Committee for Risk Assessment

RAC

Annex 3
Records
of the targeted consultation following the submission of documents containing additional information on carcinogenicity

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

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

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

The proposal for the harmonised classification and labelling (CLH) of 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 - ; CAS 1472649-01-6) was submitted by the dossier submitter and was subject to a consultation, from 9 January 2023 to 10 March 2023. The comments received by that date are compiled in Annex 2 to the opinion.

After the consultation, eight documents containing additional information on carcinogenicity were submitted to ECHA. These documents were the subject of the ad hoc consultation that was launched from 26 June 2023 to 10 July 2023 and the comments received are listed below.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: 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
Dossier submitter: France

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
10.07.2023	Germany		MemberState	1
Comment received				
The applicant submitted new data for the endpoint carcinogenicity. The newly submitted data comprise: a) the previously incomplete histopathological evaluation of the intermediate dose groups of the two available carcinogenicity studies in rats and mice; b) a histopathological re-evaluation by an external company ("third-party peer review") as well as an "expert panel", which led to changes in the reported tumour incidences; c) a statistical re-evaluation based on the updated incidences. Due to the amount of data submitted and the short time we were given, we were only able to conduct a cursory review, which, however, revealed issues that cast doubt on the appli-cant's approach and the conclusions drawn. The conclusion of the applicant that the tumours found in the studies are not to be consid-ered as treatment-related depends largely on the question of which historical control data are used for comparison and which significance levels are applied for statistical testing. Ac-cording to the applicant's argumentation, the historical control data do not show any drift over time, which is why it uses all available data as a basis for comparison and not only those of a 5-year time window, as the RMS does in the Draft Assessment Report. However, a look at the calculated HCD shows that some of the HCD used by the applicant deviate con-siderably from the HCD used by the RMS, so that the impression of a drift over time is given. It is therefore not sufficiently comprehensible why the applicant deviates from the common practice of using a 5-year time window. The citation given in this context does not refer to an official guidance document, but to a study conducted by industry, which moreover only refers to a single tumour type that did not occur at all in the studies considered here. In ad-dition, the RMS had already noticed in the Draft Assessment Report that the applicant in-cluded data obtained from studies with deviating application routes in its historical control data. In the newly submitted documents, we could not find any evidence that such data were not again included in the HCD used. In summary, for the				

reasons stated above, there is doubt about the validity of the HCD used and, therefore, about the conclusion drawn that the tu-mours observed are not treatment-dependent.
RAC's response
As already reported in the first CLH_RCOM (Annex 2), RAC supports the DS position regarding the historical control data set to be considered: 1) 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. The available experimental evidence overall indicates that the tumours reported in rats and mice are treatment dependent.

Date	Country	Organisation	Type of Organisation	Comment number
10.07.2023	France		MemberState	2
Comment received				
(Please see document (.docx) attached for better display of the tables) Metyltetraprole – Ad hoc consultation 2023-07-10 DS/RMS comments Carcinogenicity potential in mice Several new documents have been provided by the applicant regarding neoplastic findings observed in mice: - Additional histopathological investigations in animals from the low and mid-dose groups (report No TST-0181). - Peer review on selected findings and organs from the carcinogenicity study (report No TST-0178). - Statistical analysis on selected pathology findings from the carcinogenicity study and the additional histopathological investigations, taking also into account results of the peer reviewed histopathological analysis (report No TST-0175) - Position of an expert panel on selected neoplasms (report No TST-0179) - Position paper of the applicant (report No TST-0131) The aim of the additional histopathological investigations (TST-0181) was as follows: "this study was initiated to add histopathological investigation of the spleen, lymph nodes (axillary, submandibular, mesenteric), bone marrow, thymus, liver, lung, and uterus collected from CD-1 mice following treatment at 700 or 2000 ppm" and it was stated in the report that "only tissues from animals terminated at the schedule sacrifice and not examined in VRY0055" (i.e. reference of the mouse carcinogenicity study) were analysed. Regarding the peer reviewed histopathological examination (TST-0178), the following is reported: "All previously diagnosed cases of malignant lymphoma and histiocytic sarcoma were reviewed. Furthermore, spleen, lymph node (axillary, submandibular, mesenteric), bone marrow, thymus, liver, uterus, and lung from all animals underwent Peer Review in order to confirm the absence or presence of systemic neoplasms". The DS noted that a summary table with tumour incidences taking into account: i) the main study; ii) the additional histopathological investigation conducted on animals from the low and mid-dose level; iii) the peer review histopathological analysis was available in the position paper from the applicant and in the report on statistics. However, individual data were not reported and it is quite difficult to follow the reasons for the discrepancies observed with the main study. The DS therefore checked all the provided documents, including the raw data of the main carcinogenicity study. The uncertainties and limitations described below were noted. In the additional histopathological examination, animals killed or dying during the study were not examined (this was already done in the main study). However, it seems that data				

