	6000	0000
Haematopoietic system	n=	50	20	*17*	50	50	14	*16*	50
	Malignant lymphoma	0	0	**3** ***(18%)***	**6%**	0	0	**1** ***(6%)***	**2** **4%**
	Pairwise comparison against control (1-tailed)				p=0.132				p=0.234
	Cochran-Armitage trend test (1-sided)[1]	p=0.02639				p=0.06442			
	HCD 5 studies 2010-2016, diet	*0%, 0%, 3.8%, 4%, 4%* *Mean: 2.4%; range: 0-4%*				*0%, 0%, 0%, 0%, 4 2%* *Mean: 0.8 0.4%; range: 0-4 2%*			
	HCD 6 studies 2010-2018, diet	*0%, 0%, 1.9%, 3.8%, 4%, 4%* *Mean: 2.3%; range: 0-4%*				*0%, 0%, 0%, 0%, 0%, 4%* *Mean: 0.7%; range: 0-4%*			
	HCD 4 studies 2014-2018, diet	*0%, 1.9%, 3.8%, 4%* *Mean: 2.4%; range: 0-4%*				*0%, 0%, 0%, 0%* *Mean: 0.0%; range: 0-0%*			

[1] DS/RMS assessment

Malignant uterine schwannomas in rats:

Organ/Tissue	Finding	Dose level (ppm)							
		Male				Female			
		0	2000	6000	20000	0	2000	6000	0000
Uterus	n=	-	-	-	-	50	24	22	50
	M-schwannoma, malignant	-	-	-	-	0	1 ***(4%)***	0	**3** **6%**
	Pairwise comparison against control (1-tailed)								p=0.110
	Cochran-Armitage trend test (1-sided)[1]					p=0.05432			
	HCD 5 studies 2010-2016, diet					*0%, 0%, 0%, 0%, 2%* *Mean: 0.4%; range: 0-2%*			
	HCD 6 studies 2010-2018, diet					*0%, 0%, 0%, 0%, 0%, 2%* *Mean: 0.3%; range: 0-2%*			
	HCD 4 studies 2014-2018, diet					*0%, 0%, 0%, 0%* *Mean: 0.0%; range: 0-0%*			

[1] DS/RMS assessment

Mammary tumors in rats:

Organ/Tissue	Finding	Dose level (ppm)							
		Male				Female			
		0	2000	6000	20000	0	2000	6000	0000
Mammary gland	n=	50	20	17	50	50	39	41	50
	Mammary adenoma	0	0	0	0	1	1	2	**4** **8%**
	Pairwise comparison against control (1-tailed)								p=0.180
	Cochran-Armitage trend test (1-sided)[1]					p=0.06233			
	HCD 5 studies 2010-2016, diet					*0%, 0%, 2%, 3.8%, 4%* *Mean: 2%; range: 0-4%*			
	HCD 6 studies 2010-2018, diet					*0%, 0%, 2%, 3.8%, 3.8%, 4%* *Mean: 2.3%; range: 0-4%*			
	HCD 4 studies 2014-2018, diet					*0%, 0%, 3.8%, 3.8%* *Mean: 1.9%; range: 0-3.8%*			
	Mammary adenocarcinoma	0	0	0	0	4	1	3	**7** **14%**

	Pairwise comparison against control (1-tailed)								p=0.236
	Cochran-Armitage trend test (1-sided)[1]					p=0.1053			
	HCD 5 studies 2010-2016, diet					*6%, 7.7%, 12%, 14%, 17.3% Mean: 11.4%; range: 6-17.3%*			
	HCD 6 studies 2010-2018, diet					*6%, 7.7%, 12%, 14%, 17.3%, 23.1% Mean: 13.4%; range: 6-23.1%*			
	HCD 4 studies 2014-2018, diet					*6%, 7.7%, 17.3%, 23.1% Mean: 13.5%; range: 6-23.1%*			
	Total (adenoma and adenocarcinoma)	0	0	0	0	5	2	5	**11 22%**
	Cochran-Armitage trend test (1-sided)[1]					p=0.02357			
	HCD 5 studies 2010-2016, diet					*6%, 11.5%, 16%, 16%, 17.3% Mean: 13.4%; range: 6-17.3%*			
	HCD 6 studies 2010-2018, diet					*6%, 11.5%, 16%, 16%, 17.3%, 26.9% Mean: 15.6%; range: 6-26.9%*			
	HCD 4 studies 2014-2018, diet					*6%, 11.5%, 17.3%, 26.9% Mean: 15.4%; range: 6-26.9%*			

