Committee for Risk Assessment

RAC

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

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14 March 2024

CLH-O-0000007431-80-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 March 2024** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical 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**

Rapporteur, appointed by RAC: **Gabriele Aquilina**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

Administrative information on the opinion

France has submitted on **9 December 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 January 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 March 2023**.

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposals, RAC opinions and potential Annex VI entries if agreed by the Commission.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	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	-	1472649-01-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=1	
RAC opinion	TBD	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	-	1472649-01-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=1	
Resulting Annex VI entry if agreed by COM	TBD	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	-	1472649-01-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Metyltetraprole is a new active substance for plant protection purposes. It is a fungicide to be used in agriculture and horticulture. In the first application for the European market, the intended representative uses are winter and spring wheat and barley, as well as cucumber.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification was proposed for physical hazards.

Metyltetraprole is solid. Consequently, the Dossier Submitter (DS) concluded that the following hazard classes are considered not to be applicable for metyltetraprole: flammable gases (including chemically unstable gases), oxidising gases, gases under pressure, flammable liquids, pyrophoric liquids, and oxidising liquids. No classification for the other hazard classes was based on the following:

Explosives

Metyltetraprole was tested for explosive properties using EC Method A.14 and was found not to be explosive. However, the EC Method A.14 is not sufficient on its own to conclude on explosive properties. The DS concluded that the substance does not contain chemical groups with explosive properties and no classification is warranted.

Flammable solids

The A.10 test (Comb, A.L. 2016d) result showed that the substance is not highly flammable, and no classification is warranted.

Self-reactive substances

The substance does not contain chemical groups associated with explosive or self-reactive properties (Tables A6.1 and A6.3 in Appendix 6 of UN RTDG) and no classification is warranted.

Pyrophoric solids

According to the DS experience in manufacture or handling shows that the substance does not ignite spontaneously in contact with air at normal temperatures and no classification is warranted.

Self-heating substances

The DS considers that self-heating test is not considered as the melting point (130-134 °C) is lower than 160 °C and no classification is warranted.

Substances which in contact with water emit flammable gases

Based on handling of the substance it is not expected to emit flammable gases in contact with water. No classification is warranted.

Oxidising solids

The chemical structure contains oxygen and chlorine, but these elements are bond to carbon only. No classification is warranted.

Organic peroxides

The hazard class if not applicable as the substance does not contain peroxides.

Corrosive to metals

The melting point of the substance is 130-134 °C. No classification is warranted because the melting point is above the cut-off criteria of 55 °C.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

Comparison with the criteria

On the basis of the proposed classification by the DS, RAC concluded **no classification is warranted for**:

- **hazard class of explosives**. However, RAC disagrees with the DS conclusion on metyltetraprole not containing functional groups associated with explosive properties (Table A6.1 in Appendix 6 of UN RTDG). There is no data available to conclude if the screening criteria (CLP Annex I, 2.1.4.3 (a-c)) are fulfilled. According to the CLP Regulation (Annex I, 2.1.2.3), explosive properties are tested using UN test series 2 to 8. Corresponding UN test result was not available. Consequently, RAC concludes that data is not sufficient for classification.

- **flammable solid**. The A.10 test result showed that the substance is not highly flammable which complies with the ECHA Guidance R.7a.

- **self-reactive substance**. However, RAC disagrees with the DS conclusion that there are no chemical groups present in the molecule associated with self-reactive properties. Metyltetraprole contains functional groups associated with explosive properties or functional groups indicating self-reactive properties. There is no data available to assess if the screening criteria is fulfilled (CLP Annex I, 2.8.4.2). According to the CLP Regulation, self-reactive properties are tested using UN test series A to H. Since no corresponding UN test results are available and the substance contains the above-mentioned groups, it cannot be excluded that the substance has self-reactive properties. RAC concludes that data is not sufficient for concluding on classification.

- **pyrophoric solid**. Experience in manufacture or handling shows that the substance does not ignite spontaneously in contact with air at normal temperatures (CLP Annex I, 2.10.4.1).

- **self-heating substance**. The melting point (130-134 °C) is lower than 160 °C (CLP guidance 2.11.4.2).

- **substance which in contact with water emits flammable gases**. Based on handling of the substance it is not expected to emit flammable gases in contact with water (CLP Annex I, 2.12.4.1 (b)).

- **oxidising solid**. The chemical structure contains oxygen and chlorine, but these elements are bonded to carbon only (CLP Annex I (2.14.4.1 (b)).

- **organic peroxide**. There is no chemical peroxide group present in the chemical structure (CLP Annex I (2.15.1)).

- **corrosive to metals**. The melting point of the substance is 130-134 °C. No classification is warranted because the melting point is above the cut-off criteria of 55 °C (CLP Guidance 2.16.4.1).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

One study is available for oral acute toxicity. The test substance, metyltetraprole, was suspended in 0.5 % (w/v) methyl cellulose (aqueous), the stability of test compound was confirmed by analysis prior to, and after dosing. In particular, for the oral study the stability at 2-250 mg/mL in 0.5 % methylcellulose for 14 days under refrigeration followed by 24 hours at room temperature was also confirmed. The suspension was administered to a group of 6 female rats by oral gavage at a dose level of 2000 mg/kg bw. Since no mortality was observed in the study, the oral LD_{50} is >2000 mg/kg bw, therefore no classification is required according to Regulation 1272/2008.

Acute dermal toxicity

One study is available for acute dermal toxicity. The test substance was moistened with 0.4 mL of water, an amount sufficient to allow good contact with skin. The stability of the test compound was confirmed by analysis prior to, and after dosing. Then, the substance was administered to the shaved skin of 5 male and 5 female rats at 2000 mg/kg bw and held in place by an occlusive dressing for 24 hours. The observation period was 14 days post-exposure. No mortality was observed in the rat study. The LD_{50} of the test substance in rats was found to be greater than 2000 mg/kg bw, therefore no classification is required according to Regulation 1272/2008.

Acute inhalation toxicity

One study is available for acute inhalation toxicity. A nose-only inhalation exposure study was performed in male and females WIST rats at 2.52 mg/L (the maximum attainable concentration). The test substance atmosphere was generated by the turntable of a dust feeder. The stability of test compound was confirmed by analysis prior to, and after dosing. Air concentrations were determined 5 times gravimetrically and 4 times by chemical analysis (method for glass filters TSA-0031). No mortality was observed. Under the conditions of this study the rat acute inhalation 4-hour nose only LC_{50} of metyltetraprole was >2.52 mg/L both in males and females. It should be noted that the concentration was below the upper concentration for classification as acute tox 4 (5 mg/L). Based on the results of this study, no classification is required according to Regulation 1272/2008.

Comments received during consultation

During the consultation, one comment was received from one MSCA, supporting the proposal that classification of metyltetraprole for acute oral, dermal and inhalation toxicity is not required.

Assessment and comparison with the classification criteria

The cut-off value of acute oral toxicity is 2000 mg/kg bw. The LD_{50} of metyltetraprole is >2000 mg/kg bw (oral administration) and therefore, according to CLP criteria, no classification for acute oral toxicity is warranted.

The cut-off value of acute dermal classification is 2000 mg/kg bw. The dermal LD_{50} of metyltetraprole in rats is greater than 2000 mg/kg bw (dermal administration), therefore according to CLP criteria, no classification for acute dermal oral toxicity is warranted.

The cut-off value of acute inhalation toxicity is 5 mg/L. The LC_{50} of metyltetraprole is >2.52 mg/L (the maximum attainable concentration) and therefore, according to CLP criteria, no classification for inhalation toxicity is required.

Overall, RAC concludes that **no classification is warranted for acute oral, dermal and inhalation toxicity.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

In acute oral, dermal and inhalation toxicity studies, there was no evidence of specific target organ toxicity (see also acute toxicity section).

In an acute neurotoxicity study, rats were dosed orally (by gavage) with 500, 1000 and 2000 mg/kg bw of metyltetraprole and observed for 14 days. No mortality was observed. From the dose level of 1000 mg/kg bw slight effects were noted:

- increased incidence of tremors in males, above historical control data (HCD) incidence on Day 15 but with no clear dose relationship;
- increased landing foot splay in males on Day 8, above HCD.

The DS concluded that there was no evidence from the single exposure studies to trigger a classification of metyltetraprole for specific target-organ toxicity Category 1, 2 or 3.

Comments received during consultation

One comment was received during the consultation by one MSCA.

The MSCA was in agreement with the DS on no classification for STOT SE of the test substance, considering the slight incidences and transient nature of the findings reported in the acute neurotoxicity study in rats. MSCA also requested the DS to clarify why the data of a 90-day study in rats was not considered for the classification as STOT SE. The DS did not consider appropriate the use of data from a 90-day study for STOT SE classification and confirmed that such data were considered for STOT RE classification.

Assessment and comparison with the classification criteria

According to CLP criteria, specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed.

STOT-SE Categories 1 and 2 are assigned on the basis of clear evidence of significant or severe toxicity to a specific target organ arising from a single exposure to a substance. STOT SE Category 3 is assigned for the transient effects of respiratory tract irritation and narcotic effects.

In the acute neurotoxicity study, there were neurotoxic effects (tremors, landing foot splay) observed at 1000 mg/kg bw, a dose level that would be relevant for Category 2 classification (the guidance value range for Category 2 is 2000≥C>300 mg/kg bw for oral exposure in rat). However, although a treatment-relationship cannot completely be excluded for these effects, given the slight incidences and transient nature of these findings, a classification is not warranted. According to the Guidance on the Application of the CLP Criteria' (2017), Category 3 covers

'transient effects' occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE). The transient effects reported in the study cannot be associated with respiratory tract irritation nor to narcotic effects. Therefore, the requirements for Category 3 are not fulfilled.

Therefore, RAC concludes that **no classification is warranted for STOT SE** (in agreement with the DS proposal).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation based on a study performed according to OECD 404 in rabbit and a 28-day repeat dose dermal toxicity study performed in Han Wistar rat according to OECD 410.

In the first study, 3 male New Zealand White rabbits received dermal treatments with 0.5 g of the test substance moistened with 0.4 mL of distilled water for 4 hours under semi-occlusive conditions. At the end of the exposure period, the patches were removed from the skin and the treated areas were wiped with absorbent cotton dipped in tap water to clean off any remaining test substance. The irritation reactions were observed 1, 24, 48 and 72 hours after removal of the test substance.

No signs of ill health or toxicity were observed in any animals during the experimental period. Skin irritation reactions were not observed in any animals during the observation period of 72 hours after the removal of the patches.

In the second experiment (28-day repeat dose dermal toxicity study in rats) the test substance was administered via the dermal route to 10 male and female Han Wistar rats for each group, at constant dose levels of 0, 100, 300 and 1000 mg/kg bw/d 6 hours/ d for 28 days. The control group was sham dosed, using approximately 0.2 mL of purified water. There was no indication that metyltetraprole caused irritation at the dermal application sites in treated rats.

Based on these data the DS concluded that metyltetraprole is not irritating to the skin.

Comments received during consultation

One comment was received in the consultation by a MSCA that supported the non-classification proposed by DS.

Assessment and comparison with the classification criteria

As there is no evidence of skin irritation in the relevant studies, RAC concludes that **classification as skin corrosive or skin irritant is not warranted** (in agreement with the DS proposal).

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

One eye irritation study on metyltetraprole performed in rabbits according to EC No 440/2008 is available. Three animals were used for the unwashed group, and three for the washed group. In

the unwashed group, a volume of 0.1 mL (0.060 g) of the test substance was applied to one eye of each rabbit. In the washed group, the test substance was applied in a similar manner to the unwashed group, except that the treated eyes were irrigated for 30 seconds with saline 30 seconds after application of the test substance. In the unwashed group, redness (grade 1) and chemosis (grade 1) in the conjunctiva were observed in 2 out of 3 rabbits after application. In 1 out of 3 rabbits, discharge (grade 1) in the conjunctiva was observed after application. These reactions disappeared 48 hours after application. In the washed group, redness (grade 1) and chemosis (grade 2) in the conjunctiva in 1 out of 3 rabbits were observed after application. These reactions disappeared 48 hours after application. Based on the results of the unwashed group metyltetraprole was minimally irritating to the rabbit eye. DS concluded that no classification for serious eye damage/eye irritation is warranted for metyltetraprole.

Comments received during consultation

No comments were received in the consultation.

Assessment and comparison with the classification criteria

According to CLP criteria, substances are classified as irritating to eyes (Category 2) if, when applied to the eye of an animal, it produces at least in 2 of 3 tested animals, a positive response of:

- corneal opacity $\geqslant$ 1 and/or
- iritis $\geqslant$ 1, and/or
- conjunctival redness $\geqslant$ 2 and/or
- conjunctival oedema (chemosis) $\geqslant$ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

In the available study, mean scores did not meet the criteria for classification and metyltetraprole is considered not to be an eye irritant.

RAC concludes that, **no classification for serious eye damage/eye irritation is warranted** for metyltetraprole (in agreement with the DS proposal).

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No data was available in the CLH-dossier.

Comments received during consultation

No comments were received in the consultation.

Assessment and comparison with the classification criteria

RAC concludes **no classification is warranted on respiratory sensitisation** due to lacking data.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

For skin sensitisation one study was available performed on guinea pigs (Guinea Pig Maximization Test, GPMT) according to OECD TG 406. Forty animals were used in the main study (10 animals in the metyltetraprole control group, 20 in the metyltetraprole sensitized group, 5 animals in the α-Hexylcinnamaldehyde (HCA) control group, and 5 animals in the HCA sensitized group). Based on the dose-finding study, the concentrations of the test substance in the main study were selected to be 5 % for the first induction (intradermal injection) and 50 % for the second induction (topical application) as a mildly irritating and maximum concentration, and 2.5 % for the challenge as a maximum non-irritating concentration. Metyltetraprole was found to be non-sensitiser in the maximisation assay.

Comments received during consultation

One comment by a MSCA was received in the consultation.

MSCA supported the proposal that classification for skin sensitisation is not required for metyltetraprole. The available GPMT assay on guinea pig was considered sufficient to conclude on skin sensitisation although the LLNA assay was not conducted.

Assessment and comparison with the classification criteria

As none of the tested animals showed a positive response in the guinea pig maximisation test, according to CLP criteria classification as skin sensitiser is not applicable.

RAC concludes that **no classification for skin sensitisation is warranted** for metyltetraprole (in agreement with the DS proposal).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Two 13-week oral (diet) and a 28-day dermal toxicity studies have been provided in the rat. 13-week and 52-week oral (capsules) toxicity studies have been provided in the dog. A 13-week oral (diet) toxicity study has been provided in the mouse. Moreover, the effects after repeated exposure observed in reproductive or long-term studies were also considered.

The liver (rat, mouse, and dog), kidneys, thymus, lungs, heart (rat) and haematological system (rat, dog) were identified as the target organs/systems following oral administration of metyltetraprole.

The studies assessed by the DS are the following:

- In the rat, following 13-week dietary dosing, there were findings in the liver and kidneys, as well as in the haematological parameters, at 20000 ppm (equivalent to 1508 mg/kg bw/d in males and 1715 mg/kg bw/d in females). Liver weights were high in both sexes and associated with histopathological findings (centrilobular hypertrophy) in the males. In the kidneys, there were eosinophilic droplets accumulation in the proximal convoluted tubule epithelium in 7 males out of 10. Immunohistochemistry examination of a number of these animals revealed α2μ-globulin accumulation. Although this finding was

considered male rat-specific and not relevant to humans, it should be taken into account in the NOAEL setting. Increased white blood cell counts, due to an increase in lymphocyte counts, were considered treatment-related and adverse in both sexes. The NOAEL for short-term exposure in the rat was 6000 ppm (equivalent to 438 mg/kg bw/d in males and 509 mg/kg bw/d in females).