for animals terminated at the schedule sacrifice were (re-)examined. This re-assessment of scheduled terminated animals included those already examined by the main study where 'abnormalities' were found and is in contradiction with the statement in the study TST-0181: "only tissues from animals terminated at the schedule sacrifice and not examined in VRY0055". Therefore, for these animals, it seems that histopathological examination was conducted twice.
However, it is surprising that for some animals, results of this additional histopathological investigation are not consistent with the results obtained in the main study and in the peer review analysis report. For example, for male #120 (mid-dose level), and females #0383 (low dose) and #0405 (mid dose): no histopathological finding was found for hemopoietic system in the additional histopathological investigation, whereas M-lymphoma was noted in the main study and was not invalidated by the peer review analysis.
Example of inconsistencies between the main mouse carcinogenicity study and the additional histopathological investigations for male #120
Main study:
Report TST-0181:
Another example is for female #0426 (mid-dose level), no histopathological finding was found for hemopoietic system or uterus in the additional histopathological investigation, whereas 'Hemopoietic system: M-sarcoma, histiocytic' and 'Uterus: N-sarcoma, histiocytic, metastasis' were reported in the main study and were not invalidated by the peer review analysis.
These discrepancies raised serious doubts on the assessments provided in the different reports.
Another consequence of having re-examined the same animals than in the main study is that all animals identified to show lymphoma in the additional histopathological investigation were already previously identified in the main study (and therefore in the original CLH/DAR). The thorough check of raw data of study reports was therefore essential, as this was not described by the applicant.
In addition, in the position paper of the applicant, it is reported that one additional female of the control group showed malignant lymphoma. The RMS had to check the raw data of the different reports to understand this point: 3 additional females were diagnosed with M-lymphoma (#0314, 0331 ,0350), 1 female (#0301) was not confirmed to bear M-lymphoma and 1 female (#317) was diagnosed with histiocytic sarcoma at peer review and for this female the DS assumed that the malignant lymphoma stated in the main study report was a misdiagnose. No such detailed assessment is available in the different documents and the lack of transparency and clarity in the assessment of the applicant is a critical point.
In addition, the high number of new cases of lymphoma diagnosed after the peer review analysis, particularly in the male and female control groups, seems very doubtful and raised uncertainties in either the first assessment available in the study report or the peer review assessment.
Furthermore, it is not understood why, in the statistical analysis and in the position paper, the number of animals examined in the low and mid-dose males and low-dose females is 50, whereas 51 animals were included in each tested groups.
The DS has only limited time to do this exercise and any other eventual inconsistencies may not have been captured.
For each type of tumour considered treatment-related in the CLH report, the tables below describe the incidences reported in the different reports. These tables are provided by the DS/RMS.
Tumours of the haematopoietic system in mice
Lymphomas:In the additional histopathological investigation, the males and females of the low and mid-dose groups showing lymphoma were already reported in the main study. As noted above, 1 mid-dose male #120, 1 low-dose female #0383 and 1 mid-dose female

#0405 were reported with lymphoma in the main study but not in the additional analysis. This inconsistency was not explained by the applicant.
Following the peer review of histopathological findings, an important number of animals were diagnosed with lymphoma whereas they were not in the initial study, particularly in the control groups in both sexes. There was 2 control males #0013 and 0014 and 3 control females #0314, 0331 and 0350. On the other hand, 2 control females were considered misdiagnosed in the main study, i.e. #0301 consider by the peer review experts as a mistake because malignant lymphoma was included without any organ noted to be affected and #0317 diagnosed with a histiocytic sarcoma instead of a lymphoma. In addition, 2 females from the mid-dose group (#0423 and 0443) were diagnosed with lymphoma in the peer review, not in the main study. The high number of discrepancies between the initial study report and the peer-review analysis questioned about the validity of the histopathological investigations in this study (either initial or peer-review).
The statistical analysis conducted by the applicant in its position paper TST-0131 were conducted considering 50 animals in the low and mid-dose male groups and in the low-dose female groups, whereas the number of animals per groups was 51 and it is not explained why only 50 animals were included in these groups.
Statistical analysis conducted by the DS/RMS are presented in the table.
Finding Dose level (ppm)
Male Female
0 700 2000 7000 0 700 2000 7000
Incidences reported in the main study report
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) 2 - - - p=0.323 - - - p=0.477
Cochran-Armitage trend test (1-sided)1 p=0.1717 p=0.3324
Killed or dying during the study 3/17 4/17 6/13 7/14 4/14 5/12 8/15 5/14
Animal identification 0004WE
0009FD
0033FD 0072FD
0079FD
0084WE
0092WE 0107WE
0111WE
0127WE
0131FD
0134WE
0135WE 0161FD
0168FD
0170WE
0176WE
0178FD
0185WE
0190FD 0302FD