[1] DS/RMS assessment

Tumours of the haematopoietic system in mice:

Organ	Finding	Dose level (ppm)							
		Male				Female			
		0	700	2000	7000	0	700	2000	7000
Haematopoietic system	n=	51	20	17	51	51	16	20	51
	Lymphoma	5 9.8%	6 *(30%)*	**8 *(47%)***	**8 16%**	8 16%	8 *(50%)*	12 *(60%)*	9 18%
	Pairwise comparison against control (1-tailed)				p=0.323				p=0.477
	Cochran-Armitage trend test (1-sided)[1]	p=0.1717				p=0.3324			
	HCD 9 studies 2010-2016, diet	*Mean 5.0% Range 0.0-11.8%*				*Mean 12.9% Range 0.0-23.5%*			
	HCD 10 studies 2010-2018, diet	*Mean 4.9% Range 0.0-11.8%*				*Mean 13.1% Range 0.0-23.5%*			
	HCD 5 studies 2014-2018, diet	*Mean 4.7% Range 0.0-11.8%*				*Mean 11.4% Range 0.0-21.6%*			
	n=	51	20	17	51	51	16	20	51
	Histiocytic sarcomas	0	0	0	0	1 2%	0 *(0%)*	**3 *(15%)***	**3 6%**
	Pairwise comparison against control (1-tailed)								p=0.300
	Cochran-Armitage trend test (1-sided)[1]					p=0.09714			
	HCD 9 ~~8~~ studies 2010-2016 ~~2015~~, diet					*Mean 1.3% Range 0.0-3.9%*			
	HCD 10 studies 2010-2018, diet					*Mean 1.8% Range 0.0-5.9%*			
	HCD 5 studies 2014-2018, diet					*Mean 1.96% Range 0.0-5.9%*			

[1] DS/RMS assessment

RAC's response

RAC supports the DS position regarding the historical control data set to be considered. In particular: 1) The application of regression analysis to historical data from 2010 is considered inappropriate and there is no reason to deviate from the common practice of

considering data from the last 5 years; 2) The historical data should include only studies conducted by dietary administration, also considering the possible impact of gavage on the animal physiology and therefore on the tumour background incidence.
RAC also takes note of the additional statistical analyses provided by the DS.

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	United Kingdom	<confidential>	National Authority	2

Comment received

Carcinogenicity
Page 56 of the CLH report states:
'the RMS considered that the HCD provided by the applicant are not fully relevant as a period of 5 years around the study was not used and also, studies conducted by several routes of administration have been included in the HCD. As the study was conducted in 2015-2017, HCD from studies conducted before 2010 should be disregarded. In addition, only HCD from studies conducted by dietary administration should be considered'.
Some of the discarded HCD could be informative for the assessment of carcinogenicity (i.e., the data from other routes of administration).
Would it be possible to see the full HCD set? We note reference to a position paper 'TST-0100' in the DAR which appears to contain this information.

Dossier Submitter's Response

The full HCD set is available in Volume 3CA B6, which is an Annex of the DAR-CLH Volume 1 (pages 211-23 for rats, page 230 for mice). The analysis of the HCD by the applicant is not considered relevant (in terms of route of administration and contemporaneity of the studies), but since data for each individual study is available, the DS/RMS has proposed HCD set considering only relevant studies (please also refer to comment 1 above, where the applicant provided updated position paper on HCD – document TST-0100).
Please note that we do not consider appropriate to consider historical control data from other routes/methods of administration (please also refer to comment 1 above).

RAC's response

Noted. See the comment above.

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	3

Comment received

Prior to the discussion on classification for carcinogenicity, further statistical analysis is required. As shown below on one example, classification in category 1B, H350 may be more appropriate.
Justification: The statistical analysis of tumour incidences presented in the dossier does not meet current OECD recommendations. A trend test can be performed despite the smaller number of animals at low and medium doses. Tumour findings should be reconsidered based on appropriate statistical analysis.
We have carried out a few analyses as examples:
• The trend test (Cochran-Armitage test for trend one-sided (one-tailed)) was significant (alpha = 0.05) for the endpoints mammary adenocarcinoma (female rats) (p-value: 0.049) and mammary adenoma and carcinoma (female rats) (p-value 0.0098). For the endpoint mammary adenomas female rats, the p-value was 0.0594 for the trend test.
• For the endpoint malignant lymphoma (both male and female mice), both the lower (m: 82.2 mg/kg bw/d; f: 103.4 mg/kg bw/d) (p-value males = 0.044; p-value females =