- In the 13-week rat dietary study, the liver was also considered a target organ and in addition, other target organs were identified at the LOAEL of 20000 ppm (equivalent to 1609 mg/kg bw/d in males and 1769 mg/kg bw/d in females). Adverse effects on thymus (decreased weight and cysts in females), lungs (inflammatory cell infiltrates in males) and heart (myocardial degeneration in males) were observed in addition to liver effects (increased weights in females). In this study, the NOAEL was therefore 10000 ppm (equivalent to 786 mg/kg bw/d in males and 850 mg/kg bw/d in females). This study was conducted using a batch of the substance which was considered by the applicant as not representative of the proposed specifications.
- In the dog, two dietary studies are available (13-week and 52-week). The test substance was weighed directly into gelatine capsules without modification. Test substance was stored refrigerated (2 to 8°C), in the dark. In both studies the liver findings were observed at the LOAEL of 1000 mg/kg bw/d. They consisted in increased weights, modification of the clinical chemistry parameters (decreased cholesterol and albumin) and, in the 52-week study only, histopathological findings (minimal diffuse hepatocellular hypertrophy). At 1000 mg/kg bw/d, decreased body weight gains were observed in the male dogs treated for 13 weeks, and decreased haematocrit, haemoglobin concentration and erythrocyte counts, as well as haemosiderin in Kupfer cells, were noted in the 52-week study. The short-term NOAEL in the dog was 300 mg/kg bw/d in the 13- and 52-week studies.
- In the dietary study in the mouse the test substance was dispersed in the diet following procedures suitable to assure an adequate homogeneity and a correct dosing. In this study the target organ was the liver. At the dose level of 7000 ppm (equivalent to 1057 mg/kg bw/d in males and 1358 mg/kg bw/d in females), increased weights and histopathological findings (centrilobular hypertrophy) were observed in the liver of males. The short-term NOAEL in the mouse was therefore 3500 ppm (equivalent to 521 mg/kg bw/d in males and 644 mg/kg bw/d in females).
- Following daily 6-hour semi-occluded dermal application of metyltetraprole to Han Wistar rats for 4 weeks, a slight increased incidence of eschar formation was observed at 1000 mg/kg bw/d. No systemic effects were observed. Therefore, the local LOAEL was 300 mg/kg bw/d whereas the systemic NOAEL was 1000 mg/kg bw/d.
- In a chronic toxicity/carcinogenicity study, Han Wistar rats received metyltetraprole in the diet up to 20000 ppm (equivalent to 852 and 1190 mg/kg bw/d in males and females, respectively) for up to 2 years. In this study, target organs were the liver, the kidneys, the adrenals and the heart at the highest dose level of 20000 ppm. From the dose level of 6000 ppm onwards, statistically and biologically significant increased leucocyte counts were observed in females. Therefore, the NOAEL of this study was considered to be 2000 ppm in females (equivalent to 111.8 mg/kg bw/d) and 6000 ppm in males (equivalent to 83.9 mg/kg bw/d).
- In a carcinogenicity study, CD-1 mice received metyltetraprole in the diet up to 7000 ppm (equivalent to 820 and 1012 mg/kg bw/d in males and females, respectively) for up to 78 weeks. Target organ was the liver in males with statistically significant increased liver weights observed from 2000 ppm onwards. Although not associated with histopathological findings, this effect is considered adverse because of the magnitude of its change (12 to 20% compared to the control group). The NOAEL of this study was therefore 700 ppm in

males (equivalent to 82.2 mg/kg bw/d). In females, in the absence of adverse effect, the NOAEL is the highest dose level of 7000 ppm (equivalent to 1012 mg/kg bw/d).

- In a rat 2-generation reproductive toxicity study conducted according to the latest version of the OECD TG 416 (2001), the parental NOAEL was 6000 ppm (409 mg/kg bw/d), based on findings observed in the dams: increased absolute and relative liver and thyroid weights in F0 and F1 generations, as well as decreased absolute and relative uterus weights in F0 generation. The reproductive NOAEL of this study is considered to be 20000 ppm (equivalent to 1385 mg/kg bw/d), in the absence of treatment-related effects on reproductive parameters. In the offspring, at the dose level of 20000 ppm, decreased body weights (F2) and body weight gains (F1 and F2) during late lactation as well as decreased thymus weight (F1 and F2) and spleen weight (F2) were observed. The offspring NOAEL was therefore 6000 ppm (equivalent to 409 mg/kg bw/d).
- In a developmental toxicity study in the rats, there was no evidence of maternal toxicity up to the top dose tested (1000 mg/kg bw/d).The developmental NOAEL was 500 mg/kg bw/d, based on skeletal findings observed at the highest dose level. Increased incidence of misaligned hemicentres of the sternebrae and misaligned costal cartilage were observed in pups treated at 1000 mg/kg bw/d. In the DevTox database, misaligned sternebral hemicenters and costal cartilages are classified in the Grey Zone (i.e. no consensus on whether they should be considered as variations or malformations). They were considered by the study author as minor skeletal abnormalities.
- In a developmental toxicity study in the rabbits, clear maternal toxicity was observed at the top dose of 750 mg/kg bw/d, consisting of scant or no feces, decreased body weight gains, decreased food consumption from midgestation and increased number of dams with markedly decreased food consumption (20 g/d or less), as well as abortions. At the mid dose level of 250 mg/kg bw/d occurred scant or no feces, decreased food consumption, increased number of dams with food consumption at 20 g/d or less and one abortion likely due to decreased food consumption. All these findings were also observed in a preliminary study at the dose levels of 500 (including 1 abortion) and 1000 mg/kg bw/d. Thus, it cannot be excluded that these findings are treatment related at the dose level of 250 mg/kg bw/d. Therefore, the maternal NOAEL of this study was considered to be 100 mg/kg bw/d. In the absence of adverse effects in the pups, the offspring NOAEL was considered to be 750 mg/kg bw/d.

Based on all the above reported data, the DS concluded that no classification for STOT-RE was warranted for metyltetraprole.

Comments received during consultation

One comment by one MSCA was received during the consultation. MSCA expressed its agreement for no classification as STOT RE.

Assessment and comparison with the classification criteria

Substances are classified as specific target organ toxicants following repeated exposure on the basis of "significant" or "severe" toxicity. In this context "significant" means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound or serious than "significant" effects and are of a considerably adverse nature which significantly impact on health.

In accordance with the guidance on the application of the CLP criteria, the following effects might be indicative of significant or severe toxicity and thus merit classification for STOT-RE*:*

a) Morbidity or death resulting from repeated or long-term exposure.

b) Significant functional changes in the central or peripheral nervous systems or other organ systems

c) Any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters

d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination

e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity

f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in liver)

g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

To help to reach a decision whether a substance shall be classified or not, and to what degree it shall be classified (Category 1 or Category 2), dose/concentration 'guidance values' (see table below) are provided for consideration of the dose/concentration which has been shown to produce significant health effects.

Adjusted guidance values (mg/kg bw/d) for Categories 1 and 2 after administration by oral route are in the table below:

Duration	Cat.1	Cat.2
28-day	30	300
90-day	10	100
1-year	2.5	25
18-month	1.7	17
2-year	1.25	12.5

No adverse effect has been observed at doses below the cut-off values for Category 2 in the toxicity studies conducted on metyltetraprole, the lowest LOAEL being 1000 mg/kg bw/d in the 90-day studies, 225 mg/kg bw/d in the long-term studies, 1385 mg/kg bw/d in the 2-generation study and 250 mg/kg bw/d in the developmental studies.

Therefore, RAC concludes that **no classification for STOT-RE is warranted** for metyltetraprole (in agreement with the DS proposal).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The following genotoxicity/germ cell mutagenicity tests *in vitro* were available:

- A study was performed according to OECD TG 471 in the S. typhimurium (strains TA1535; TA1537; TA98 and TA100) and E. coli WP2 uvrA strains both with and without metabolic activation. Dose levels -/+ S9 in the main test were: 0, 156, 313, 625, 1250, 2500, 5000 µg/plate in triplicate. In the main test there was no dose related increases in revertant colonies in any of the tested strains either with or without S9 up to 5000 µg/plate. Positive control substances gave appropriate results.

- A Mammalian Gene Mutation Assay in Chinese Hamster V79 cells was performed according to OECD TG 476 to reveal gene mutation in mammalian cells. In the assay no relevant and reproducible increase in mutant colony numbers/10^6 viable cells were observed in the main experiments up to the maximum concentration both with and without S9. EMS and DMBA, used as positive controls, showed a distinct increase in induced mutant colonies. Under the experimental conditions metyltetraprole did not induce gene mutations at the HPRT locus in V79 cells. The maximum concentration applied was limited by the solubility of the test substance, but induced an evident cytotoxic effect, with a relative cloning efficiency between 10 and 20 %, as recommended by OECD TG 476.
- A Chromosome Aberration Assay in Chinese Hamster Lung Cells (CHL/IU) was performed according to OECD TG 473. The test substance induced no increases in the incidence of structurally aberrant cells or polyploid cells in any treatment group in the presence and absence of S9 mix. All the negative control cultures gave values of chromosome aberrations within the expected range. Positive control chemicals, MMC (without metabolic activation) and CP (with metabolic activation) showed the expected responses in the incidence of cells with structural aberrations. The maximum concentration applied caused an evident cytotoxicity with a Relative Increase in Cell Count between 37.5 and 48 %, in line with the recommendation of the OECD TG. Therefore, under the experimental conditions reported metyltetraprole did not show an increase in the incidence of chromosomal aberrations in Chinese hamster lung cells.

The following test *in vivo* was available:

- A Micronucleus Test in mice was performed according to OECD TG 474. Based on a preliminary dose range finding study, the maximum tolerated dose (MTD) was set at 2000 mg/kg bw. As no sex differences were reported, only male mice were used for this micronucleus study. The test substance was administered once to five mice per dose per sampling time by gavage. The bone marrow cells were obtained 24 and 48 hours after dosing. At 48 hours after dosing, sampling bone marrow cells from the 500 and 1000 mg/kg bw groups was not carried out. No increase in the mean frequencies of micronucleated polychromatic erythrocytes (PCE) in the metyltetraprole treated groups was observed in any experimental condition. A statistically significant increase in the frequency of micronucleated polychromatic erythrocytes was noted in the positive control group treated with MMC. The PCE ratio was not changed at 24 and 48 hours after treatment, therefore there was no evidence of bone marrow toxicity. In conclusion, under the conditions of this study, metyltetraprole did not induce an increase in micronuclei in bone marrow cells of mice following acute exposure by oral gavage at the dose level of 500, 1000 and 2000 mg/kg bw. However, in the absence of bone marrow toxicity, no proof of bone marrow exposure was available in this micronucleus test. The applicant provided a pharmacokinetic study in the CD-1 mouse that showed the presence of metyltetraprole in the plasma of animals treated with a single oral gavage dose of 2000 mg/kg bw, to show the systemic exposure to the test substance.

Based on the above reported data, the DS concluded that metyltetraprole is not mutagenic and therefore it does not require classification for germ cell mutagenicity.

Comments received during consultation

One comment by one MSCA was received in the consultation.

The MSCA noted that the *in vitro* data do not address aneugenic effects. In fact, the CA aberration test is not designed to measure numerical aberrations, while the micronucleus assay would be appropriate for this aim. However, considering the available *in viv*o micronucleus assay, the

MSCA agreed with the DS that metyltetraprole does not require classification for germ cell mutagenicity.

Assessment and comparison with the classification criteria

As reported above, the *in vitro* data obtained on metyltetraprole addressed:

- Gene mutation (both in bacteria and in mammalian cells) and
- Structural chromosomal aberration in mammalian cells

No information is available on the potential aneugenicity of the test substance. In fact, as also reported in the comment of one MSCA (see above), the chromosomal aberration assay in mammalian cells (OECD TG 473) is not designed to reveal aneugenicity, while the appropriate test is the micronucleus test, with centromere analysis (FISH or CREST) in case of positive result.

In the *in vivo* micronucleus study available the target exposure was not demonstrated (PCE/NCE not changed), even though the test was conducted up to the limit dose.

To assess target exposure a single dose toxicokinetics study has been provided. The test was designed to assess the pharmacokinetic characteristics and potential toxicity of metyltetraprole following single administration by oral gavage to male mice at a dose level of 2000 mg/kg bw. Plasma samples were analysed for metyltetraprole content using a validated LC-MS/MS method. The T_{max} and C_{max} of metyltetraprole were estimated to be approximately 2 hours and 411 ng/mL, respectively, with estimated systemic exposure over the first 24 hours after dosing (AUC_{24}) and when extrapolated to infinite time (AUC_{inf}) of 2310 and 2320 ng.h/mL, respectively. The terminal half-life ($t_{1/2}$) was approximately 3 hours. From the result of the toxicokinetic study, it is concluded that the animals were systemically exposed to metyltetraprole and that the substance reached the bone marrow.

However, it should be noted that bone marrow exposure is a quantitative, not a qualitative parameter. RAC notes that the toxicokinetic study mentioned may provide evidence that some bone marrow exposure occurred, but it cannot be considered a demonstration that the substance could not induce aneuploidy at a higher local concentration, that could be reached, for example, in the first site of contact. The plasma levels detected in the toxicokinetic studies are at least an order of magnitude lower than the concentration that could be tested *in vitro*. In conclusion, while a clastogenic effect was excluded by the reliable negative outcome of the *in vitro* CA assay, to definitely rule out the concern for aneugenicity an *in vitro* micronucleus test with an adequate concentrations range would be needed.

The classification criteria for germ cell mutagenicity takes into account test results from mutagenicity or genotoxicity tests *in vitro* and from studies with mammalian somatic and germ cells *in vivo*.

Therefore, considering that there is no experimental evidence of mutagenic activity, RAC concludes that **no classification for germ cell mutagenicity is warranted** for metyltetraprole.

Additionally, RAC notes that aneugenic effects for metyltetraprole are not adequately examined.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

A chronic toxicity/carcinogenicity study performed in Han Wistar rats by diet administration at 0, 2000, 6000 and 20000 ppm of metyltetraprole (the high dose is equivalent to 852 and 1190 mg/kg bw/d in males and females, respectively) for 104 weeks is available. The test substance was dispersed in the diet following a procedure suitable to assure an adequate homogeneity and a correct dosing. In this study, histopathology was performed only in control, high dose group, animal decedents or animals with gross lesions at 2000 and 6000 ppm, then a trend analysis was not feasible. In addition to the effects mentioned in the above STOT RE section, the following carcinogenic effects were reported in rats:

- malignant lymphoma in males and females at 6000 and 20000 ppm
- malignant uterine schwannomas at 20000 ppm
- mammary gland adenoma/adenocarcinoma at 20000 ppm

The increased incidences were not statistically significant according to one-tailed pairwise comparisons, no information on low and mid dose group was available but the DS considered the findings of biological relevance. Moreover, a Cochran-Armitage trend test (1-sided) provided by the DS showed a significant trend in males (p=0.02639) for malignant lymphomas, a borderline significance in females for malignant uterine schwannomas (p=0.05432) and a significant trend in females for total adenomas and adenocarcinomas (p=0.02357) (see next three tables below).

A carcinogenicity study performed according to OECD TG 451 in CD-1 mice after oral administration (diet) with 0, 700, 2000 and 7000 ppm of metyltetraprole is also available. Increased incidence of hematopoietic cancers was observed in males (lymphoma) and females (lymphoma and histiocytic sarcomas) from 2000 ppm onwards. Lymphoma was the most common factor leading to death and occurred earlier in the treated group. The DS considered that HCD provided by the applicant were not appropriate, as a period longer than 5 years before the study was used. As the study was conducted in 2015-2017 and HCD from studies conducted before 2010 should be disregarded. Therefore, the following relevant HCD are available: 9 studies conducted in 2011-2016 with the following incidences:

- Malignant lymphoma: 0.0 to 11.8 %, mean of 5.0 % for males; 0.0 to 23.5 %, mean of 12.9 % for females.
- Histiocytic sarcoma: 0.0 to 3.9 %, mean of 1.3 % for females.