0304WE
0312WE
0315WE
0359FD
0371WE
0392FD
0393FD
0402FD 0404FD
0409WE
0422FD
0429FD
0435FD
0438WE
0439FD
0453WE 0457FD
0459WE
0462WE
0489FD
0499WE
Killed after 78-wk 2/34 2/3 2/4 1/37 4/37 3/4 4/5 4/37
Animal identification 0026
0028 0056
0074 0118
0120 0194 0301
0317
0320
0329 0370
0382
0383 0405
0418
0441
0451 0474
0490
0492
0502
Incidences reported in the additional histopathological investigation (TST-0181)
Killed after 78-wk - 2/34 1/38 - - 2/39 3/36 –
Animal identification – 0056
0074 0118 - - 0370
0382 0418
0441
0451 -
Incidences following the peer review on selected findings and organs (TST-0178)
Differences with the initial analysis +2 - - - +1 - +2 –
Animal identification 0013 a
0014 b 0314 c
0331 d
0350 e
Not confirmed: 0301 f
0317 g 0423 h
0433 i

Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 51 51 51 51 51 51 51 51
Lymphomas 7 6 8 8 9 8 14 9
% 13.7% 11.8% 15.7% 15.7% 17.6% 15.7% 27.5% 17.6%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.723 p=0.500 p=0.500 - p=0.702 p=0.1717 p=0.602
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.327 p=0.318
One-tailed PETO pairwise test (applicant)2 - p=0.4207 p=0.4091 p=0.4368 - p=0.3859 p=0.1404 p=0.4781
One-tailed PETO trend test (applicant)2 p=0.3594 p=0.4745
HCD 5 studies
2014-2018, diet Mean 4.7%
Range 0.0-11.8% Mean 11.4%
Range 0.0-21.6%
1 DS/RMS assessment
2 Applicant assessment – n=50 for the low and mid-dose males and low-dose females without explanations
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report:
a "Animal no. 13: malignant lymphoma (thymus, spleen), was not diagnosed"
b "Animal no. 14: malignant lymphoma (thymus), was diagnosed wrong (atypical hyperplasia is not a tumor entity in CD-1 mice)"
c "Animal no. 314: malignant lymphoma (thymus, spleen, kidney), was diagnosed as hyperplasia in thymus and spleen"
d "Animal no. 331: malignant lymphoma (thymus, spleen), was diagnosed as hyperplasia in thymus and spleen"
e "Animal no. 350: malignant lymphoma (thymus, spleen, lymph nodes, lung), was mentioned as hyperplasia in thymus and increased cellularity in lymph nodes"
f "In animal no. 301, malignant lymphoma was included without any organ noted to be affected. In this case, the tumor was probably entered by mistake"
g "Animal no. 317: histiocytic sarcoma (spleen, lymph nodes, kidney), was not mentioned"
h "Animal no. 423: malignant lymphoma (thymus, lymph nodes), was diagnosed as hyperplasia in thymus"
i "Animal no. 433: malignant lymphoma (thymus, spleen, lymph nodes) was diagnosed as hyperplasia in thymus"
Histiocytic sarcomas
In the additional histopathological investigation, no animals of the low and mid-dose levels killed at the terminal sacrifice were reported to show histiocytic sarcomas. However, for female #0426 (mid-dose level, killed after 78-week and analysed in the main study already), no histopathological finding was found for hemopoietic system or uterus in the additional histopathological investigation, whereas 'Hemopoietic system: M-sarcoma, histiocytic' and 'Uterus: N-sarcoma, histiocytic, metastasis' were reported in the main study and were not invalidated by the peer review analysis. This inconsistency was not explained by the applicant.
Following the peer review of histopathological findings, histiocytic sarcoma was diagnosed in one additional female mouse from the control group #0317 (originally diagnosed with lymphoma).
The statistical analysis conducted by the applicant in its position paper TST-0131 were conducted considering 50 animals in the low and mid-dose male groups and in the low-