0.0086) as well as the second highest dose group (m: 225 mg/kg bw/d; f: 291 mg/kg bw/d) (p-value males= 0.002; p-value females = 0.0004) were statistically significant according to the Fisher's exact test. Lack of statistical significance at the top dose remains to be discussed in the context of systemic toxicity. According to OECD GD 116 (2012), paragraph 345, "A trend test is more powerful than the pair-wise test. A complication is that a trend test may fail to detect curvi-linear responses such as might arise from non-linear effects such as complications from saturation. In such situations the pair-wise tests may give more appropriate results". For pair wise comparisons, "Fisher's exact test is now preferred […] (paragraph 342)."
Dossier Submitter's Response
Further statistical analysis has been performed by the DS/RMS for each tumor types. This consisted of Cochran-Armitage trend test (one-sided). The results are reported in the update tables in the comment number 1 above. Please note that the applicant commented (Comment number 1) that additional histopathological examination on low and intermediate dose groups of the rat and mouse carcinogenicity studies will be conducted, as well as further statistical analysis. These data should be provided to ECHA as soon as available and should be taken into account by RAC members to conclude on classification for carcinogenicity.
RAC's response
Noted. Further considerations, taking into account also the new documents provided by the Applicant in the later additional consultation, are reported in the other Annex document (CLH_targeted_PC_RCOM).

Date	Country	Organisation	Type of Organisation	Comment number
23.02.2023	The Netherlands		MemberState	4
Comment received				
The RMS proposes a Carc. Cat 2 (H351) classification based on a weight of evidence approach described on pages 64 to 66. Based on the available evidence most factors seem to favor a Cat 1B classification (same type of tumors in both sexes and in both species, malignant tumors, multisite response/other tumors in different organs (in female rats), MoA unknown and no evidence of a confounding effect of excessive toxicity). The classification seems to have been downgraded to a Cat 2 based on the arguments that 1) the tumor incidences are relatively low and not suggestive of a clear effect and 2) that statistical significance was not reached. However, a firm justification should be added. The statement on the relatively low tumor incidence seems rather subjective and this should be further elaborated. A low tumor incidence is normally considered relevant/sufficient for classification when the background incidence is low or the tumor type is relatively rare. Obtaining statistical significance is sometimes not possible with low tumor incidences even though they are relevant. In this case it seems the difference with the HCD should be considered more important. In addition, we propose to more extensively discuss the weight of evidence specified for tumor type (i.e. 1) malignant lymphomas observed in male and female rats and male mice, 2) uterine schwannomas in rat, 3) mammary gland tumors in rat and 4) histiocytic sarcomas in female mice) as these tumors occurred in different organs, have different cells of origin and different natural background incidences. Based on the current argumentation the NL-CA leans more towards supporting a classification as Carc. 1B H350, but this may be changed with a better justification for downgrading to category 2.				
Dossier Submitter's Response				