The DS considered relevant only the HCD from dietary studies conducted in the period 2014-2018 and included a new dietary study (2018) provided by the Applicant.

The incidences of malignant lymphomas in males (16 %) and histiocytic sarcomas in females (6 %) observed in the study with metyltetraprole were above the relevant HCD at 7000 ppm (top dose). It should be noted that the animals of the low and intermediate dose groups were not examined, except for those with abnormalities. Nevertheless, as the incidences (in terms of number of animals affected) of lymphoma in males and histiocytic sarcomas in females were the same in the intermediate and high dose groups (8/17 and 8/51 for lymphoma, 3/20 and 3/51 for histiocytic sarcomas in 2000 ppm and 7000 ppm groups, respectively), these findings should also be considered treatment-related at 2000 ppm. It is noted that the increased incidences of these tumours were not statistically significant according to one-tailed pairwise comparisons performed by the Applicant. A Cochran-Armitage trend test (1-sided) provided by the DS confirmed the absence of statistical significance (see fourth table below).

DS noted that investigation of neoplastic lesions in animals from the low- and mid-dose groups would have been of value. Nevertheless, considering the biological relevance of these findings (comparison to respective control groups and to relevant HCD), DS considered that it cannot be excluded that the following tumours were related to metyltetraprole administration:

- lymphomas in males at 2000 ppm and 7000 ppm
- histiocytic sarcomas in females at 2000 ppm and 7000 ppm

A new statistical analysis was performed by the DS on the updated position paper on HCD provided by the Applicant (TST-0100). The DS proposed two additional sets of HCD compared to HCD presented in the CLH-report/Draft Assessment Report (DAR), then the sets are:

- **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 centred as closely as possible to the date of the study within a 5-year period. As both experimental parts of the carcinogenicity studies were conducted in the period 2015-2017, the DS considered that HCD from years 2014 to 2018 are the most relevant.

The DS updated the tables with malignant effects as reported below. No significant differences are seen in the three sets of HCD, except that no incidence at all for malignant lymphomas and malignant uterine schwannomas were reported in female rats in HCD from 2014 to 2018.

In summary, the DS rejected 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) based on the results of the provided linear regression between incidence of neoplasms and years of the studies because the approach was considered not appropriate and the recommendations regarding methodologies reported in EFSA Journal 2018;16(1):5122 and EFSA Journal 2018;16(1):5123 were not followed. Moreover, DS does not consider appropriate the use HCD from other routes/methods of administration, because 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. The DS analysed the updated position paper on HCD provided by the Applicant. The updated tables of neoplastic findings, including the new HCD-set and the Cochran-Armitage trend test performed by DS are reported below. The updated information which is not available in the original Background document (CLH/DAR) is highlighted in yellow.

Table: *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%)	3 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

Table: *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

Table: *Mammary tumours 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

Table: *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 studies 2010-2016, 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

To conclude whether metylteraprole triggers Category 1B, Category 2 or no classification for carcinogenicity, the DS has taken into consideration the following factors:

(a) Tumour type and background incidence: As described above, increased incidences of several type of tumours were observed in the rat and mouse long-term toxicity studies. These incidences were above relevant HCD range calculated from contemporary studies (approx. 5-year around the date of the study) conducted in the same strain of rodents, in the same laboratory and by the same route of administration (diet) for the following neoplastic findings at the following dose levels:

In the rat:

- malignant lymphomas in males and females at 6000 ppm and 20000 ppm
- malignant uterine schwannomas at 20000 ppm
- mammary gland adenomas and combined mammary gland adenomas/adeno- carcinomas at 2000 ppm
- moreover, the Cochran-Armitage trend test was statistically significant for different tumour types (see above).

In the mouse:

- lymphomas in males at 2000 ppm and 7000 ppm

- histiocytic sarcomas in females at 2000 ppm and 7000 ppm

The same conclusions are applicable also when the HCD 2014-2018 (4 studies for diet) was used by the DS after the analysis of the update HCD provided by the Applicant as reported in the last table above.

(b) Multi-site responses: Evidence of multi-site responses was reported in rats but not in mice. In female rats, increased incidences of malignant lymphoma, malignant uterine schwannomas as well as mammary gland tumours were observed. In male rats, in male mice and in female mice, only one type of tumours was noted in each of them, i.e. lymphomas in male rats and mice, and histiocytic sarcomas in female mice.

(c) Progression of lesions to malignancy: In mammary gland in female rats, both adenoma and adenocarcinoma were observed. All other tumours were malignant. However, there was a lack of pre-neoplastic or related non-neoplastic lesions which could have indicated a progression to malignancy.

(d) Reduced tumour latency: No statistical significance using Peto's mortality-prevalence method was observed. Although, it is important to note that most of the tumours were already apparent in decedent animals (killed or dying during the study).

(e) Whether responses are in single or both sexes: Both sexes were affected by neoplastic lesions. Malignant lymphomas were observed in both sexes. Other tumour types were seen in either male or female rats/mice.

(f) Whether responses are in a single species or several species: Both tested species were affected by neoplastic lesions. Malignant lymphomas were observed in rats and mice. Other tumour types were seen in either rats or mice.

(g) The possibility of a confounding effect of excessive toxicity at test doses: No evidence of a confounding effect of excessive toxicity.

(h) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity: No structural similarity with substances that have carcinogenic potential.

(i) Routes of exposure: Only experimental studies by oral route (dietary administration) are available.

(j) Comparison of ADME between test animals and humans: No human data are available. The *in vitro* metabolism data suggest a similarity between experimental animals and humans.

(k) Mode of action and its relevance for humans: Metyltetraprole did not show genotoxic potential in *in vitro* and *in vivo* assays. It is therefore unlikely that carcinogenic effects of metyltetraprole were the consequence of a genotoxic mode of action. There is no available toxicity data supporting a specific MoA for carcinogenicity, therefore the human relevance of the observed tumours cannot be excluded.

Dossier submitter's overall weight of evidence analysis

Factors that may be in favour of a Category 1 classification:

- Several types of tumours were observed at several sites in both sexes and both species following metyltetraprole administration.
- Most of the tumours were malignant.
- The MoA underlying these neoplastic lesions is 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.

Factors that are rather in favour of a Category 2 classification:

- Although the incidences of tumours were above the range of HCD, they remained relatively low and are not suggestive of a clear effect.
- No statistical significance was noted according to one-tailed pairwise comparisons using Peto's method (comparison to the high dose group vs. control group), provided that one-tailed analysis is more conservative than two-tailed one. It is nevertheless noted that a trend analysis could not be conducted because of the study design.

Moreover, in the final conclusion on classification it should also be considered that a Cochran-Armitage trend analysis performed by the DS revealed a significant trend only in rats, for malignant lymphoma in males and for total adenomas and adenocarcinomas in females, and a borderline significance in females for malignant uterine schwannomas.

Overall, the DS considered not sufficiently convincing to propose a classification as Carc Category 1 (the incidences for each tumours remain slight when compared to concurrent control group and/or the HCD). The DS considered a classification as carcinogen Category 2 for metyltetraprole more appropriate.

Comments received during consultation

Comments during the first consultation

During the first consultation, the Applicant questioned the appropriateness of the carcinogenicity classification proposed by the DS for metyltetraprole. In particular the Applicant claimed: the tumours observed are within the HCD; no clear evidences of multi-site responses were observed;

- no evidence of progression of lesions to malignancy are reported;
- a reduced tumour latency was not observed;
- no clear evidence that the responses were observed in any sexes;
- no clear evidence that the responses were observed in any species;
- not structurally similar to substances for which there is good evidence of carcinogenicity potential;
- the relevant route of exposure is the oral route (dietary administration);
- the ADME between animals and human is similar;
- no evidence of a confounding effect of excessive toxicity;
- no toxicity data support particular MoAs for carcinogenicity.

The Applicant provided an updated position paper on the HCD (TST-0100).

In summary, the paper provides elements to support the Applicant's position that a 5-years window for HCD is not the period relevant in this evaluation. To perform their evaluation the Applicant refers to a study (Nolte et al. 2011) where the linear regression was used to quantify the strength of the relationship between the response variable (tumour incidence) and the explanatory variables (year of study start) in this analysis. If this analysis reveals that explanatory variables (year of study start) have no linear relationship with the response (tumour incidence), it can be concluded that there is no time-related shift in tumour incidence and thus the HCD is stable during the relevant time-period. Based on the result of these analyses, Nolte et al. (2011) concluded that a time window should be defined for each neoplastic lesion after analysis of the dependency between tumour incidence and year of study start, and the use of a "fixed moving time window" may lead to loss of important information or the reference to inappropriate HCD.

Then, in the position paper the correlation between incidence of the following neoplastic lesions and year of study start was evaluated. As shown in the TST-0100 report, there was no statistically significant correlation between year of study start and incidence of neoplastic lesions when all

studies (rats; 2008-2018, mice; 2002-2018) were included, thus indicating stability of the background incidence over time, and therefore the Applicant concludes that there is no need to apply a window of 5 years when defining the reliability of HCD.

Therefore, the Applicant claims that significant correlation between year of study start and incidence of neoplastic lesions when all studies are included (rats; 2008-2018, mice; 2002-2018) is indicative of stability of the background incidence over time, and therefore there is no need to apply a window of 5 years to HCD as used in a common practice.

Three MSCA commented the carcinogenicity.

One MSCA commented that some of the discarded HCD could be informative for the assessment of carcinogenicity and requested to see the full data of the HCD.

One MSCA suggested to re-evaluate the statistical analysis of the carcinogenic data before reaching a conclusion on carcinogenicity Category 2, proposing that Category 1B is more appropriate. In addition, MSCA questioned the appropriateness of the pairwise test used by the Applicant instead of the trend test (not used by the Applicant and suggested by OECD GD 116 as more powerful). The MSCA pointed out that in the situation where there might be non-linear effects (for example due to saturation of the test substance) pairwise tests might give most appropriate results, but Fisher's exact test is the preferred test.

One MSCA was in favour of Category 1B classification instead of Category 2 based on the following observations: 1) lymphomas are observed in male and female rats and in male mice; 2) uterine schwannomas are observed in rat; 3) mammary gland tumours are observed in rat; 4) histiocytic sarcomas are observed in female mice. These tumours occurred in different organs, have different cells of origin and different natural background incidences, then a classification as Carc. 1B H350 seemed more appropriate in the absence of a better justification for downgrading to the Category 2.

A comment of one NGO supporting the Category 1B was submitted. The NGO commented that there are more factors in favour of a classification in Category 1B than in Category 2.

Comments during the second, targeted consultation

In the first consultation, the Applicant provided to ECHA eight new documents containing additional information on carcinogenicity. These documents were subject to an *ad hoc* consultation that was launched from 26/06/2023 to 10/07/2023 and the comments received are as follows:

One MSCA raised doubts on the HCD and the statistical testing used by the Applicant in the evaluation of carcinogenicity data and about the conclusion drawn by the Applicant that the tumours observed are not treatment related.

The DS submitted its evaluation of the new data provided by the Applicant. The DS observed a high number of discrepancies between the initial study reports in rats and mice, the additional histopathological investigations and the peer-review analysis that raised uncertainties either for the first assessment in the study reports or the peer review assessment.

Following the submission by the Applicant to ECHA of the eight new documents containing additional information on carcinogenicity the DS evaluated them. A summary of the studies and their evaluation by the DS is reported in the Appendix 1 of this opinion.

Taking note of EFSA's additional requests

During the review of the active substances for the plant protection product use (PPR process), on 25th of September 2023, EFSA requested the Applicant to provide additional information. Due to the possible impact of these data on the harmonised classification and labelling in accordance

with Regulation (EC) No 1272/2008 and the assessment ongoing by RAC, the Applicant submitted these responses also to ECHA on 22nd of December 2023. The following responses to EFSA requests were related to carcinogenicity studies and mode of action: **Q34, Q35, Q36, Q37, Q38, Q48, Q49** and **Q51.**

Moreover, the following three new reports were submitted by the Applicant:

- Eniola, S., Stewart, J, 2023. VRY0054: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0188
- Mowat, V., Stewart, J, 2023. VRY0055: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0189
- The updated position paper of the Applicant, Report No TST-0190

It should be noted that the RAC assessment, at this stage of the procedure, was already based on updated data, because the tables reported in the document TST-190 are the same as those available earlier after the second, targeted consultation.

In addition, Appendix 2 provides a summary of the Applicant's responses to EFSA requests related to carcinogenicity studies.

Assessment and comparison with the classification criteria

According to (EC) No 1272/2008 a substance is classified for carcinogenicity:

Category 1: Known or presumed human carcinogen on the basis of epidemiological and/or animal data.

- Category 1A, known to have carcinogenic potential for human, classification is largely based on human evidence, or
- Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

Category 2: Suspected human carcinogen on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional consideration.

As regards metyltetraprole in the absence of human data, Category 1A is not considered applicable.

In order to assess the strength of evidence from the experimental animal studies, RAC took into account all the available data:

- the main carcinogenicity studies (both in rats and mice);
- the additional histopathological investigation in animals from the low and mid dose group commissioned by the Applicant (both in rats and mice);
- the peer review evaluation of the findings of the main studies commissioned by the Applicant (both for rats and mice);
- the statistical analysis of the findings in the main study and in the additional histopathological evaluation (taking into account also the results of the peer review and Expert Panel evaluation, both in rats and mice, see Appendix 1);
- the Applicant's responses to EFSA's questions in the PPR procedure regarding carcinogenicity (see Appendix 2).

RAC also considered two critical points in its assessment:

1) Which historical control data are used for comparison, and

2) Which p-levels are applied to establish the statistical significance.

Regarding the first point, RAC supports the DS position on the historical control data set to be considered. In particular:

- The application of regression analysis to historical data from year 2010 is considered inappropriate since it would deviate from the 5-year temporal window recommended by the relevant CLP guidance.
- 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. Moreover, it should be noted that according to OECD GD 116 the concurrent control group is always the most important consideration in the testing for increased tumour rates, while the historical control data should mainly be used in evaluating the validity of the data from the concurrent control groups.
- With regard to the p-levels, RAC observes that the value of $p<0.05$ is most commonly used to define the statistical significance. Moreover, in line with OECD GD 35 and 116, statistical significance should not be considered the only criterion when evaluating the outcome of a carcinogenicity study, as the overall biological relevance is also to be taken into account.

The following paragraphs provide a detailed assessment of the factors (CLP Annex I, 3.6.2.2.6) RAC has considered to support the conclusion on classification.

Tumour type and background incidence (in rats and mice)

The tumour incidences in rats

The incidences of the following tumours observed in rats were above the historical control data:

- malignant lymphomas in males and females at 6000 ppm and 20000 ppm;
- malignant uterine schwannomas at 2000 and 20000 ppm;
- mammary gland adenomas at 20000 ppm.

Moreover, for malignant lymphomas in male rats both the Cochran-Armitage trend test (1-sided) and the Peto's trend test showed a statistically significant trend ($p=0.012$ and $p=0.0102$, respectively) and the one-tailed Peto's pairwise test was significant at mid and high dose ($p<0.05$).

A significant trend (Cochran-Armitage test, $p=0.0342$) was reported for total schwannomas (uterus, uterine cervix and abdominal cavity).