dose female groups, whereas the number of animals per groups was 51 and it is not explained why only 50 animals were included in these groups.
Statistical analysis conducted by the DS/RMS are presented in the table.
Finding Dose level (ppm)
Female
0 700 2000 7000
Incidences reported in the main study report
n= 51 16 20 51
Histiocytic sarcomas 1
2% 0
(0%) 3
(15%) 3
6%
Pairwise comparison against control (1-tailed) 2 - - - p=0.300
Cochran-Armitage trend test (1-sided)1 p=0.09714
Killed or dying during the study 1/14 0/12 2/15 1/14
Animal identification 0328WE 0410WE
0414WE 0485WE
Killed after 78-wk 0/37 0/4 1/5 2/37
Animal identification 0426 0467
0479
Incidences reported in the additional histopathological investigation (TST-0181)
Killed after 78-wk - 0/39 0/36 –
Animal identification
Incidences following the peer review on selected findings and organs (TST-0178)
Differences with the initial analysis +1 - - -
Animal identification 0317 a
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 51 51 51 51
2 0 3 3
% 3.9% 0% 5.9% 5.9%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=1 p=0.500 p=0.500
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.167
One-tailed PETO pairwise test (applicant)2 - - p=0.3632 p=0.3121
One-tailed PETO trend test (applicant)2 p=0.1003
HCD 5 studies
2014-2018, diet Mean 1.96%
Range 0.0-5.9%
1 DS/RMS assessment
2 Applicant assessment – n=50 for the low and mid-dose males and low-dose females without explanations
WE: Euthanized for welfare reasons
Peer review report
a "Animal no. 317: histiocytic sarcoma (spleen, lymph nodes, kidney), was not mentioned"
Carcinogenicity potential in rats
Several new documents have been provided by the applicant regarding neoplastic findings observed in rats:
- Additional histopathological investigations in animals from the low and mid-dose groups (report No TST-0180)
- Peer review on selected findings and organs from the carcinogenicity study (report No TST-0177)

- Statistical analysis on selected pathology findings from the carcinogenicity study and the additional histopathological investigations, taking also into account results of the peer reviewed histopathological analysis (report No TST-0176)
- Position of an expert panel on selected neoplasms (report No TST-0179)
- Position paper of the applicant (report No TST-0131)

The aim of the additional histopathological investigations (TST-0180) was as follows: "this study was initiated to add histopathological investigation of the spleen, lymph nodes (axillary, submandibular, mesenteric), bone marrow, thymus, liver, lung, mammary gland and uterus with cervix collected from Han Wistar rats following treatment at 2000 or 6000 ppm" and it was stated in the report that "only tissues from animals terminated at the schedule sacrifice and not examined in VRY0054" (i.e. reference of the rat carcinogenicity study) were analysed.

Regarding the peer reviewed histopathological examination (TST-0177), the following is reported: "All previously diagnosed cases of malignant lymphoma, malignant schwannoma and mammary gland neoplasms from animals scheduled for the 104-Week sacrifice were reviewed. Furthermore, spleen, lymph node (axillary, submandibular, mesenteric), bone marrow, thymus, liver, and lung, and uterus and mammary glands from all animals these animals underwent Peer Review in order to confirm the absence or presence of systemic neoplasms".

The DS noted that a summary table with tumour incidences taking into account

iv) the main study;

v) the additional histopathological investigation conducted on animals from the low and mid-dose level;

vi) the peer review histopathological analysis was available in the position paper from the applicant and in the report on statistics. However, individual data were not reported and it is quite difficult to follow the reasons for the discrepancies observed with the main study. The thorough check of raw data of study reports was therefore essential, as this was not described by the applicant. The DS therefore checked all the provided documents, including the raw data of the main carcinogenicity study. The uncertainties and limitations described below were noted.

In the additional histopathological examination, animals killed or dying during the study were not examined (this was already done in the main study). However, it seems that data for animals terminated at the schedule sacrifice were (re-)examined. This re-assessment of scheduled terminated animals included those already examined by the main study where 'abnormalities' were found and is in contradiction with the statement in the study TST-0180: "only tissues from animals terminated at the schedule sacrifice and not examined in VRY0054". Therefore, for these animals, it seems that histopathological examination was conducted twice.

However, it is surprising that results of this additional histopathological investigation could be not consistent with the results obtained in the main study and in the peer review analysis report. For example, for male #187 (mid-dose level): no histopathological finding was found for spleen in the additional histopathological investigation, whereas 'N-lymphoma, metastasis, originating finding: Hemopoietic system, Lymphoma' was noted in the main study and was not invalidated by the peer review analysis.

Another example is for female #0301 (low-dose level), no histopathological finding was found for mammary gland in the additional histopathological investigation, whereas mammary gland adenoma was reported in the main study and was not invalidated by the peer review analysis. The same contradiction is noted for females #0485 and #500 (mid-dose level), reported with mammary adenocarcinoma in the main study and the mention "No histopathology findings" in the additional investigations.

These discrepancies raised serious doubts on the assessments provided in the different reports.