It is noted that NL, as co-RMS for the active substance metyltetraprole, agreed with the category 2 classification for carcinogenicity during the commenting period before submission of the DAR/CLH Vol 1 to EFSA and ECHA. Factors contributing to classification in either category 1 or category 2 are available in the DAR/CLH Vol 1. In addition, the applicant has commented (Comment number 1) that additional histopathological examination on low and intermediate dose groups of the rat and mouse carcinogenicity studies will be conducted, as well as further statistical analysis. These data should be provided to ECHA as soon as available and should be taken into account by RAC members to conclude on classification for carcinogenicity.
RAC's response
The conclusions regarding the proposed classification requires an overall evaluation taking into account all the available data, including the new information provided by the Applicant. This evaluation is reported in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
10.03.2023	France	Générations Futures	National NGO	5
Comment received				
this substance is proposed by the RMS to be classified as suspected carcinogen (category 2). However, there are much more factors in favor of a classification in category 1B than in category 2: - Several types of malignant tumors were observed at several sites in both sexes and both species following metyltetraprole administration. - The incidences of tumours were slightly but above the range of HCD. - The MoA underlying these neoplastic lesions were unknown and therefore human relevance cannot be excluded. - There is no evidence of confounding effect of excessive toxicity. Indeed, although the tumours were generally observed at high dose levels, the systemic toxicity at these doses remain low. The only element in favor of a classification in category 2 is the slight incidence of tumors reported when compared to controls or HCD. However, according to the guidance on the application of the CLP criteria (p.383/647) , "if a substance causes tumours at multiple sites and/or in more than one species then this usually provides strong evidence of carcinogenicity. Typically such a tumour profile would lead to a classification in category 1B." Metyltetraprole should therefore be classified in category 1B. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Générations Futures_metyltetraprole.pdf				
Dossier Submitter's Response				
Noted. Factors contributing to classification in either category 1 or category 2 are available in the DAR/CLH Vol 1. The final decision on the category applicable to classification for carcinogenicity is now the responsibility of ECHA and RAC members.				
RAC's response				
The conclusions regarding the proposed classification requires an overall evaluation taking into account all the available data, including the new information provided by the Applicant. This evaluation is reported in the RAC opinion.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	6
Comment received				
We agree with the DS that based on the available data, no classification for mutagenicity is warranted for metyltetraprole. Nevertheless, we noted that the in vitro chromosome aberration assay is not designed to measure aneugenic effects and an in vitro micronucleus assay may have been more informative. However, taking into account the available *in vivo* micronucleus test, the data is considered conclusive.				
Dossier Submitter's Response				
Agreed				
RAC's response				
RAC agrees on the consideration that *in vitro* chromosome aberration (CA) assay is not designed to detect aneugenicity. However, the *in vivo* micronucleus assay should be considered inconclusive, because there is no evidence of bone marrow toxicity. The mentioned TK-study may provide an evidence that some bone marrow exposure occurred, but cannot be considered a demonstration that the substance could not induce aneuploidy at a higher local concentration, for example, in the first site of contact. In fact, the plasma levels detected in the TK-studies are at least an order of magnitude lower than the concentration that could be tested *in vitro*. While gene mutation induction is ruled out by the negative Ames test and a clastogenic effect is excluded by the reliably negative outcome of the *in vitro* CA assay, in order to definitely rule out the concern for aneugenicity an *in vitro* MN assay performed with an adequate concentrations range would be needed. RAC agrees with the DS on the conclusion that no classification for germ cell mutagenicity is warranted for metyltetraprole, considering that there is no experimental evidence of mutagenic activity. However, it is noted that aneugenic effects were not adequately assessed.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	7
Comment received				
Adverse effects on sexual function and fertility. We agree with the DS that based on the available data from a 2-generation study in rats (Anonymous, 2017), the effects are not sufficient for classification of metyltetraprole as toxic for sexual function and fertility. Adverse effects on development Overall, we could support non-classification for developmental toxicity. However, skeletal findings in the rat were observed at a dose without maternal effects and although incidences were low, they exceeded the HCD. According to the DevTox database, these findings are considered grey zone. The following information might support the proposed conclusion that the observed findings are not sufficient for a classification for developmental toxicity, and could be provided by the DS: • Was a statistical analysis performed to calculate possible significances? • Considering the different skeletal findings, were there always different fetuses affected, or were there fetuses with multiple findings? • Were the historical control data appropriately reported?				

Dossier Submitter's Response
In the rat developmental study, as stated in the DAR/CLH, the same 4 foetuses (from 3 litters) presented both findings misaligned hemicentres of sternebrae and misaligned costal cartilage at the high dose (i.e. fetus#9 from dam#2, fetuses#4 and 10 from dam#3, fetus#8 from dam#10). One additional fetus (from a different litter) presented misaligned costal cartilage only (fetus#6 from dam#15). In the other groups (control group, 250 and 500 mg/kg bw/d), 2 fetuses from 2 litters in each group were also affected by both findings. There was no dose-related increase in the total number of foetuses and litters affected by skeletal findings ("minor skeletal abnormalities" as stated in the study report) (tabulated in the DAR Vol 3CA B6 and last line of the table below).