Overall evaluation in rats				
Tumour type	**Findings**	**Comparison with HCD**	**Statistical analysis**	
			Trend test	**Pairwise**
M-lymphoma	M mid and high dose	Over HCD	p=0.012[1] p=0.0102[2]	Mid dose p=0.0409[2] High dose p=0.058[1] p=0.0146[2]
	F mid and high dose	Over HCD	NS	NS

M-uterine schwannoma	F low and high dose	Over HCD	NS	NS
Combined M-schwannomas	F all doses	NA	p=0.0342[1]	NS
Mammary Adenoma	F high dose	Over HCD	NS	NS
Mamm. Adenocarc.	F high dose	Within HCD	NS	NS
Mammary Adenoma/adenocarc	F high dose	Within HCD	p=0.053[1]	NS

[1] DS Assessment
[2] Applicant Assessment
HCD-historical control data

Regarding mammary tumours in female rats, only the incidences of adenomas were over the historical control data, but they were not statistically significant. However, the analysis of tumour onset showed a reduced latency for these benign tumours (see table below).

Doses (ppm)	Time of onset					
	< 104 weeks			104 weeks		
	Adenoma	Fibroadenoma	Adenocarc	Adenoma	Fibroadenoma	Adenocarc
0	-	2 (99; 102) (4%)	1 (99) (2%)	1 (2%)	10 (20%)	3 (6%)
2000	-	2 (89; 93) (4%)	1 (91) (2%)	2 (4%)	7 (14%)	-
6000	2 (80; 97) (4%)	3(88; 91; 99) (6%)	1 (80) (2%)		15 (30%)	2 (4%)
20000	4 (88; 97; 97; 101) (8%)	1 (97) (2%)	3 (42; 97; 103) (6%)	-	11 (22%)	4 8%

The tumour incidences in mice

In mice lymphomas were reported in males at 2000 ppm and 7000 ppm, and in females, only at 2000 ppm, however these increases were not statistically significant.

Overall evaluation in mice			
Tumour type	**Findings**	**Comparison with HCD**	**Statistical analysis**
M-lymphoma	M mid and high dose	Over HCD	NS
	F **only** mid dose	Over HCD	NS

Histiocytic sarcoma	none	Within HCD	NS

NS-not significant

Multi-site responses

Evidence of multi-site responses were reported in female rats, i.e. increased incidences of malignant lymphoma, malignant uterine schwannomas as well as mammary gland benign tumours were observed.

Progression of lesions to malignancy

In mammary gland in female rats, both adenoma and adenocarcinoma were observed. All other tumours were malignant. However, no pre-neoplastic or related non-neoplastic lesions were observed, which could have indicated a progression to malignancy.

Reduced tumour latency

Before 104-weeks adenomas were observed only at mid and high dose with an apparent dose-response trend (no statistical analysis available). Moreover, it was noted that most of the tumours were already apparent in decedent animals (killed or dying during the study). However, no statistical significance was observed using Peto's mortality-prevalence method.

Whether responses are in single or both sexes

Both sexes were affected by neoplastic lesions. Malignant lymphomas were observed in both sexes. Other tumour types were only seen in female rats.

Whether responses are in a single species or several species

Neoplasm incidences, both over historical control data and statistical significant, were reported only in rats. Increased incidence of the same type of neoplasm was reported over historical control datain mice too, however without statistical significance. This supports the conclusion that in mice there is no clear evidence of carcinogenicity.

Structural similarity to a substance(s) for which there is good evidence of carcinogenicity

Metyltetraprole shows no structural similarity with substances that have carcinogenic potential.

Routes of exposure

Only experimental studies by oral route (dietary administration) are available.

Comparison of absorption, distribution, metabolism and excretion between test animals and humans

No human data are available. The *in vitro* metabolism data suggest a similarity between experimental animals and humans.

The possibility of a confounding effect of excessive toxicity at test doses

No evidence of a confounding effect of excessive toxicity was reported.

Mode of action and its relevance for humans

Metyltetraprole did not show genotoxic potential in *in vitro* and *in vivo* assays. RAC notes that aneugenicity was not adequately investigated, however this data gap has no impact on the carcinogenicity assessment, as an aneugenic effect of the substance is unlikely and the role of aneugenicity in carcinogenesis remains unclear. No changes indicating effects on immune system were observed in spleen, thymus, lymph nodes, bone marrow or mucosa associated lymphoid tissue, and therefore, an immunotoxic MoA for the observed malignant lymphomas

is unlikely. A mammalian cell-based luciferase reporter gene assay provided by the Applicant showed no effect. However, RAC notes that other mechanisms involving oestrogen or androgen pathways were not investigated.

Therefore, a plausible mechanism to explain the increased tumour incidence reported in the available experimental studies is not identified so far. On the other hand, RAC notes that in the absence of an identified MoA, the human relevance of these tumours cannot be excluded.

RAC's overall conclusions on carcinogenicity

- Despite some types of tumours observed at several sites in both sexes in rats, the incidences of these tumours were low.
- Statistical significance (pairwise as well as in a trend test) was reported only for a single tumour type (M-lymphoma, at the mid- and high dose) in male rats.
- In female rats M-schwannomas and M-lymphoma tumours can be considered rare as no cases were reported in the HCD from 5 years before the study; therefore, these findings should be considered biologically relevant also in the absence of statistical significance.
- In mice, values above the historical control data were reported for lymphomas (a relatively common tumour type in this species and strain, as reflected in the historical control data, but without statistical significance or any dose response relationship. Therefore, there is no clear evidence of carcinogenic effect in mice.
- The substance is not genotoxic and there is no evidence of hormonal (androgen or estrogen pathways) or immunotoxic effects. However, in the absence of an identified MoA, the human relevance of the observed tumours cannot be excluded.

In consideration of all the reasons summarised above and on the basis of the applicable CLP criteria, RAC concludes that metyltetraprole warrants a **classification as carcinogen in Category 2 (H351)**(in agreement with the DS proposal).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

A 2-generation reproductive study in the rat has been provided.

This study was conducted according to the latest version of the OECD TG 416 (2001), and the parental NOAEL was 6000 ppm (409 mg/kg bw/d) based on findings observed in the dams: increased absolute and relative liver and thyroid weights in F0 and F1 generations as well as decreased absolute and relative uterus weights in F0 generation.

The reproductive NOAEL of this study was considered to be 20000 ppm (equivalent to 1385 mg/kg bw/d). It is noted that a slight statistically significant decrease in the gestation length was observed in F1 females only at the highest dose level of 20000 ppm (mean gestation length was the same in each group but a higher percentage of animals had a gestation length of 22 days and a lower percentage had a gestation length of 23 days compared to controls). Nevertheless, considering all the available information (including historical control data), this effect was not considered treatment-related and adverse.

In the offspring, at the dose level of 20000 ppm, decreased body weights (F2) and body weight gains (F1 and F2) during late lactation as well as decreased thymus weight (F1 and F2) and

spleen weight (F2) were observed. The offspring NOAEL was therefore 6000 ppm (equivalent to 409 mg/kg bw/d).

In the 2-generation rat study no adverse effects on sexual function or fertility were observed.

DS concluded that no classification for adverse effects on sexual function or fertility was warranted for metyltetraprole.

Developmental toxicity

Developmental toxicity studies in rats and rabbits have been provided.

In the rat study there was no evidence of maternal toxicity up to the top dose tested (1000 mg/kg bw/d). The maternal NOAEL was therefore 1000 mg/kg bw/d. The developmental NOAEL was 500 mg/kg bw/d based on skeletal findings observed at the highest dose level. Increased incidence of misaligned hemicentres of the sternebrae and misaligned costal cartilage were observed in pups treated at 1000 mg/kg bw/d.

In the DevTox database, misaligned sternebral hemicenters and costal cartilages are classified in the Grey Zone (i.e. no consensus on whether they should be considered as variations or malformations). They were considered by the study author as minor skeletal abnormalities.

In the rabbit study clear maternal toxicity was observed at the top dose of 750 mg/kg bw/d consisting of scant or no faeces, decreased body weight gain, decreased food consumption from mid-gestation and increased number of dams with markedly decreased food consumption (20 g/d or less) as well as abortions. At the mid dose, 250 mg/kg bw/d, scant or no faeces, decreased food consumption, increased number of dams with food consumption (20 g/d or less) and one abortion likely due to decreased food consumption occurred. All these findings were also observed in a preliminary study at the dose levels of 500 (including 1 abortion) and 1000 mg/kg bw/d. Therefore, it cannot be excluded that these findings are treatment related at the dose level of 250 mg/kg bw/d. Therefore, the maternal NOAEL of this study is considered to be 100 mg/kg bw/d. In the absence of adverse effects in the pups, the offspring NOAEL is considered to be 750 mg/kg bw/d.

In the rat developmental toxicity study skeletal alterations were noted in the pups in the absence of maternal toxicity at the highest tested dose of 1000 mg/kg bw/d. Skeletal alterations consisted in misaligned sternebral hemicentres and costal cartilage. Although metyltetraprole may warrant classification based on these skeletal findings which cannot be explained by maternal toxicity, the DS considered that a classification for developmental toxicity is not appropriate for the following reasons:

<u>Magnitude of the increase in the incidence of skeletal alterations</u>

1) The incidence of misaligned sternebral hemicentres (4 foetuses in 3 litters, 290/0 foetuses, 12.5 % litters) was at the upper limit of HCD (relevant in terms of dates of the study, strain and source of rats, laboratory, route of administration), but above the mean of the HCD (max. 3 foetuses and 3 litters, foetuses: mean 0.9 % range 0.0-28 %, litters: mean 4.2 % range 0.0-15.0 %).
2) The incidence of misaligned costal cartilage (5 foetuses in 4 litters, 3-7 % foetuses, 16.7 % litters) slightly exceeded relevant HCD (max. 3 foetuses and 3 litters, foetuses: mean 1.1 %, range 0.0-28 % - litters: mean 5.1 %, range 0.0-15 %).

For both findings, it is also noted that they occurred in the control group at incidences already above the mean of HCD (1.7 % foetuses, 8.7 % litters for both findings in the control group vs in the HCD: 0.9 % foetuses, 4.2 % litters for misaligned sternebral hemicentres and 1.1 % foetuses, 0.1 % litters for misaligned costal cartilage).

In terms of number of litters affected (litters are the relevant unit to conclude on developmental toxicity potential), the same number of litter (for misaligned sternebral hemicentres) or one additional litter (misaligned costal cartilage) were affected in the study with metyltetraprole when compared to studies included in HCD (maximal range). When compared to control group, only one more litter was affected for misaligned sternebral hemicentres and two more litters for misaligned costal cartilage.

These findings were not considered as very rare since they occurred in 9 and 11 out of 17 studies included in the HCD for misaligned sternebral hemicentres and misaligned costal cartilage, respectively (i.e. 53 % and 65 % of the studies).

Overall, the incidences were therefore only slightly increased when compared to HCD maximal range and when compared to control group. In addition, DS noted that both findings were present in the same four foetuses (from three litters), and another foetus showed misaligned costal cartilage only.

Type of skeletal alterations

According to the DevTox database misaligned sternebral hemicentres and costal cartilages are classified in the Grey Zone (i.e. no consensus on whether they should be considered as variations or malformations). They were considered by the study author as minor skeletal abnormalities and not as malformations. The study author defined minor abnormalities as follows: *"minor differences from normal that are deleted relatively frequently considered to have little detrimental effect and may be a transient stage in development e.g. bipartile centrum, dilated ureter".*

Overall, these skeletal alterations (misaligned sternebral hemicenters and costal cartilages) were not considered as malformations and their increased incidences were slight compared to concurrent controls and HCD. Therefore, DS considered that they did not represent a teratogenic effect.

In the rabbit developmental toxicity study, no adverse effects were observed in the offspring.

DS concluded that no classification for adverse effects on development was warranted for metyltetraprole.

Lactation

There was no indication of impaired nursing behaviour or decreased pup viability during lactation. Results of the study did not indicate any direct, adverse effect on the offspring due to transfer of the substance via the milk or on the quality of the milk. Therefore, DS concluded that no classification was warranted for effects on or via lactation.

Comments received during consultation

One comment was received in the consultation from one MSCA.

The MSCA was in agreement with the DS on no classification for reproductive classification.

The MSCA noted that based on the available data from a 2-generation study in rats the effects are not sufficient for classification of metyltetraprole as toxic for sexual function and fertility. For developmental effects the MSCA noted that 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. Furthermore, the MSCA was of the opinion that a statistical analysis is necessary to calculate the possible significance of the data and clarify whether the different skeletal findings were always affected by different foetuses or whether there were foetuses with multiple findings and asked if HCD had been adequately reported.

Assessment and comparison with the classification criteria

Fertility

The reproductive toxicity concerning adverse effects on sexual function and fertility of metyltetraprole was investigated in a 2-generation reproductive study in the rats (DAR B.6.6 and in 2.6.6 of the updated Volume 1/CLH report version of October 2022). In this reproduction study (conducted according to the latest version of the OECD TG 416 - 2001) metyltetraprole was administered in the diet to male and female Han Wistar rats (24 rats/sex/concentration for F0 and F1 generations) at 0, 2000, 6000 or 20000 ppm: equivalent to 0, 132, 409 and 1385 mg/kg bw/d for F0 and 0, 187, 551 and 1872 mg/kg bw/d for F1 generation.

Based on findings observed in the dams at 20000 ppm (increased absolute and relative liver and thyroid weights in F0 and F1 generations, as well as decreased absolute and relative uterus weights in F0 generation) the parental NOAEL is 6000 ppm (409 mg/kg bw/d).

In the absence of treatment-related effects on reproductive parameters the reproductive NOAEL of this study is considered to be 20000 ppm (equivalent to 1385 mg/kg bw/d).

It is noted that a slight statistically significant decrease in gestation length was observed in F1 females only at the highest dose level of 20000 ppm (mean gestation length was the same in each group but a higher percentage of animals had a gestation length of 22 days and a lower percentage had a gestation length of 23 days compared to controls). Nevertheless, considering all the available information (including historical control data) this effect is considered treatment-related and adverse.

In the offspring, at the dose level of 20000 ppm, decreased body weights (F2) and body weight gain (F1 and F2) during late lactation as well as decreased thymus weight (F1 and F2) and spleen weight (F2) were observed.

The offspring NOAEL was therefore 6000 ppm (equivalent to 409 mg/kg bw/d).

According to the CLP Regulation (Annex I, 3.7.1.3. Adverse effects on sexual function and fertility) any effect of substances that has the potential to interfere with sexual function and fertility must be considered. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

Category 1 classification

"Known or presumed human reproductive toxicant substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)."

Category 1A

"Known human reproductive toxicant. The classification of a substance in Category 1A is largely based on evidence from humans."

Category 1B

"Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse

effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

RAC concludes that no classification for Repr. Category 1A for fertility is warranted as no human data are available. Also, no classification for Repr. Category 1B or Category 2 for fertility is warranted as the available 2-generation study on rats did not show any adverse effects related to fertility. Therefore, RAC considers that no classification is warranted for Reproductive toxicity for fertility.

Developmental toxicity

Developmental toxicity studies in rats and rabbits are available.

In the developmental toxicity study in rats (conducted according to the OECD TG 414 (2001)) metyltetraprole was administered orally by gavage to male and female Han Wistar rats (24 rats/sex/concentration) at 0, 250, 500 and 1000 mg/kg bw/d from day 6 to day 19 of gestation. There was no evidence of maternal toxicity up to the top dose tested (1000 mg/kg bw/d) and the maternal NOAEL was therefore 1000 mg/kg bw/d.

The developmental NOAEL was 500 mg/kg bw/d based on skeletal findings observed at the highest dose level. Increased incidence of misaligned hemicentres of the sternebrae and misaligned costal cartilage were observed in pups treated at 1000 mg/kg bw/d.

RAC notes that misaligned sternebral hemicenters and costal cartilages are classified in the Grey Zone (i.e. no consensus on whether they should be considered as variations or malformations). They are considered as minor skeletal abnormalities according to the DevTox database.