No detailed assessment is available in the different documents and the lack of transparency and clarity in the assessment of the applicant is a critical point.
The DS has only limited time to do this exercise and any other eventual inconsistencies may not have been captured.
For each type of tumour considered treatment-related in the CLH report, the tables below describe the incidences reported in the different reports. These tables are provided by the DS/RMS.
Malignant lymphomas in rats
In the additional histopathological investigation, one male of the mid-dose group (#0187) was shown to present malignant lymphoma and was already reported in the main study.
Following the peer review of histopathological findings, 1 male of the low dose group (#0033) and 1 male of the high dose group (#132) were diagnosed with lymphoma in the peer review, not in the main study. In females, one female from the high dose group (#429) seems to have been misdiagnosed in the main study. According to the peer review experts, malignant lymphoma was included without any organ noted to be affected and they considered that this was probably entered by mistake. The DS/RMS considers this assumption as an uncertainty.
Statistical analysis conducted by the DS/RMS are presented in the table, in addition to the one conducted by the applicant.
Finding Dose level (ppm)
Male Female
0 2000 6000 20000 0 2000 6000 20000
Incidences reported in the main study report
n= 50 20 17 50 50 14 16 50
Malignant lymphoma 0 0 3
(18%) 3
6% 0 0 1
(6%) 2
4%
Pairwise comparison against control (1-tailed) 2 - - - p=0.132 - - - p=0.234
Cochran-Armitage trend test (1-sided)1 p=0.02639 p=0.06442
Killed or dying during the study 0/14 0/20 2/16 3/15 0/17 0/14 1/16 1/20
Animal identification 0176FD
0188WE 0130WE
0142WE
0146WE 0497WE 0438WE
Killed after 104-wk 0/36 0/0 1/1 0/35 0/33 0/0 0/0 1/30
Animal identification 0187 0429
Incidences reported in the additional histopathological investigation (TST-0180)
Killed after 104-wk - 0/30 1/34 - - 0/36 0/34 –
Animal identification 0187
Incidences following the peer review on selected findings and organs (TST-0177)
Differences with the initial analysis - +1 - +1 - - - -1
Animal identification 0033 a 0132 a 0429 b
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 50 50 50 50 50 50 50 50
Malignant lymphoma 0 1 3 4 0 0 1 1
% 0% 2% 6% 8% 0% 0% 2% 2%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.500 p=0.121 p=0.058 - - p=0.5 p=0.5
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.012 p=0.102

One-tailed PETO pairwise test (applicant)2 - p=0.1401 p=0.0409 p=0.0146 - - p=0.1587 p=0.1515
One-tailed PETO trend test (applicant)2 p=0.0102 p=0.1314
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
2 Applicant assessment
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report
a "Leukemia in animals #0033 and #0132 changed to lymphoma, LGL-type";
b "In animal no. 429, malignant lymphoma was included without any organ noted to be affected. In this case, the tumor was probably entered by mistake".
Malignant uterine schwannomas in rats
In the additional histopathological investigation, no animals of the low and mid-dose levels killed at the terminal sacrifice were reported to show histiocytic sarcomas.
Following the peer review of histopathological findings, 2 females of the high dose group (out of the 3 initially diagnosed with malignant schwannoma in the uterus) were considered to have been misdiagnosed in the original study. The peer review experts concluded that malignant schwannomas were rather found in the abdominal cavity (large mass adjacent to the uterus) for female #0404 and in the uterine cervix ('cervix' with metastasis in the vagina) for female #0429.
The DS/RMS notes that malignant schwannomas are tumors originated from nerve sheath and can arise from different organs. It could therefore be considered appropriate to combine malignant schwannomas from different organs, especially because in females treated with metyltetraprole, reproductive organs are especially affected.
Statistical analysis conducted by the DS/RMS are presented in the table, in addition to the one conducted by the applicant. The DS/RMS conducted also statistics on combined incidence of malignant schwannomas.

Finding Dose level (ppm)
Female
0 2000 6000 20000
Incidences reported in the main study report
n= 50 24 22 50
Uterus: M-schwannoma, malignant 0 1 (4%) 0 3 6%
Pairwise comparison against control (1-tailed) 2 - - - p=0.110
Cochran-Armitage trend test (1-sided)1 p=0.05432
Killed or dying during the study 0/17 1/14 0/16 2/20
Animal identification 0324WE 0404FD 0433FD
Killed after 104-wk 0/33 0/10 0/6 1/30
Animal identification 0429
Incidences reported in the additional histopathological investigation (TST-0180)
Killed after 104-wk - 0/36 0/34 –
Animal identification
Incidences following the peer review on selected findings and organs (TST-0177)
Differences with the initial analysis - - - -2
Animal identification 0404 (abdominal cavity) a 0429 (cervix) b.

Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 50 50 50 50
Uterus: M-schwannoma, malignant 0 1 0 1
0% 2% 0% 2%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.500 p=1 p=0.500
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.2625
One-tailed PETO pairwise test (applicant)2 - p=0.1685 - p=0.1539
One-tailed PETO trend test (applicant)2 p=0.2119
Uterus: HCD 4 studies
2014-2018, diet
[HCD not available for other organs] 0%, 0%, 0%, 0%
Mean: 0.0%; range: 0-0%
Uterine cervix: M-schwannoma, malignant 0 0 0 1 (#0404FD)
Abdominal cavity: M-schwannoma, malignant 0 0 0 1 (#0429)
Ovary: M-schwannoma, malignant 0 0 1 (#0496FD) 0
Combined incidence of M-schwannoma, malignant 0 1 1 3
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.500 p=0.500 p=0.121
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.0342
1 DS/RMS assessment
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report
a " Malignant schwannoma in female no. 404: uterus and vagina are not affected. This tumor is from an uncertain location but was present as a large mass adjacent to the uterus, and hence is considered a malignant schwannoma from the abdominal cavity during the PR".
b " Malignant schwannoma in female no. 429: uterus has an endometrial stromal polyp (Figure 14). The mass diagnosed as a malignant schwannoma is not present in the uterus but is located in the cervix and with invasion into the vagina. It should, therefore, be reported under 'cervix' with metastasis in the vagina"
Mammary tumors in rats
In the additional histopathological investigation, no animals of the low and mid-dose levels killed at the terminal sacrifice were reported to show mammary tumours.
However, for female #0301 (low-dose level, killed after 104-week and analysed in the main study already), no histopathological finding was found for mammary gland in the additional histopathological investigation, whereas mammary adenoma was reported in the main study and was not invalidated by the peer review analysis. Similarly, females #0485 and #500 (mid-dose level) were reported with mammary adenocarcinoma in the main study and the mention "No histopathology findings" was noted in the additional investigations. These inconsistencies were not explained by the applicant.
Following the peer review of histopathological findings, mammary gland adenoma was diagnosed in one additional female rat from the low dose group #0343 (originally diagnosed with fibroadenoma).
Statistical analysis conducted by the DS/RMS are presented in the table, in addition to the one conducted by the applicant.
Finding Dose level (ppm)
Female
0 2000 6000 20000
Mammary adenoma Incidences reported in the main study report
n= 50 39 41 50

Mammary adenoma 1 1 2 4
8%
Pairwise comparison against control (1-tailed) 2 - - - p=0.180
Cochran-Armitage trend test (1-sided)1 p=0.06233
Killed or dying during the study 0/17 0/14 2/16 4/20
Animal identification 0471WE
0487WE 0402FD
0410WE
0425WE
0438WE
Killed after 104-wk 1/33 1/25 0/25 0/30
Animal identification 0351 0301
Incidences reported in the additional histopathological investigation (TST-0180)
Killed after 104-wk - 0/36 0/34 –
Animal identification
Incidences following the peer review on selected findings and organs (TST-0177)
Differences with the initial analysis - +1 - -
Animal identification 0343 a
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 50 50 50 50
Mammary adenoma 1 2 2 4
% 2% 4% 4% 8%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.500 p=0.500 p=0.181
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.085
One-tailed PETO pairwise test (applicant)2 - p=0.3050 p=0.1949 p=0.0885
One-tailed PETO trend test (applicant)2 p=0.0968
HCD 4 studies
2014-2018, diet 0%, 0%, 3.8%, 3.8%
Mean: 1.9%; range: 0-3.8%
Mammary adenocarcinoma Incidences reported in the main study report
n= 50 39 41 50
Mammary adenocarcinoma 4 1 3 7
14%
Pairwise comparison against control (1-tailed) 2 - - - p=0.236
Cochran-Armitage trend test (1-sided)1 p=0.1053
Killed or dying during the study 1/17 1/14 1/16 4/20
Animal identification 0366WE 0334WE 0487WE 0402FD
0403WE
0417WE
0426WE
Killed after 104-wk 3/33 0/25 2/25 3/30
Animal identification 0355
0379
0382 0485
0500
0414
0429
0431
Incidences reported in the additional histopathological investigation (TST-0180)
Killed after 104-wk - 0/36 0/34 –

Animal identification
Incidences following the peer review on selected findings and organs (TST-0177)
Differences with the initial analysis - - - -
Animal identification
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 50 50 50 50
Mammary adenocarcinoma 4 1 3 7
% 8% 2% 6% 14%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.972 p=0.782 p=0.262
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.093
One-tailed PETO pairwise test (applicant)2 - p=0.0764 p=0.3483 p=0.1469
One-tailed PETO trend test (applicant)2 p=0.212
HCD 4 studies
2014-2018, diet 6%, 7.7%, 17.3%, 23.1%
Mean: 13.5%; range: 6-23.1%
Mammary adenoma AND adenocarcinoma Incidences reported in the main study report n= 50 39 41 50
Mammary adenocarcinoma 5 2 5 11
22%
Cochran-Armitage trend test (1-sided)1 p=0.02357
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs
n= 50 50 50 50
Mammary adenoma AND adenocarcinoma 5 3 4 10
Animal #0487 showed both adenoma and adenocarcinoma Animal #0402 showed both adenoma and adenocarcinoma
% 10% 6% 8% 20%
DS/RMS: Fisher pairwise comparison against control (1-tailed) 1 - p=0.866 p=0.756 p=0.131
DS/RMS: Cochran-Armitage trend test (1-sided)1 p=0.053
HCD 4 studies
2014-2018, diet 6%, 11.5%, 17.3%, 26.9%
Mean: 15.4%; range: 6-26.9%
1 DS/RMS assessment
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report
a "Adenoma in mammary gland in female no. 343: one fibroadenoma changed to adenoma"