Group	:	2	4	3	1
Compound	:	Control	S-2367 TG	S-2367 TG	S-2367 TG
Dose (mg/kg/day)	:	0	250	500	1000

		Fetuses				Litters			
Group		**2**	**4**	**3**	**1**	**2**	**4**	**3**	**1**
Number Examined		121	141	130	136	23	24	24	24
Total Number Normal		31	25	22	29	14	16	12	16
Minor skeletal abnormalities									
Cranial	misshapen basisphenoid	0	2	0	0	0	2	0	0
Vertebral element abnormality	thoracic	1	0	0	0	1	0	0	0
Ribs	medially thickened/kinked	1	4	3	2	1	2	2	2
	fused	0	1	0	0	0	1	0	0
Sternebrae	bipartite ossified	0	0	0	1	0	0	0	1
	misaligned ossification sites	2	4	4	2	2	3	4	2
	misaligned hemicentres	2	2	2	4	2	2	2	3
Costal cartilage	misaligned	2	2	2	5	2	2	2	4
Total affected by one or more of the above		6	12	8	9	6	8	7	7

In the study report, a statistical analysis was performed on number of affected litters using two-tailed Fisher's exact test. There was no statistical significance ($p>0.05$).

Regarding historical control data, HCD available in the study report as well as an update provided by the applicant at the request of the DS in order to cover a period of 5 years around the date of the study, were taken into account. Although studies using different routes of administration were presented, the DS only included studies conducted by gavage. Therefore, the HCD presented in the DAR/CLH are considered relevant in terms of dates of the study, strain and source of rats, laboratory, route of administration, type of study. HCD included incidences expressed on litter and fetus basis, and the number of litter and foetuses examined was given for each study.

RAC's response
RAC agrees with the DS and German MSCA that no classification neither on sexual function and fertility, nor for developmental effects is warranted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	8
Comment received				
DE-CA supports the proposal that classification for acute toxicity (oral, dermal and inhalation) is not required for metyltetraprole.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	9
Comment received				
DE-CA supports the proposal that classification for skin corrosion/irritation is not required for metyltetraprole.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	10
Comment received				
DE-CA supports the proposal that classification for skin sensitisation is not required for metyltetraprole. Although the LLNA was not conducted, the available GPMT is acceptable.				
Dossier Submitter's Response				
Agreed.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	11
Comment received				
DE-CA supports the proposal that specific target organ toxicity after single exposure relevant for classification of metyltetraprole was not observed. However, neurobehavioural and neuropathological findings observed in the acute neurotoxicity study as well as in the second 90-d study in rats require further consideration. In Vol. 1, section 2.6.2.10.1 (STOT SE), only FOB findings observed in the acute neurotoxicity study are presented. Based on histopathologic examinations of the peripheral nerves of females at the highest dose, minor effects were observed although in some cases the findings are within the range of reported HCD. An overall discussion of other studies should be provided, especially the second 90-d study in rats.				
Dossier Submitter's Response				
In the acute neurotoxicity study, histopathological examination revealed no clear treatment-related effects (Table B.6.7.1.2-9 in Vol 3CA B6 – Annex to the CLH report). Some findings in the peripheral nerve have a higher incidence in the high dose female compared to the concurrent control group, i.e. axonal degeneration at the sciatic notch (1/5F in the control versus 2/5 in the high dose group) and tibial axonal degeneration at knee (0/5F in the control versus 1/5 in the high dose group). The incidence of axonal degeneration at the sciatic notch remains within HCD (0 to 3/5 affected), although the incidence of tibial axonal degeneration at knee is above HCD (0 incidence). Nevertheless, the HCD are quite limited (only 3 studies), the severity is minimal and there is no pattern				

of axonal degeneration in the other areas of sciatic and tibial nerves. There was also no histopathological treatment-related effect in the brain, spinal cord and skeletal muscles in this study. The treatment-relationship of these findings is therefore questionable and are not considered to support a STOT SE classification. Regarding the proposal in the comment to discuss the second 90-d study in rats, the DS does not consider appropriate to use this study for STOT SE classification, since no observation was conducted following a single administration of the test substance and if findings were observed, they could not be attributed to a single exposure. Results of repeated-dose toxicity studies were discussed for a potential STOT RE classification.
RAC's response
RAC agrees with the DS on considering the available acute study not supportive of classification. The outcome of the 90-d study is not relevant to STOT SE classification and should be discussed in the STOT RE section.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	Germany		MemberState	12
Comment received				
Classification for specific target organ toxicity after repeated exposure is not indicated for metyltetraprole.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
09.03.2023	United Kingdom	<confidential>	National Authority	13
Comment received				
Metyltetraprole (ISO) (EC: -; CAS: 1472649-01-6 Relevant bioaccumulation information is presented in the CLH report. However, it is currently unclear if the substance meets the bioaccumulation criteria for hazard classification under CLP. While this does not impact the classification proposal, it would be useful for the DS/RAC to present a conclusion. We note that an OECD TG 229 Fish Short Term Reproduction Assay (Pimephales promelas) study is available resulting in a 21-day NOEC of 0.0092 mg a.s./L (mm) based on mean eggs per female per reproductive day. The study endpoint is considered reliable and we consider it potentially relevant to hazard classification given the endpoint reflect population effects. This endpoint leads to a more stringent hazard classification of Aquatic Chronic 1 with a Chronic M-factor of 10. Additional information is also available for amphibians although this does not appear to impact the hazard classification proposal. We note that chronic data are not available for the most acutely sensitive fish species (Oncorhynchus mykiss). Using the surrogate approach would also result in Aquatic				

Chronic 1 with an M-factor of 10.