Table from DAR Vol 3CA B6 - Table B.6.6-1 Summary of reproductive and developmental toxicity studies

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 using two-tailed Fisher's exact test was performed on number of affected litters. There was no statistical significance ($p>0.05$).

HCD are available in the study report as well as in the update provided by the Applicant at the request of the DS to cover a period of 5 years around the date of the study.

Although studies using different routes of administration were presented, the DS only included studies conducted by gavage. HCD presented in the DAR/CLH-report are considered relevant in terms of dates of the study, strain and source of rats, laboratory, route of administration and type of study. Incidences expressed on litter and foetus basis, and the number of litter and foetuses examined was given for each study in the included HCD. The developmental NOAEL of this study is considered to be 500 mg/kg bw/d based on skeletal findings observed at the highest

dose level (i.e. increased incidence of misaligned hemicentres of the sternebrae and misaligned costal cartilage) observed in pups treated at 1000 mg/kg bw/d.

Moreover, the increased incidences of some alterations in the skeletal findings were compared to incidences observed in HCD coming from 2013-2016 study reports and from an additional paper containing information from years 2016-2018. The incidence of misaligned sternebral hemicentres (4 foetuses in 3 litters, 2.9 % foetuses, 12.5 % litters) was at the upper limit of relevant HCD (in terms of dates of the study, strain and source of rats, laboratory, route of administration) but far above the mean of the HCD (max. 3 foetuses and 3 litters, foetuses: mean 0.9 %, range 0.0-2.8 % - litters: mean 4.2 %, range 0.0-15.0 %). The incidence of misaligned costal cartilage (5 foetuses in 4 litters, 3.7 % foetuses, 16.7 % litters) exceeded relevant HCD (max. 3 foetuses and 3 litters, foetuses: mean 1.1 %, range 0.0-2.8 % - litters: mean 5.1 %, range 0.0-15.0 %). Although it cannot be excluded that these findings are treatment-related, it should be noted that in the DevTox database, misaligned sternebral hemicenters and costal cartilages are classified in the Grey Zone (i.e. no consensus on whether they should be considered as variations or malformations). So, they are considered as minor skeletal abnormalities.

In the rat developmental study the same four foetuses (from three litters, also reported in the DAR/CLH) presented both findings misaligned hemicentres of sternebrae and misaligned costal cartilage treated at the highest tested dose 1000 mg/kg bw/d (i.e. foetus number 9 from dam number 2, foetus numbers 4 and 10 from dam number 3, foetus number 8 from dam number 10). One additional foetus (from a different litter) presented misaligned costal cartilage only (foetus number 6 from dam number 15). In the other groups (control, 250 and 500 mg/kg bw/d), two foetuses from two litters in each group were also affected by both findings. In the table below minor skeletal abnormality and variant findings with group incidences and HCD are presented.

Table B.6.6.2.1.2-4 Fetal examinations - minor skeletal abnormality and variants findings - group incidences

Parameter/Findings		Group Incidences (Group mg/kg bw/day)							
		Fetuses				Litters			
		0	250	500	1000	0	250	500	1000
Number Examined		121	141	130	136	23	24	24	24
Total Number Normal		31	25	22	29	14	16	12	16
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** ***2.9%***	2	2	2	**3** ***12.5%***
	HCD 17 studies conducted by oral gavage on Wistar rats from the same source Harlan-Envigo (2013-2018)	*mean 0.9%, range 0.0-2.8%* *max nb of foetus affected = 3*				*mean 4.2%, range 0.0-15.0%* *max nb of litter affected = 3*			
Costal cartilage	Misaligned	2	2	2	**5** ***3.7%***	2	2	2	**4** ***16.7%***
	HCD 17 studies conducted by oral gavage on Wistar rats from the same source Harlan-Envigo (2013-2018)	*mean 1.1%, range 0.0-2.8%* *max nb of foetus affected = 3*				*mean 5.1%, range 0.0-15.0%* *max nb of litter affected = 3*			
Total affected by one or more of the above		6	12	8	9	6	8	7	7
Rib and vertebral configuration									
Cervical rib	Short supernumerary	2	1	1	1	2	1	1	1
13th rib	Short	0	0	1	1	0	0	1	1
Number of 14th ribs	Short supernumerary	25	37	33	27	13	15	16	16

Parameter/Findings		Group Incidences (Group mg/kg bw/day)							
		Fetuses				Litters			
		0	250	500	1000	0	250	500	1000
	Full supernumerary	0	0	1	0	0	0	1	0
	Total	25	37	33	27	13	15	16	16
Thoracolumbar vertebra(e)	20	2	4	0	1	2	2	0	1
Pelvic girdle	Unilateral caudal shift	3	3	4	3	3	3	3	2
Delayed/Incomplete ossification/unossified									
Vertebrae	Cervical	2	0	1	2	2	0	1	2
	Thoracic	2	1	1	3	2	1	1	3
	Lumber	0	1	0	0	0	1	0	0
	sacrocaudal	0	4 *2.8%*	3 *2.3%*	2 *1.5%*	0	3 *12.5%*	1 *4.2%*	2 *8.3%*
	HCD 17 studies conducted by oral gavage on Wistar rats from the same source Harlan-Envigo (2013-2018)	*mean 1.1%, range 0.0-5.4%*				*mean 4.1%, range 0.0-15.0%*			

In a developmental toxicity study in the rabbits, (conducted according to the OECD TG 414 (2001)) metyltetraprole was administered orally by gavage to male and female NZW rabbits at 0, 100, 250, 750 mg/kg bw/d from day 6 to day 28 of gestation.

Clear maternal toxicity was observed at the top dose 750 mg/kg bw/d. Critical effects at this dose were decreased in body weight gains (-18 % GD 6-29), decreased food consumption and increased number of females (47.6 % vs. 13 % in the control group) with markedly decreased food consumption (20 g/d or less), scant or no faeces related to decreased food consumption (61.9 % vs. 21.7 % in the control group) and two abortions (GD 25) likely due to decreased food consumption.

At the mid dose 250 mg/kg bw/d, critical effects were decreased food consumption and increased number of females (22.7 % vs. 13 % in the control group) with markedly decreased food consumption (20 g/d or less), scant or no faeces related to decreased food consumption (31.8 % vs. 21.7 % in the control group) and one abortion (GD 26) likely due to decreased food consumption.

All these findings were also observed in a preliminary study at the dose levels of 500 (including 1 abortion) and 1000 mg/kg bw/d. Thus, it cannot be excluded that these findings are treatment related at the dose level of 250 mg/kg bw/d.

Therefore, the maternal NOAEL of this study is 100 mg/kg bw/d. In the absence of adverse effects in the pups, the offspring NOAEL is 750 mg/kg bw/d.

According to the CLP Regulation (Annex I, 3.7.1.4. Adverse effects on development of the offspring) developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects

induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

Category 1 classification

"Known or presumed human reproductive toxicant. Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)."

Category 1A

"Known human reproductive toxicant. The classification of a substance in Category 1A is largely based on evidence from humans."

Category 1B

"Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Category *2*

"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

In addition, according to CLP Regulation Annex I, 3.7.2.3.3: *"If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments".*

RAC concludes that no classification for Repr. Category 1A for development is warranted as no human data are available. Also, no classification for Repr. Category 1B for development is warranted due to lack of clear evidence on adverse effects on development in the absence of other toxic effects.

Further, no classification for Repr. Category 2 for development is warranted due to the fact that only minor developmental effects, considered of low toxicological significance were reported in rats, while in rabbit no adverse effects were reported in the offspring.

Therefore, RAC considers that no classification is warranted for Reproductive toxicity for Development.

Lactation

RAC agrees with the DS assessment that no classification is warranted for effects on or via lactation.

Overall conclusion

RAC considers that **no classification is warranted for Reproductive toxicity** (in agreement with the DS proposal).

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed to classify metyltetraprole (S-2367) to Aquatic Acute Category 1 based on the 96-hour LC_{50} of 0.048 mg/L for *Oncorhynchus mykiss* and to Aquatic Chronic Category 1 based on the 28-day NOEC of 0.015 mg/L for *Pimephales promelas*. M-factor of 10 was warranted ($0.01 < LC_{50} \leq 0.1$ mg/L) for the Acute Category and M-factor of 1 for the Chronic Category ($0.01 < NOEC \leq 0.1$) for a not rapidly degradable substance.

Degradation

In the ready biodegradation test (OECD TG 301B, GLP) 15 % biodegradation after 28 days was observed (CA-B.8.2.2.1) and metyltetraprole could not be considered as readily biodegradable.

Metyltetraprole did not degrade over the course of 5 days in solution at pH 4, 7 and 9 at 50 °C (CA-8.2.1.1.) in an OECD TG 111 hydrolysis test (GLP). Therefore, metyltetraprole was considered to be hydrolytically stable.

Metyltetraprole was stable in a natural surface water system in the OECD TG 309 (GLP) aerobic mineralisation study (CA-8.2.2.2).

In a water-sediment study (GLP, OECD TG 308) the behaviour of metyltetraprole was studied using radio-labelled [pyrazolyl-3-^{14}C]S-2367 and [benzyl ring-U-^{14}C]S-2367 in two systems (river, pond) (CA-8.2.2.3). Metyltetraprole was mainly adsorbed to sediment in both systems and then degraded very slowly. No major or minor non-transient metabolites were formed. Non-extractable residues reach a maximum of 9.7 % at 100 days. The DT_{50} values in the total system ranged from 428 to 715 days. The $DissT_{50}$ values in the surface compartment ranged from 1.6 to 1.95 days (CA-8.2.2.3.2).

The DS concluded that the substance was not rapidly degradable.

In the aquatic photodegradation study (OECD TG 316, GLP), metyltetraprole degraded very rapidly, with DT_{90} less than 12 hours. Numerous major metabolites (ISS7, S-2367-R1, S-2367-R2, S-2367-R6, de-ClPh-S-2367, OHTM and carbon dioxide) were formed (CA-8.2.1.2.).

Bioaccumulation

The measured log P_{ow} for metyltetraprole was 4.16 at 20 °C (Comb, A.L. 2015f). In the OECD TG 305 (GLP) test the steady state fish bioconcentration factors (BCF) based on measured ^{14}C-S-

2367 were 526 and 433 L/kg in the whole fish (*Oncorhynchus mykiss*) for the low (0.200 µg/L) and high exposure (2.00 µg/L), respectively (CA-B.9.2.4.1).

Aquatic toxicity

Acute

Table: *Reliable acute toxicity data on metyltetraprole (S-2367)*

Test protocol	Species	Test result (mg a.s./L)	Reference
Fish			
In six studies the LC_{50} values were > 0.15 to > 0.19 mg/L as measured concentrations. It was noted in several studies that these concentrations represent the functional limit of solubility of the test substance under test conditions.			
OECD TG 203, OPPTS 850.1075, GLP	*Oncorhynchus mykiss*	**96-h LC_{50} 0.048 (mm)**	CA-B.9.2.1.1
OECD TG 203, OPPTS 850.1075, GLP	*Pimephales promelas*	96-h LC_{50} 0.061 (mm)	CA-B.9.2.1.3
OECD TG 203, OPPTS 850.1075, GLP	*Menidia beryllina*	96-h LC_{50} 0.102 (mm)	CA-B.9.2.1.9
OECD TG 203, OPPTS 850.1075, GLP	*Pseudorasbora parva*	96-h LC_{50} 0.158 (mm)	CA-B.9.2.1.10
OECD TG 203, OPPTS 850.1075, GLP	*Tribolodon hakonensis*	96-h LC_{50} 0.114 (mm)	CA-B.9.2.1.8
Invertebrates			
OECD TG 202, OCSPP 850.1010, GLP, static	*Daphnia magna*	48-h EC_{50} 0.34 (mm)	CA-B.9.2.5.1
Algae			
OECD TG 201, OCSPP 850.4500, EC.C3, GLP, static	*Pseudokirchneriella subcapitata*	96-h E_rC_{50} > 0.32 (mm) (highest TWA concentration tested)	CA-B.9.2.11.1

mm= mean measured concentrations

Altogether 11 reliable acute fish studies were available on the active substance. The studies followed OECD TG 203/OPPTS 850.1075 and GLP. Tests were semi-static and a solvent was used. A solvent was used also in the *Daphnia* and algae studies.

There were reliable acute toxicity data available for fish, *Daphnia* and algae. The DS, the Rapporteur MS under the PPP process, considered the algae test not reliable for risk assessment in relation to the ongoing PPP process 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. The lowest acute toxicity value was a 96-hour LC_{50} of 0.048 mg a.s./L for *Oncorhynchus mykiss*.

Chronic

Table: *Reliable chronic toxicity data on metyltetraprole (S-2367)*

Test protocol	Species	Test result (mg a.s./L)	Reference
Fish			
OECD TG 210 (ELS), OCSPP Draft Guideline	*Pimephales promelas*	28-day:	CA-B.9.2.2.1

850.1400, GLP, flow-through		**$NOEC_{total\ length}$ 0.015 (mm)** EC_{10}, total length 0.030 (mm) EC_{10}, wet weight 0.016 (mm)	
OECD TG 210 (ELS), OCSPP Draft Guideline 850.1400, GLP, flow-through	*Cyprinodon variegatus*	28-day: $NOEC_{length,\ wet\ weight)}$ 0.071 (mm)	CA-B.9.2.2.2
Invertebrates			
OECD TG 211, OCSPP 850.1300, GLP, static-renewal	*Daphnia magna*	21-day, reproduction: NOEC 0.11 (mm) EC_{10} 0.11 (mm)	CA-9.2.7.1
Algae			
OECD TG 201, OCSPP 850.4500, EC.C3, static	*Pseudokirchneriella subcapitata*	96-h: E_rC_{10} 0.25 (mm) NOE_rC 0.16 (mm)	CA-B.9.2.11.1

mm= mean measured concentrations

There were reliable data available on fish, *Daphnia* and algae. Solvent was used in all studies. In addition, the DS presented a *Chironomus* study (OECD TG 218) where the substance was applied to sediment. Based on the results of the sediment, pore water, and overlying water analyses, the majority of metyltetraprole applied remained associated with the sediment throughout the exposure and, thus, the study is not relevant for aquatic hazard classification.

The DS considered the 28-day NOEC for total length of 0.015 mg a.s./L to be the lowest chronic toxicity value.

Comments received during consultation

A National Authority (NA) commented the proposal. First, they noted that relevant bioaccumulation information was presented in the CLH Report but no conclusion on bioaccumulation was made. The DS answered by referring to the bioaccumulation criterion (B) classification limit of 2000 for the bioconcentration factor (BCF) used in PBT hazard classification. RAC notes that for aquatic hazard classification the limit for BCF is 500.

The NA also considered the OECD TG 229 Fish Short Term Reproduction Assay study potentially relevant for hazard classification given the endpoint, 21-day NOEC of 0.0092 mg a.s./L based on mean eggs per female per productive day, reflect population effects. This endpoint would lead to a more stringent hazard classification (Aquatic Chronic 1, M-factor of 10). The DS answered that 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 study protocols listed in the guidance, such studies for endocrine disruptors assessment should not be used for hazard classification. 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[1], it is only used as supportive information by RAC, and other available long-term tests were considered to be of higher relevance.

The NA commented also that additional information was available for amphibians although this does not appear to impact the hazard classification proposal. RAC notes that there is a valid OECD TG 231 study available in the CLH report. Mean measured NOEC of 0.019 mg a.s./L for *Xenopus laevis* was determined based on the hind-limb length normalised with snout-vent length. This effect is not considered relevant in aquatic hazard classification.