Overall conclusion on rat and mouse carcinogenicity
OThe DS/RMS considers that the new data provided by the applicant do not change the initial assessment provided by the DS/RMS in the CLH report/vol 1 of the DAR.
As detailed above, some inconsistencies were reported in the different documents regarding tumor incidences in the original study report and in the additional histopathological investigation and peer review histopathology reports. The assessment provided also lacks of transparency. There are full disagreement for low and mid doses in the additional histopathology examination which raises concerns (as shown above in details).
In addition, important discrepancies were noted between the incidences of some tumours reported by the original pathologist in the study report and by the peer-reviewers pathologists. This gives rise to uncertainties and doubts in the various assessments

provided by the applicant. In conclusion on the additional peer review, DS/RMS has concern on the methodology followed. Post hoc examination of data is necessarily biased, since the new panel will likely understand that there are existing doubts from the applicant point of view and be more subject to selection bias. It is also unclear why such post hoc peer review was undertaken at late stages in the process if applicant had already doubts at the termination of the main study.
Regarding statistics, the DS/RMS would like to recall that statistical analysis is only a line of evidence in an overall weight of evidence approach. Of course, biological significance should still be considered and comparison to concurrent control group (and to HCD, if appropriate) is taken into account.
In any case, DS/RMS still considers to observe treatment-related trends for some histopathological data, based on the additional statistics performed (as shown above in details). Furthermore, based on TK data (see below) there are demonstrated issues of oral exposure of the animals at the high dose, which likely jeopardizes a full appreciation of the dose-response, the latter may be minimized by the absence of proportional increase of Cmax/AUC compared to the increase of oral dose. Based on the additional data, observed trends confirmed and considering the aforementioned uncertainties, RMS maintains its initial appreciation of carcinogenicity data.
Additional considerations on toxicokinetics (TK)
In the 2-year rat study, blood samples were obtained from the toxicity phase animals on weeks 4, 13, 26 and 52 from 4 males and 4 females per group (please refer to Vol 3CAB6, B.6.5.1, pages 204-206). Metyltetraprole was analysed using a LC-MS/MS bioanalytical method that was considered validated by the DS/RMS.
Maximum plasma concentration (Cmax) of metyltetraprole and areas under the mean plasma concentration-time curves estimated over a 24-hour interval (AUC24) are reported in the tables below.
Dose
Level
(ppm) Cmax (ng/mL) (mean ± SD)
Week 4 Week 13 Week 26 Week 52
Males Females Males Females Males Females Males Females
2000 34.4 138 15.3 63.2 14.8 96.3 28.6 64.7
+ 5.5 + 34 + 3.0 + 9.3 + 3.7 + 48.9 + 30.2 + 11.5
6000 129 320 28.8 137 23.6 144 25.2 142
+ 139 + 212 + 10.1 + 34 + 6.7 + 57 + 9.0 + 38
20000 156 260 40.6 156 36.1 146 43.9 231
+ 76 + 55 + 4.4 + 53 + 3.6 + 37 + 5.6 + 158
Dose
Level
(ppm) AUC24 (ng.h/mL) (mean ± SD)
Week 4 Week 13 Week 26 Week 52
Males Females Males Females Males Females Males Females
2000 668 2710 316 1290 301 1490 379 1270
+ 71 + 650 + 63 + 170 + 72 + 360 + 171 + 180
6000 1630 4990 621 2910 508 2600 529 2890
+ 920 + 1720 + 228 + 720 + 152 + 460 + 236 + 590
20000 2280 4830 885 2990 735 2680 902 3700
+ 290 + 1490 + 106 + 680 + 42 + 320 + 123 + 1690
The relationships between the Cmax of metyltetraprole, AUC24 and achieved dietary intake during Week 4, 13, 26 and 52 are presented below:
Dose
Level