Finally, while we recognise it will not impact the hazard classification, we are unclear why the algal growth inhibition study with metyltetraprole is not considered reliable.

Please can the DS provide further information to clarify.

Dossier Submitter's Response

Bioaccumulation

Concerning the bioaccumulation summary of effects are available in Table 2.9.2-1 and information are available in section 3.1.3. $BCF_{KL,TRR}$ ranged from 1076 to 1423 L/kg, according to Regulation (EC) No 1272/2008 an active substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is greater than 2000.
Consequently taking into account the data available, the active substance does not meet the bioaccumulation criteria.

OECD TG 229 Fish Short Term Reproduction Assay (FSTRA)

FSTRA study is a level 3 CF screening test according to the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. Taking into account the specificity of all studies protocole listed in the guidance, such studies for endocrine disruptors assessment should not be used for hazard classification. Indeed for the purposes of these tests, the highest test concentration should be set by the maximum tolerated concentration (MTC) determined from a range finder or from other toxicity data, or 10 mg/L, or the maximum solubility in water, whichever is lowest. The concepts of MTC are then useful for highest concentration selection to be sure to have tested the highest possible concentration without excessive toxicity. Indeed, if endocrine-related adverse effects are only observed in combination to an excessive systemic toxicity it is not possible to consider that the endocrine adversity is indicative of endocrine disruption.
Moreover, the results of this study do not indicate an endocrine activity in Fathead minnows (*Pimephales promelas*) at all tested concentration.

Chronic M-factor

The lowest LC_{50} value obtained for fish is 0.048 mg a.s./L based on *Oncorhynchus mykiss* and no chronic data are available for this species.
The lowest NOEC value obtained for fish is 0.015 for *Pimephales promelas* and acute chronic data is available for this species with an LC_{50} value of 0.061 mg a.s./L (please refer to Table 2.9.2-2).
Based on acute toxicity data LC_{50} values obtained for both fish are in the same range (0.048 mg a.s./L vs 0.061 mg a.s./L), consequently the lowest NOEC value of 0.015 for *Pimephales promelas* could be used as surrogate.

RAC's response

Bioaccumulation

The DS answered by referring to the bioaccumulation criterion (B) classification limit of 2 000 for the bioconcentration factor (BCF) used in PBT hazard classification. RAC notes that for aquatic hazard classification the limit for BCF is 500. RAC is of the opinion the metyltetraprole has potential for bioaccumulation based on the BCF for fish of 526 for the whole fish in the OECD TG 305 test and on the measured log P_{ow} for metyltetraprole of 4.16.

OECD TG 229 Fish Short Term Reproduction Assay (FSTRA)

RAC notes that endocrine disruption per se is of no relevance for aquatic hazard classification according to the current EU system, whereas the observed effects on reproduction (number of eggs) are relevant. However, as the test followed the method of a screening assay, it is only used as supportive information by RAC, and other available long-term tests were considered to be of higher relevance.

Chronic M-factor

RAC agrees with the DS. There are data for all three trophic levels and RAC is of the opinion that this case does not require the approach mentioned on page 505 of the CLP Guidance 'Chronic toxicity data (ECx or NOEC) would normally override acute data for long-term hazard classification. However, when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor.'

Algal growth inhibition study

The DS considered the algae test not reliable for risk assessment because the measured values of the highest concentration dropped below the value of the lowest nominal one. RAC, however, is of the opinion that the study is reliable for hazard classification purposes.

PUBLIC ATTACHMENTS
1. Générations Futures_metyltetraprole.pdf [Please refer to comment No. 5]
2. TST-0100 revised February 2023 (final)_Redacted.pdf [Please refer to comment No. 1]

CONFIDENTIAL ATTACHMENTS
1. TST-0100 revised February 2023 (final).pdf [Please refer to comment No. 1]