The NA also brought up that chronic data was 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. The DS answered that the lowest LC_{50} value obtained for fish was 0.048 mg a.s./L based on *Oncorhynchus mykiss* and no chronic data were available for this species. The lowest chronic NOEC value of 0.015 mg/L, however, was obtained for *Pimephales promelas* to which also an LC_{50} value of 0.061 mg a.s./L was available. Based on acute toxicity data LC_{50} values obtained for both fish being in the same range (0.048 mg a.s./L vs 0.061 mg a.s./L), the DS considers that the lowest NOEC value of 0.015 for *Pimephales promelas* could be used as surrogate. 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 which states that *"Chronic toxicity data (EC_x 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 strictest classification and M-factor."*

The NA was also unclear why the algal growth inhibition study was not considered reliable. 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.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS conclusion to consider metyltetraprole as not rapidly degradable based on:

- metyltetraprole was not readily biodegradable (15 % degradation in the OECD TG 301B test is below the 60 % pass level of the test)
- metyltetraprole was stable in the surface water mineralization study (OECD TG 309)
- metyltetraprole was hydrolytically stable (no degradation) in the OECD TG 111 test
- DT_{50}s in the water/sediment study (OECD TG 308) were from 428 to 715 days in the total system and does not therefore fulfill the cut-off criteria for the primary degradation half-life < 16 days in the aquatic environment.

[1] OECD TG 229 Chapter 3: This bioassay serves as an *in vivo* reproductive screening assay and its application should be seen in the context of the "OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals". In this Conceptual Framework the Fish Short Term Reproduction Assay is proposed at Level 3 as an *in vivo* assay providing data about selected endocrine mechanism(s)/pathway(s) (30).

Bioaccumulation

The DS did not conclude on bioaccumulation. RAC is of the opinion the metyltetraprole has potential for bioaccumulation based on:

- the BCF for fish of 526 L/kg for the whole fish in the OECD TG 305 test was greater than the cut-off for the BCF of 500.
- the measured log P_{ow} for metyltetraprole was 4.16 which is greater than the cut-off log Pow of 4

Aquatic toxicity

There are reliable acute toxicity data for fish, *Daphnia* and algae. RAC agrees with the DS conclusion that the 96-hour LC50 of 0.048 mg/L for *Oncorhynchus mykiss* was the lowest acute toxicity value.

There are also reliable chronic data for fish, *Daphnia* and algae. RAC agrees with the DS to consider the OECD TG 210 test with *Pimephales promelas* to give the lowest toxicity values. However, instead of using the 28-day NOEC (total length) of 0.015 mg/L for classification, RAC considers more appropriate to refer to the EC_{10} (wet weight) of 0.016 mg/L. The use of EC_{10} results is preferable to the use of NOECs for determining chronic aquatic toxicity since these are statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

Overall, RAC concludes that metyltetraprole warrants classification as **Aquatic Acute 1, M=10 and Aquatic Chronic 1, M=1**.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Metyltetraprole is very unlikely to undergo transport via air due to its low volatility (vapour pressure 3.6 x 10^{-9} Pa at 20 °C) and its fast degradation in air (DT_{50} of 0.052 days). The DS considers that therefore the compound will not be subject to significant concerns related to long range atmospheric transport and atmospheric accumulation and concludes that there is no evidence that metyltetraprole may present a danger to the structure and/or functioning of the stratospheric ozone layer.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS conclusion that the available evidence concerning the properties and the predicted or observed environmental fate and behaviour of metyltetraprole do not indicate that it may present a danger to the structure and/or functioning of the stratospheric ozone layer. Consequently, **no classification is warranted for hazards to the ozone layer**.

Additional references

- Additional histopathological investigations in animals from the low and mid-dose groups in rats (Report No TST-0176).

- Additional histopathological investigations in animals from the low and mid-dose groups in mouse (Report No TST-0181).
- Peer review report on selected findings and organs from the carcinogenicity study conducted in Han Wister rats (Report No TST-0177).
- Peer review report on selected findings and organs from the carcinogenicity study conducted in mice (Report No TST-0178).
- Statistics on selected pathology findings from the main study and from the additional histopathological investigations in animals from the low and mid-dose groups in Han Wistar rats (Report No TST-0176).
- Statistics on selected pathology findings from the main study and from the additional histopathological investigations in animals from the low and mid-dose groups in CD-1 mice (Report No TST-0175).
- Expert Panel assessment on selected neoplasms from dietary carcinogenicity studies performed with metyltetraprole (Report No TST-0179).
- Eniola, S., Stewart, J, 2023. VRY0054: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0188
- Mowat, V., Stewart, J, 2023. VRY0055: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0189

The updated position paper of the Applicant, Report No TST-0190 (original position paper No TST-0131)

Appendices

Appendix 1: Summary of additional data submitted by Applicant after the first consultation, and reviewed by the Dossier Submitter in the second, targeted consultation

After the first consultation, the following eight new documents containing additional information on carcinogenicity, were submitted by the Applicant to ECHA. A summary of the studies and their evaluation by the DS is reported below:

1) **Additional histopathological investigations in animals from the low- and mid-dose groups in rats (Report No TST-0176).**

In the carcinogenic study performed in rat via dietary route for 104 weeks and described in the CLH report, the histopathology was performed on groups 1 (control) and 4 (high dose, 20000 ppm) as well as on decedents and gross lesions from animals at 2000 and 6000 ppm (groups 2 and 3, respectively). In this new report the remaining animals and target tissues from groups 2 and 3 have been analysed to evaluate the possible effects of the test item on the incidences of malignant lymphoma and histiocytic sarcoma, malignant schwannoma in the uterus, and neoplasms of the mammary glands. The purpose of this study was to provide a standard statistical evaluation, i.e., Peto test. This evaluation was performed as an independent study according to GLP.

In the study, 20 animals per sex and group have been used for a 52-week toxicity testing period; 50 animals per sex and group have been used for 104-week toxicity testing period. The animals were allocated in the following way (Table below):

Table 1: Animal Allocation

Allocation and Dose Levels ppm	**Group 1 control 0**	**Group 2 2000**	**Group 3 6000**	**Group 4 20000**
Males				
52-week sacrifice	221-240	201-220	261-273, 275, 277-280, 282, 283	241-260
104-week sacrifice	51-100	1-50	151-200	101-150
Allocation and Dose Levels ppm	**Group 1 control 0**	**Group 2 2000**	**Group 3 6000**	**Group 4 20000**
Females				
52-week sacrifice	521-540	501-520	561-580	541-560
104-week sacrifice	351-400	301-350	451-500	401-450

The number of systemic neoplasms per sex and group are listed in the table below. None of the evaluated neoplasms were considered as a rare tumour, hence, only findings that revealed P values $p<0.005$ in a trend test and $p<0.01$ in a pairwise test were considered to be of statistical relevance. The Peto trend and pairwise test analysis did not reveal any statistically significant difference for any evaluated neoplasm.

The Armitage trend test did not reveal any statistical difference for mammary alveolar or atypical hyperplasia. Negative trend values were not judged (e.g., Hyperplasia alveolar, Trend144000.000- with a P-value 0.0122). Furthermore, the Fisher's Exact Test revealed no statistical significance for any of the tested tumour entities.

Table 2: Number of Systemic Neoplasms per Sex and Group (104-Week Animals only)

Dose Levels (ppm)	0		2000		6000		20000	
Number of Animals	50 M	50 F	50 M	50 F	50 M	50 F	50 M	50 F
Combined Schwannomas*	0	0	1	1	1	1	1	3
Oral cavity: malignant schwannoma	0	0	1	0	0	0	0	0
Skin: malignant schwannoma	0	0	0	0	1	0	1	0
Ovary: malignant schwannoma	0	0	0	0	0	1	0	0
Uterus: malignant schwannoma	0	0	0	1	0	0	0	1
Cervix: malignant schwannoma	0	0	0	0	0	0	0	1
Abdominal cavity: Malignant schwannoma	0	0	0	0	0	0	0	1
Combined systemic Neoplasms*	2	0	1	0	3	1	4	1
Malignant lymphoma	0	0	1	0	3	1	4	1
Histiocytic sarcoma	2	0	0	0	0	0	0	0
Combined mammary tumors (benign only)*	0	13	0	12	1	20	0	15
Mammary: hyperplasia, alveolar	1	38	0	43	0	42	0	30
Mammary: hyperplasia, atypical	0	2	0	6	0	5	0	0
Mammary: lipoma	0	0	0	0	1	0	0	0
Mammary: adenoma	0	1	0	2	0	2	0	4
Mammary: fibroadenoma	0	12	0	11	1	18	0	12
Combined mammary tumors (benign+malignant)*	0	16	0	12	1	21	0	19
Mammary: adenocarcinoma	0	4	0	1	0	3	0	7

*combined tumors may more than one primary tumor per animal, hence, this value is not simply an addition of all tumors, but on tumor bearers

2) Additional histopathological investigations in animals from the low and mid-dose groups in mouse (Report No TST-0181).

In the carcinogenic study performed in mice by diet for 78 weeks as described in the CLH report, the histopathology was performed as follows:

This additional study was conducted 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.

The study design was as follows:

Group	Treatment	Dietary concentration (ppm)*	Number of animals		Animal numbers*	
			Male	Female	Male	Female
2	S-2367 TG	700	51	51	52-102	352-402
3	S-2367 TG	2000	51	51	103-153	403-453

* only tissues from animals terminated at the schedule sacrifice and not examined in the study already reported in the CLH

In the study, only tissues from animals terminated at the scheduled sacrifice and not examined in the original study were reported. In summary, no metyltetraprole related neoplastic or non-neoplastic findings were noted following additional histopathological evaluation of the spleen, lymph nodes (axillary, submandibular, mesenteric), bone marrow of the sternum and femur, thymus, liver and lung in males and females and the uterus with cervix of females given 700 or 2000 ppm as reported in the two following tables:

Labcorp Study Number 8496120

Table 5.1: Histopathology – group distribution of neoplastic findings for animals killed after 78 weeks of treatment: Carcinogenicity phase (Additional Investigation dose group 700 or 2000 ppm)

TABLE 5.1 Histopathology - group distribution of neoplastic findings for animals killed after 78 weeks of treatment: Carcinogenicity phase (Additional Investigation dose group 700 or 2000 ppm) Request ID: 5496126

	Control	S-2367 TG		
Dose Group	1	2	3	4
Level (ppm)	0	700	2000	7000

		Number of animals affected			
	Group/Sex	2M	3M	2F	3F
Tissue/Organ and Findings	No. of animals	34	38	39	36
Bone, Femur Including Joint	No. examined	34	38	39	36
N-lymphoma	Total	1	1	2	3
Bone, Sternum Including Marrow	No. examined	34	38	39	36
N-lymphoma	Total	1	1	2	3
Hemopoietic System	No. examined	34	38	39	36
M-lymphoma	Total	2	1	2	3
Liver	No. examined	34	38	39	36
N-lymphoma	Total	2	1	1	3
M-carcinoma, hepatocellular	Total	1	1	0	0
B-adenoma, hepatocellular	Total	0	1	0	0
Lungs and Bronchi	No. examined	28	38	39	35
N-lymphoma	Total	1	1	2	2
M-adenocarcinoma, bronchioloalveolar	Total	0	1	2	0

Labcorp Study Number 8496120

TABLE 5.1 (cont) Histopathology - group distribution of neoplastic findings for animals killed after 78 weeks of treatment: Carcinogenicity phase (Additional Investigation dose group 700 or 2000 ppm) Request ID: 5496126

	Control	S-2367 TG		
Dose Group	1	2	3	4
Level (ppm)	0	700	2000	7000

		Number of animals affected			
	Group/Sex	2M	3M	2F	3F
Tissue/Organ and Findings	No. of animals	34	38	39	36
Lungs and Bronchi	No. examined	28	38	39	35
B-adenoma, bronchioloalveolar	Total	0	1	2	1
Lymph Node Axillary Lt	No. examined	32	34	32	32
N-lymphoma	Total	0	0	0	2
Lymph Node, Mandibular	No. examined	32	37	37	35
N-carcinoma	Total	1	0	0	0
N-lymphoma	Total	1	0	2	2
Lymph Node, Mesenteric	No. examined	33	38	39	36
N-lymphoma	Total	2	1	1	1
Spleen	No. examined	34	38	39	36
N-lymphoma	Total	2	0	1	3
M-hemangiosarcoma	Total	1	1	0	0

Labcorp Study Number 8496120

TABLE 5.1 (cont) Histopathology - group distribution of neoplastic findings for animals killed after 78 weeks of treatment: Carcinogenicity phase (Additional Investigation dose group 700 or 2000 ppm) Request ID: 5496126

	Control	S-2367 TG		
Dose Group	1	2	3	4
Level (ppm)	0	700	2000	7000

		Number of animals affected			
	Group/Sex	2M	3M	2F	3F
Tissue/Organ and Findings	No. of animals	34	38	39	36
Thymus	No. examined	34	38	38	27
N-lymphoma	Total	1	1	2	1
Uterine Cervix	No. examined	-	-	31	32
Uterus	No. examined	-	-	31	32
M-sarcoma, endometrial, stromal	Total	-	-	0	1
B-polyp, glandular	Total	-	-	2	0

3) Peer review report on selected findings and organs from the carcinogenicity study conducted in Han Wister rats (Report No TST-0177).

The purpose of the peer review was to confirm the presence or absence of previously recorded neoplasms in the main carcinogenicity study in rats. Then, 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, lung, and uterus and mammary glands from all animals were peer reviewed in order to confirm the absence or presence of systemic neoplasms.

The author of the peer review reported that there were little differences between the evaluation performed by the peer review panel and the one performed by pathologists in the main study. However, differences in terminology of two previously diagnosed cases of leukemia (change to malignant lymphoma, LGL type), one case of histiocytic sarcoma instead of fibrosarcoma in the skin, one case of fibroadenoma instead of adenoma in the mammary glands, and locations for malignant schwannomas different than previously described changed the outcome of the study.

The new list of cases is reported below:

- Animal numbers 33 and 132: Change leukemia to lymphoma, LGL-type;
- Animal number 90: Subcutaneous tissue/preputial gland fibrosarcoma changed to histiocytic sarcoma;
- Female number 334: Fibroadenoma in mammary gland: two masses were diagnosed as adenocarcinoma in the original report. One mass represent adenocarcinoma, one mass represents fibroadenoma;
- Female number 343: One mammary gland fibroadenoma changed to adenoma;
- - Female number 395: Lobular hyperplasia in mammary gland changed to fibroadenoma;
- Female number 404: Uterus and vagina were not affected. Malignant schwannoma was from an uncertain location but was present as a large mass adjacent to the uterus, and hence was considered a malignant schwannoma from the abdominal cavity;
- Female number 429: Uterus had an endometrial stromal polyp; The mass diagnosed as a malignant schwannoma was not present in the uterus but was located in the cervix with invasion into the vagina. This malignant schwannoma should, therefore, be reported under 'cervix' with metastasis in the vagina;
- Female number 429: Originally diagnosed cystadenocarcinoma of ovary should be rediagnosed as malignant granulosa cell tumour;
- Animal number 429: Malignant lymphoma was reported without any organ. In this case, the tumour was probably entered by mistake;

- Animal number 467: Adenocarcinoma was not reported in the clitoral gland.

4) Peer review report on selected findings and organs from the carcinogenicity study conducted in mice (Report No TST-0178).