(ppm) Achieved intake ratio
Week 4 Week 13 Week 26 Week 52
Males Females Males Females Males Females Males Females
2000 1 1 1 1 1 1 1 1
6000 2.3 3.1 3.0 3.1 3.0 3.1 2.9 3.2
20000 10.1 10.2 10.5 10.8 10.7 10.6 10.7 10.9
Dose
Level
(ppm) Cmax ratio
Week 4 Week 13 Week 26 Week 52
Males Females Males Females Males Females Males Females
2000 1 1 1 1 1 1 1 1
6000 3.8 2.3 1.9 2.2 1.6 1.5 0.9 2.2
20000 4.5 1.9 2.7 2.5 2.4 1.5 1.5 3.6
Dose
Level
(ppm) AUC24 ratio
Week 4 Week 13 Week 26 Week 52
Males Females Males Females Males Females Males Females
2000 1 1 1 1 1 1 1 1
6000 2.4 1.8 2.0 2.3 1.7 1.7 1.4 2.3
20000 3.4 1.8 2.8 2.3 2.4 1.8 2.4 2.9
The following conclusions can be drawn
- Non-linearity of systemic exposure is obvious after administration of metyltetraprole in rats for 4, 13, 26 and 52 weeks. Indeed, increase of Cmax and AUC24 is not proportional with the dose.
The Cmax and AUC24 values in male and female rats were approximately 35% and 76% lower than those values predicted from a linear relationship at the mid and high nominal dietary concentrations respectively. As stated in the TK report, there was evidence of statistically significant non-proportionality ($p<0.001$).
Especially in females, the Cmax and AUC24 values are the same both at 6000 ppm and at 20000 ppm on weeks 13 and 26, or even lower at 20000 ppm than at 6000 ppm on week 4.
- The Cmax and AUC24 values of both sexes were lower during weeks 13, 26 and 52 than during week 4. These differences were statistically significant ($p<0.001$).
- There was gender differences. The Cmax and AUC24 values of females were approximately 2.4-fold higher than those in males during week 4 and approximately 3.3-fold higher than those in males during weeks 13, 26 and 52. These differences were statistically significant ($p<0.039$).

Consequences of the TK profile of metyltetraprole on the assessment of carcinogenicity studies:
The non-linearity of the internal exposure suggests a decreased in absorption rate when the administered dose increased. This was substantiated by the oral absorption values derived from the ADME studies where the oral absorption value was 64-73% at the low dose of 1 mg/kg bw and 1.3-2.1% at the high dose of 1000 mg/kg bw.
In the carcinogenicity study in rats, the overall mean achieved doses were 100.6/132.1, 301/403 and 1059/1373 mg/kg bw/d for males/females receiving 2000, 6000 and 20000 ppm respectively for the 52-week treatment period and 83.9/111.8, 255/339 and 852/1190 mg/kg bw/d for the 104-week treatment period.
The interpretation of the neoplastic findings observed in the carcinogenicity should therefore be conducted with care. Since the systemic exposure to metyltetraprole did not

increase proportionally with the dose or even did not increase at all between 6000 and 20000 ppm, neoplastic lesions that occurred with the same or lower incidence in the high dose group compared to the mid dose group could also be considered treatment-related. In this case, the usefulness of statistical trend tests is limited.
Uncertainties
- The rate and extent of systemic exposure is unknown after the first year of treatment since only animals of the toxicity phase (up to 52-week) were analysed.
- TK investigations were not conducted in the carcinogenicity study in mice. No analysis was also performed in the other study available in mice, i.e. the 90-day mouse study. There is however no reason to think that the TK profile would be different from that observed in rats. To note that in dogs (90-day and 1-year studies), non-proportionality of systemic exposure was also reported.
- Only metyltetraprole was analysed in the TK study. It is therefore unknown whether a metabolite, potentially toxic too, were major. However, the same profile was identified in the ADME study where total radioactivity was analysed. Indeed, there was also a non-linearity observed in the systemic exposure: systemic exposure of rats to radioactivity increased with increasing dose over the dose range 1 to 1000 mg/kg bw, however, these increases were less than the proportionate dose increment and the plasma Cmax and AUCt values at the highest dose level were 30-46 times higher than those at the low dose level and approx. 96% lower than those predicted from a linear relationship.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Metyltetraprole ad-hoc consultation 2023-07_v3.docx

RAC's response

RAC acknowledges the thorough and detailed examination made by the DS on the new documents provided by the Applicant regarding the carcinogenicity studies.
RAC agrees with the DS that the supplementary information does not provide substantial elements that can modify the former evaluation. The overall evaluation performed by the DS and supported by further statistical analyses indicate that the findings reported in the carcinogenicity studies conducted both in rats and in mice should be regarded as biologically relevant and treatment-related.

The considerations of the DS on the toxicokinetic measurement performed in the 2-year study in rats add further relevant information that can be useful in the interpretation of the carcinogenicity data.

The reported non-linearity of the relationship between the administered dosage and the internal exposure, indicating a decreased absorption of the test material at higher dose levels, suggests caution in the interpretation of carcinogenicity data and provides a possible explanation to the fact that a clear dose response trend was not always observed.

CONFIDENTIAL ATTACHMENTS
1. Metyltetraprole ad-hoc consultation 2023-07_v3.docx [Please refer to comment No. 2]