The purpose of the peer review was to confirm or not the presence of previously diagnosed malignant sarcoma and histiocytic sarcoma of the original study conducted in CD-1 mice after metyltetraprole administration by diet for 78 weeks (i.e. the main study reported in CLH). 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 were peer reviewed in order to confirm the absence or presence of systemic neoplasms. The animals used were allocated in the following way:

Table 1: Animal Allocation

Allocation and Dose Levels ppm	**Group 1 control 0**	**Group 2 700**	**Group 3 2000**	**Group 4 7000**
Males	1-51	52-102	103-153	154-204
Females	301-307, 309-351, 506	352-402	403-453	454-477, 479-505

The differences noted and incidences changed are reported below:

- Animal number 13: Malignant lymphoma in thymus and spleen was not diagnosed;
- Animal number 14: Malignant lymphoma in thymus was diagnosed wrongly (atypical hyperplasia is not a tumour entity in CD-1 mice);
- Animal number 28: Malignant lymphoma in thymus was originally diagnosed as hyperplasia in thymus;
- Animal number 314: Malignant lymphoma in thymus, spleen and kidney, was diagnosed as hyperplasia in thymus and spleen;
- Animal number 317: Histiocytic sarcoma (spleen, lymph nodes, kidney), was not mentioned;
- Animal number 331: Malignant lymphoma in thymus and spleen, was diagnosed as hyperplasia in both organs;
- Animal number 350: Malignant lymphoma (thymus, spleen, lymph nodes, lung), was mentioned as hyperplasia in thymus and increased cellularity in lymph nodes;
- Animal number 423: Malignant lymphoma in thymus and lymph nodes, was diagnosed as hyperplasia in thymus;
- Animal number 433: Malignant lymphoma (thymus, spleen, lymph nodes) was diagnosed as hyperplasia in thymus;
- Animal number 301: Malignant lymphoma was included without any organ. In this case, the tumour was probably entered by mistake.

5) Statistics on selected pathology findings from the main study and from the additional histopathological investigations in animals from the low and mid-dose groups in Han Wistar rats (Report No TST-0176).

The purpose of this study was to provide a standard statistical evaluation, i.e., Peto test performed under GLP at AnaPath Services GmbH to the main Han Wistar rat study and to the additional histopathological data for the dose groups 2 and 3. This evaluation was performed as an independent study. Statistical evaluation of all neoplastic lesions was carried out applying the Peto test for positive trend (Peto *et al*., 1980), with respect to dose rates. In addition to a trend test over all groups, a pairwise comparison between groups was calculated using the Peto test. All calculations were made using the PathData system. The OECD Guidance Document 116 uses "a criterion of a statistically significant difference at one-tailed p-values of $p<0.025$ for trend

tests and p<0.05 for pairwise comparisons for rare neoplasms and one-tailed p-values of p<0.005 for trend tests and p<0.01 for pairwise comparisons for common neoplasms, respectively". In the report a statistical evaluation of all non-neoplastic findings in the mammary gland was carried out with the trend test according to Armitage (1955). The respective data on non-neoplastic lesions were given in tabular form under trend test statistics. For probability levels, all findings at P ⩽0.05 were deemed to be statistically significant. In addition, a Fisher's Exact test was performed on all described lesions. For common tumours, the probability value P<0.01 was deemed to be a valid criterion.

The analysis was performed on the following animals:

Table 1: Animal Allocation

Allocation and Dose Levels ppm	**Group 1 control 0**	**Group 2 2000**	**Group 3 6000**	**Group 4 20000**
Males				
52-week sacrifice	221-240	201-220	261-273, 275, 277-280, 282, 283	241-260
104-week sacrifice	51-100	1-50	151-200	101-150
Allocation and Dose Levels ppm	**Group 1 control 0**	**Group 2 2000**	**Group 3 6000**	**Group 4 20000**
Females				
52-week sacrifice	521-540	501-520	561-580	541-560
104-week sacrifice	351-400	301-350	451-500	401-450

The number of systemic neoplasms per sex and group are listed below:

Table 2: Number of Systemic Neoplasms per Sex and Group (104-Week Animals only)

Dose Levels (ppm)	**0**		**2000**		**6000**		**20000**	
Number of Animals	**50 M**	**50 F**	**50 M**	**50 F**	**50 M**	**50 F**	**50 M**	**50 F**
Combined Schwannomas*	0	0	1	1	1	1	1	3
Oral cavity: malignant schwannoma	0	0	1	0	0	0	0	0
Skin: malignant schwannoma	0	0	0	0	1	0	1	0
Ovary: malignant schwannoma	0	0	0	0	0	1	0	0
Uterus: malignant schwannoma	0	0	0	1	0	0	0	1
Cervix: malignant schwannoma	0	0	0	0	0	0	0	1
Abdominal cavity: Malignant schwannoma	0	0	0	0	0	0	0	1
Combined systemic Neoplasms*	2	0	1	0	3	1	4	1
Malignant lymphoma	0	0	1	0	3	1	4	1
Histiocytic sarcoma	2	0	0	0	0	0	0	0
Combined mammary tumors (benign only)*	0	13	0	12	1	20	0	15
Mammary: hyperplasia, alveolar	1	38	0	43	0	42	0	30
Mammary: hyperplasia, atypical	0	2	0	6	0	5	0	0
Mammary: lipoma	0	0	0	0	1	0	0	0
Mammary: adenoma	0	1	0	2	0	2	0	4
Mammary: fibroadenoma	0	12	0	11	1	18	0	12
Combined mammary tumors (benign+malignant)*	0	16	0	12	1	21	0	19
Mammary: adenocarcinoma	0	4	0	1	0	3	0	7

*combined tumors may more than one primary tumor per animal, hence, this value is not simply an addition of all tumors, but on tumor bearers

In summary, none of the evaluated neoplasms was considered as a rare tumour, hence, only findings that reveal p<0.005 for a trend test and p<0.01 for a pairwise test was considered to be of statistical relevance. The Peto trend and pairwise test analysis did not reveal any statistically significant difference for any evaluated neoplasm.

The Armitage trend test did not reveal any statistical difference for alveolar or atypical hyperplasia. Negative trend values have not been judged (e.g. Hyperplasia alveolar, Trend 144000.000- with a P-value 0.0122). Furthermore, the Fisher's Exact test revealed no statistical significance for any of the tested tumour entities.

6) Statistics on selected pathology findings from the main study and from the additional histopathological investigations in animals from the low and mid-dose groups in CD-1 mice (Report No TST-0175).

In the main study metyltetraprole was administered by diet to CD-1 Mice for 78 weeks. Histopathology was performed on groups 1 (control) and 4 (high dose, 7000 ppm) as well as on decedents and gross lesions from animals at 700 and 2000 ppm (groups 2 and 3, respectively). Due to possible effects by the test item for incidences of malignant lymphoma and histiocytic sarcoma, the remaining animals and target tissues from groups 2 and 3 were analysed in an additional histopathological examination study. The purpose of this study was to provide a standard statistical evaluation, i.e., Peto test performed under GLP at AnaPath Services GmbH. This evaluation was performed on all animals from the study (reported under two different reports) as an independent study.

The analysis was performed on the following animals:

Table 1: Animal Allocation

Allocation and Dose Levels ppm	Group 1 control 0	Group 2 700	Group 3 2000	Group 4 7000
Males	1-51	52-102	103-153	154-204
Females	301-307, 309-351, 506	352-402	403-453	454-477, 479-505

The number of systemic neoplasms per sex and group are listed in Table below:

Table 2: Number of Systemic Neoplasms per Sex and Group

Dose Levels (ppm)	0		700		2000		7000	
Number of Animals	51 M	51 F	51 M	51 F	51 M	51 F	51 M	51 F
Malignant lymphoma	7	9	6	8	8	14	8	9
Histiocytic sarcoma	0	2	0	0	0	3	0	3

M=male, F=female

In summary, none of the evaluated neoplasms is considered as a rare tumour, hence, only findings that reveal p<0.005 for a trend test and p<0.01 for a pairwise test are considered to be of statistical relevance.

The Peto trend and pairwise test analysis did not reveal any statistically significant difference for any evaluated neoplasm. Furthermore, the Fisher's Exact test revealed no statistical significance for any of the tested tumour entities.

7) Expert Panel assessment on selected neoplasms from dietary carcinogenicity studies performed with metyltetraprole (Report No TST-0179).

An Expert Panel commissioned by the Applicant evaluated all the available information (main/original study, additional histopathological data and statistical analysis) for both rats and mice. The final data are reported below:

Table 7: Number of Systemic Neoplasms per Sex and Group in CD-1 mice

Dose Levels (ppm)	**0**		**700**		**2000**		**7000**	
Number of Animals	**51 M**	**51 F**	**51 M**	**51 F**	**51 M**	**51 F**	**51 M**	**51 F**
Malignant lymphoma	7	9	6	8	8	14	8	9
Histiocytic sarcoma	0	2	0	0	0	3	0	3

M-males, F-Females

In the statistical evaluation performed for all data, neither the Peto nor the Fisher's Exact revealed P-values $p<0.005$ for a trend test and $p<0.01$ for a pairwise test for any systemic neoplasia separately or combined.

Table 8: Summary on diagnoses on selected organs made by EP members (CD-1 mice)

Animal Number	13	14	314	317	331	350	423	433
Organ	Thymus, Spleen	Thymus	Thymus, Spleen, Kidney	Spleen, Lymph node, Kidney	Thymus, Spleen	Thymus, Spleen, Lymph node, Lung Liver	Thmyus, Lymph node	Spleen, Lymph node
Pathologist	Findings							
1	ML	ML	ML	HS	ML	ML	ML	ML
2	ML	ML	ML	HS	ML	ML	ML	ML
3	ML	ML	ML	HS	ML	ML	ML	ML
4	ML	ML	ML	HS	ML	ML	ML	ML
5	ML	ML	ML	HS	Ml	ML	ML	ML
6	ML	ML	ML	HS	ML	ML	ML	ML
7	ML	ML	ML	ML (pleomorphic)	ML	ML	ML	ML
8	ML	ML	ML	HS	ML	ML	ML	ML
Consensus	ML	ML	ML	HS	ML	ML	ML	ML

ML: Malignant Lymphoma, HS: Histiocytic Sarcoma

Table 10: Summary on diagnoses on selected organs made by EP members (RccHan™:WIST rats)

Rat	90	33	343	395	404	429	429	132
Organ	Subcutaneous tissue	Spleen	Mammary gland	Mammary gland	Mass/uncertain location	Uterus	Mass/uncertain location	Various
Pathologist	Finding							
1	HS	HS	Adenoma	Fibroadenoma	Malignant schwannoma, Uterus/cervix, not remarkable	Endometrial stromal polyp	Histiocytic sarcoma	No scan provided
2	HS	ML	Adenoma	Fibroadenoma	Schwannoma, malignant (mass only)	Polyp, endometrial	Histiocytic sarcoma	No scan provided
3	Fibrosarcoma (preputial gland)	ML	Adenocarci-noma (in situ)	Lobular hyperplasia	No definitice diagnose possible, possibly malignant schwannoma	Stroma polyp, benign (Vagina: stromal hyperplasia)	Cervix, schwannoma malignant	No scan provided
4	HS	ML	Adenoma	Fibroadenoma	Malignant schwannoma uterus/cervix: not remarkable	Endometrial stromal polyp	Malignant schwannoma (likely from vagina region)	No scan provided
5	malignant schwannoma	ML	Fibroadenoma	Fibroadenoma	Stromal sarcoma Mass: cervix/vagina?	Endometrial stromal polyp	stromal sarcoma	No scan provided

Rat	90	33	343	395	404	429	429	132
Organ	Subcutaneous tissue	Spleen	Mammary gland	Mammary gland	Mass/uncertain location	Uterus	Mass/uncertain location	Various
Pathologist	Finding							
6	HS	HS	Adenoma	Fibrodenoma	Schwannoma, malignant		HS (slide 51), Schwannoma malignant (slide 52)	No scan provided
7	Fibrosarcoma, preputial gland	Lymphoma malignant, LGL type	Adenocarci-noma (early)	Fibrodenoma	Schwannoma malignant(uterus/cervix: not remarkable)	Stroma polyp, Vagina, stomal hyerplasia	Schwannoma malignant, cervix	No scan provided
8	HS	Lymphoma malignant, LGL type	Adenoma	Fibrodenoma	Schwannoma malignant, uterus/cervix: not remarkable, likely abdominal cavity	stromal polpy	Schwannoma malignant. in vagina	Lymphoma LGL-type
Consensus	**HS**	**Lymphoma, LGL type**	**Adenoma**	**Fibroade-noma**	**Schwannoma, malignant (mass only), not in uterus**	**Polyp, endometrial, stromal**	**Malignant schwannoma (cervix or vagina)**	**Lymphoma, LGL-type**

The Expert Panel concluded:

None of the evaluated neoplasms in both studies can be considered as rare tumours. Hence, only findings that reveal P values $p<0.005$ for trend test and $p<0.01$ for pairwise comparison are considered to be of statistical relevance.

In rats for non-neoplastic findings in mammary glands, the Armitage trend test did not reveal any statistical difference for alveolar or atypical hyperplasia. Finally, the Fisher's Exact test revealed no statistical significance for any of the tested tumour entities.

The increase of neoplasms was within the HCD range. For malignant schwannomas no multi-site response was established. Sex related occurrence of neoplasia was not observed. There were no pre-neoplastic and benign neoplasms supporting a treatment-related progression to malignancy. In mammary glands in female rats, both adenoma and adenocarcinoma were observed, but lacked pre-neoplastic glandular hyperplasia.

Most neoplasms appeared at a late stage of treatment and were observed in animals sacrificed after 1.5 or 2 years of treatment. The Peto trend test and pairwise test did not reveal statistically significant differences.

Neoplasms, except for one malignant lymphoma in males, were not observed in rats at an interim sacrifice after one year of treatment. The single case was noted in one male at 20000 ppm in week 37. This tumour entity is however not unusual in young adult animals, and therefore, this single case was not considered to be treatment-related.

8) Position paper of the Applicant on CLH proposal for carcinogenicity classification of metyltetraprole (Report No TST-0131).

In a position paper, the applicant firstly scrutinised all existing data on metyltetraprole including newly available data (i.e., additional histopathological examination, statistical analysis, peer review and Expert Panel review) and published information, and secondly evaluated the biological plausibility of the slightly higher incidence of the tumours listed above, considering the strength of the evidence according to Regulation (EC) No 1272/2008 and based on the ECHA 'Guidance on the Application of the CLP Criteria' version 5.0 July 2017.

The corrected incidence of tumours in rats are shown in Tables 1 and 2 of the position paper (see below), and the Peto trend test and pairwise tests were carried out using these incidences.

There was no evidence that metyltetraprole had any effects on the tumour profile of Han Wistar rats, but the slightly higher incidences of some tumours were observed in both males and females. In both sex administered 6000 and 20000 ppm malignant lymphomas were observed, although there was no statistical significance in the Peto trend test and pairwise test. Other tumours were only noted in females given 20000 ppm lacking statistical significance in the Peto trend test and pairwise test. All tumours were not associated with pre-neoplastic or related non-neoplastic lesions, which was also supported by the lack of statistical difference in the Armitage trend test for alveolar or atypical hyperplasia in rats for non-neoplastic findings in mammary glands. Overall, the Expert Panel concluded that none of the tumours for which the DS had a concern are considered as treatment-related (Expert Panel, Ref No. TST-0179).

The results of carcinogenicity study in rats are reported in the following two tables:

Table 1. Incidence of tumour that ANSES pointed out and differences in diagnosis of tumours (male rats, hemopoietic system)

Dose (ppm)	0	2000	6000	20000	PETO trend	HCD range*
No. of animals	50	50	50	50		
Malignant lymphoma	0	0⇒**1** (0⇒**2.0%**) (P=0.1401)	3 (6.0%) (P=0.0409)	3⇒**4** (6.0⇒ **8.0%**) (P=0.0146)	0.0102	0-9.1%
Histiocytic sarcoma	1⇒**2** (2.0⇒ **4.0%**)	0	0	0	0.1003	-

Values represent the incidence and parenthesis represents the incidence in percentage in each group.
The orange-highlight indicate treatment-related findings judged by ANSES.
Bold: Corrected incidence following the peer review and the expert panel review.
P-values of the one-tailed PETO trend tests are shown in "PETO trend" columns, and p-values of the one-tailed PETO pairwise test against control are shown as "P" in parentheses in each group. There were no statistically significant changes ($p<0.005$ for trend and $p<0.01$ for pair-wise) (see section 3-A iii).
*: Historical control data (HCD) range in the test facility (2008-2018) (see section 3-A iii) (Samuels,

Table 2. Incidence of tumour that ANSES pointed out and differences in diagnosis of tumours (female rats)

Dose (ppm)	0	2000	6000	20000	PETO trend	HCD range*
No. of animals	50	50	50	50		
Hemopoietic system Malignant lymphoma	0	0	1 (2.0%) (P=0.1587)	2⇒**1** (4.0⇒**2.0%**) (P=0.1515)	0.1314	0-4.0%
Uterus Malignant Schwannoma	0	1 (2.0%) (P=0.1685)	0	3⇒**1** (6.0⇒**2.0%**) (P=0.1539)	0.2119	0-3.6%
Uterine cervix Malignant Schwannoma	0	0	0	0⇒**1** (0⇒**2.0%**) (P=0.1469)	0.0384	-
Abdominal cavity Malignant Schwannoma	0	0	0	0⇒**1** (0⇒**2.0%**) (P=0.1492)	0.0465	-
Mammary gland Adenoma	1 (2.0%)	1⇒**2** (2.0⇒**4.0%**) (P=0.3050)	2 (4.0%) (P=0.1949)	4 (8.0%) (P=0.0885)	0.0968	0-13.3%
Mammary gland Fibroadenoma	12	10⇒**11** (P=0.3745)	18 (P=0.0985)	12 (P=0.4274)	0.3632	-
Mammary gland Adenocarcinoma	4 (8.0%)	1 (2.0%) (P=0.0764)	3 (6.0%) (P=0.3483)	7 (14.0%) (P=0.1469)	0.0212	0-23.1%

Values represent the incidence and parentheses represent the incidence in percentage in each group.
The orange-highlight indicate treatment-related findings judged by ANSES.
Bold: Corrected incidence following the peer review and the expert panel review.
P-values of the one-tailed PETO trend tests are shown in "PETO trend" columns, and p-values of the one-tailed PETO pairwise test against control are shown as "P" in parentheses in each group. There were no statistically significant changes ($p<0.005$ for trend and $p<0.01$ for pair-wise) (see section 3-A iii).
*: Historical control data (HCD) range in the test facility (2008-2018) (see section-3 A iii) (Samuels, 2023)

Based on the conclusion of the peer review and the expert panel review, the corrected incidence of tumours in mice are shown in Tables 3 and 4, and the Peto trend test and pairwise test were carried out using these incidences (Ref No. TST-0175). There were no evidences that Metyltetraprole had any effects on the tumour profile of CD-1 mice. Although there were no clear dose-relationships nor statistical significance, the slightly higher incidences of two types of tumour were observed in males or females given 2000 and 7000 ppm as summarized in Tables

3 and 4, but all lacked pre-neoplastic or related non-neoplastic lesions. Overall, none of the tumours of DS concern are considered as treatment-related by the Expert Panel (Ref No. TST-0179).

The results of the carcinogenicity study in mouse are reported in the following tables:

Table 3. Incidence of tumour that ANSES pointed out and differences in diagnosis of tumours (male mice, hemopoietic system)

Dose (ppm)	0	700	2000	7000	PETO trend	HCD range*
No. of animals	51	50	50	51		
Malignant lymphoma	5⇒**7** (9.8⇒**13.7%**)	6 (12.0%) (P=0.4207)	8 (16.0%) (P=0.4091)	8 (15.7%) (P=0.4368)	0.3594	0-12.0%

Values represent the incidence and parentheses represent the incidence in percentage in each group.
Bold: Corrected incidence following the peer review and the expert panel review.
P-values of the one-tailed PETO trend tests are shown in "PETO trend" columns, and p-values of the one-tailed PETO pairwise test against control are shown as "P" in parentheses in each group. There were no statistically significant changes ($p<0.005$ for trend and $p<0.01$ for pair-wise) (see section 3-A iii).
The orange-highlight indicate treatment-related findings judged by ANSES.
*: Historical control data (HCD) range in the test facility (2002-2018) (see section 3-A iii) (Samuels, 2023).

Table 4. Incidence of tumour that ANSES pointed out and differences in diagnosis of tumours (female mice, hemopoietic system)

Dose (ppm)	0	700	2000	7000	PETO trend	HCD range*
No. of animals	51	50	51	51		
Histiocytic sarcoma	1⇒**2** (2.0⇒**3.9 %**)	0	3 (5.9 %) (P=0.3632)	3 (5.9 %) (P=0.3121)	0.1003	0-7.7 %
No. of animals	51	50	51	51		
Malignant lymphoma	8⇒9 (15.7⇒**17.6%**)	8 (16.0%) (P=0.3859)	12⇒**14** (23.5⇒**27.5%**) (P=0.1401)	9 (17.6%) (P=0.4781)	0.4745	0-23.5%

Values represent the incidence and parentheses represent the incidence in percentage in each group.
Bold: Corrected incidence following the peer review and the expert panel review.
P-values of the one-tailed PETO trend tests are shown in "PETO trend" columns, and p-values of the one-tailed PETO pairwise test against control are shown as "P" in parentheses in each group. There were no statistically significant changes ($p<0.005$ for trend and $p<0.01$ for pair-wise) (see section 3-A iii).
The orange-highlight indicate treatment-related findings judged by ANSES.
*: Historical control data (HCD) range in the test facility (2002-2018) (see section 3-A iii) (Samuels, 2023).

The Applicant concluded that, taking into account the peer review and the examination of scanned slides by the Expert Panel of experienced toxicologic pathologists, the corrected incidences must be used for the interpretation of results of the carcinogenicity studies with metyltetraprole, rather than the original reported data (main carcinogenicity study in rats and mice).

The Applicant claimed that there is no dose-dependent increase in tumour incidence as reported in a trend test (i.e. Peto trend test) that was typically used. As shown in above four tables, there was no statistically significant positive trend in all tumours in rats and mice.

In addition, the toxicokinetic (TK) investigation, which was performed only in the rat carcinogenicity study (main study), indicated that generally the rate and extent of systemic exposure of rats to metyltetraprole increased with increasing dietary concentration range from 2000 to 20000 ppm during weeks 4, 13, 26 and 52. However, these increases were less than the proportionate dose increment, which suggest saturation of absorption (see table below).

Although a trend test is more powerful than the pair-wise test, a complication is that a trend test may fail to detect curvilinear responses which might arise from non-linear effects such as complications from saturation (OECD, 2012). Given the results of TK, the systemic exposure of rats to metyltetraprole appeared to be characterised by nonlinear kinetics over the dietary concentration range from 2000 to 20000 ppm, and therefore curvilinear responses were assumed in the carcinogenicity studies. In such situations, the pair-wise tests will give more appropriate results (OECD, 2012). As stated in the above section, there was no statistically significant pairwise comparison between concurrent control and the treated groups, also ensuring the lack of dose-dependent increases for any of tumours for which the DS had a concern.

Table 5. Summary of AUC_{24} of S-2367

Level (ppm)	AUC_{24} (ng.h/mL)							
	Week 4		Week 13		Week 26		Week 52	
	Males	Females	Males	Females	Males	Females	Males	Females
2000	668 (71)	2710 (650)	316 (63)	1290 (170)	301 (72)	1490 (360)	379 (171)	1270 (180)
6000	1630 (920)	4990 (1720)	621 (228)	2910 (720)	508 (152)	2600 (460)	529 (236)	2890 (590)
20000	2280 (290)	4830 (1490)	885 (106)	2990 (680)	735 (42)	2680 (320)	902 (123)	3700 (1690)

Parentheses represent standard deviations.

As reported in the updated Report No TST-0100, the Applicant used for HCD analysis all studies performed with the test item, and in particular the new HCD:

- for rats; the year range 2008-2018,
- for mice; the year range 2002-2018.

As shown in the above tables (1-4), most of the tumours of concern were within the range of incidences seen in the performing laboratory. The incidences of malignant lymphomas in male mice (15.7 % and 16.0 %) were slightly higher than the HCD (0-12.0 %) but lacked statistical significance and clear dose-response. It is noteworthy that the incidence of malignant lymphomas in control males (7/51=13.7 %) was significantly higher than the mean value of the HCD (5.9 %) and exceeded the upper range of the HCD (12.0 %) suggesting that animals used in this study were derived from a batch susceptible to malignant lymphoma.

Based on both the strength and weight of evidence evaluation, the Applicant concluded that classification of carcinogenicity is not warranted for metyltetraprole, as summarised below:

Applicant's view on 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 tumour 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 did not have any hormonal effects, genotoxicity, or any published adverse outcome pathways (AOP);
- 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 are common tissues where such tumours occur spontaneously as well;
- Mammary gland tumours were common spontaneous tumours which occurred in association with spontaneous pituitary proliferative lesions in the female rat;
- Malignant lymphomas were common spontaneous tumours in rats and mice;
- The Expert Panel consisting of multiple worldwide expert pathologists concluded that all tumours for which DS had a concern were not treatment-related.

Applicant's comparison with CLP criteria (paragraph 3.6.2.3.2):

(a) Tumour type and background incidence: Some types of tumours were noted, but almost within the HCD range.

(b) Multi-site responses: No clear evidence of multi-site responses.

(c) Progression of lesions to malignancy: No evidence of progression of lesions to malignancy.

(d) Reduced tumour 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 males or females.

(f) Whether responses are in a single species or several species: No clear evidence that the responses were observed in any examined species.

(g) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity: metyltetraprole is 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 (MoA) and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity: No toxicity data supporting particular MoAs for carcinogenicity.

Dossier submitter's assessment of the eight new studies provided by the Applicant

In the analysis of the new available data the DS revealed several discrepancies.

Carcinogenic potential in mice

A contradiction between the declaration reported in the main carcinogenicity study and the additional histopathological investigation:

- In the additional histopathological examination, the author declared that the animals killed or dying during the study were not examined (this was already done in the main study). However, it seemed 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 Report No TST-0181: "only tissues from animals terminated at the schedule sacrifice and not examined in the main study". Therefore, for these animals, it seemed that histopathological examination was conducted twice (i.e. in the main carcinogenicity study and in the additional histopathological examination).
- DS considered surprising that for some animals, results of the additional histopathological investigation were not consistent with the results obtained in the main study and in the peer review analysis report. For example, for male number120 (mid-dose level), and female numbers 0383 (low dose) and 0405 (mid dose) no histopathological finding was noted for haematopoietic 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 number 120 are shown in the figure below.

Figure: comparison of information on the mice number 120 between the main and additional histopathological examination

Main study:

	Control	S-2367		
Dose Group	1	2	3	4
Level (ppm)	0	700	2000	7000

Group /Sex	Animal Number	Day (Week) of Death	Phase	Macropathology findings	Histopathology findings
3M	0120	556 (80)	Treatment		Hemopoietic System: M-lymphoma, unrelated
				Pancreas: Pale area(s), </=1mm, 5+ (many)	Examined, not correlated
				Preputial/Clitoral Glands: Mass(es), firm, right, pale and dark, 10-19mm, 1 (one)	= Preputial/Clitoral Glands: Abscess, moderate
				Spleen: Mass(es), mid region, firm, pale and dark, 2-9mm, 1 (one)	= Spleen: N-lymphoma, metastasis, originating finding: Hemopoietic System, Lymphoma

Tissues without comments under macropathology findings were within normal limits at necropsy. The following examined tissues had no histopathology findings, notes, or comments:
Pancreas

Report TST-0181:

	Control	S-2367 TG		
Dose Group	1	2	3	4
Level (ppm)	0	700	2000	7000

Group /Sex	Animal Number	Day (Week) of Death	Phase	Tissue	Findings
3M	0120	556 (80)	Treatment	Liver	Infiltrate, Inflammatory Cell, minimal, focal, lymphocytic Vacuolation, Hepatocellular, slight, multifocal
				Bone, Femur Including Joint; Bone, Sternum Including Marrow; Hemopoietic System; Lungs and Bronchi; Lymph Node Axillary Lt; Lymph Node, Mandibular; Lymph Node, Mesenteric; Spleen; Thymus	No histopathology findings

DS found several other discrepancies. For example, in the position paper of the Applicant (Report No TST-0131), it is reported that one additional female of the control group showed malignant

lymphoma. The DS checked the raw data of the different reports to understand this: 3 additional females were diagnosed with M-lymphoma (numbers 0314, 0331, 0350), 1 female (number 0301) was not confirmed to bear M-lymphoma and 1 female (number 317) was diagnosed with histiocytic sarcoma in the peer review (Report No TST-0178) 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.

In addition, the high number of new cases of lymphoma diagnosed after the peer review analysis, particularly in the male and female control groups, DS considered doubtful and raising uncertainties in either the first assessment available in the main study report (the main carcinogenicity study) or the peer review assessment (Report No TST-0178).

Furthermore, DS did not understand 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 group.

To facilitate the analysis of the data the DS provided additional tables (reported below). For each type of tumour considered treatment-related in the CLH report, the tables described the incidences reported in the different reports (main study, additional histopathological examination and peer review).

Tumours of the haematopoietic system in mice

Lymphomas in mice

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, one mid-dose male number 120, one low-dose female number 0383 and one mid-dose female number 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 main study, particularly in the control groups in both sexes. They were two control males (numbers 0013 and 0014) and three control females (numbers 0314, 0331 and 0350). On the other hand, two control females were considered misdiagnosed in the main study, i.e. number 0301 was considered by the peer review experts as a mistake because malignant lymphoma was included without any organ affected and number 0317 diagnosed with a histiocytic sarcoma instead of a lymphoma.

In addition, two females from the mid-dose group (numbers 0423 and 0443) were diagnosed with lymphoma in the peer review but not in the main study. The high number of discrepancies between the initial study report and the peer-review analysis in DS view questioned the validity of the histopathological investigations in these studies (either initial or peer-review analysis).

The statistical analysis conducted by the Applicant in its position paper (Report No TST-0131) was reported for 50 animals in the low and mid-dose male groups and in the low-dose female group, whereas the number of animals per group was 51 and it was not explained why only 50 animals were included in these groups.

DS prepared its own comparison of discrepancies and statistical analysis, as shown in the table below:

Table on tumours of the haematopoietic system in mice

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]		*0423* [h] *0433* [i]	
					Not confirmed: *0301* [f] *0317* [g]			
Overall incidences considering the original study report, the additional histopathological investigation and the peer review on selected findings/organs								
n=	51	51[1] 50[2]	51[1] 50[2]	51	51	51[1] 50[2]	51	51
Lymphomas	7	6	8	8	9	8	14	9
%	13,7%	11,8%[1] 12%[2]	15,7%[1] 16%[2]	15,7%	17,6%	15,7%[1] 16%[2]	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=0.1401	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 number 13: malignant lymphoma (thymus, spleen), was not diagnosed"
b "Animal number 14: malignant lymphoma (thymus), was diagnosed wrong (atypical hyperplasia is not a tumour entity in CD-1 mice)"
c "Animal number 314: malignant lymphoma (thymus, spleen, kidney), was diagnosed as hyperplasia in thymus and spleen"
d "Animal number 331: malignant lymphoma (thymus, spleen), was diagnosed as hyperplasia in thymus and spleen"
e "Animal number 350: malignant lymphoma (thymus, spleen, lymph nodes, lung), was mentioned as hyperplasia in thymus and increased cellularity in lymph nodes"
f "In animal number 301, malignant lymphoma was included without any organ noted to be affected. In this case, the tumour was probably entered by mistake"
g "Animal number 317: histiocytic sarcoma (spleen, lymph nodes, kidney), was not mentioned"
h "Animal number 423: malignant lymphoma (thymus, lymph nodes), was diagnosed as hyperplasia in thymus"
i "Animal number 433: malignant lymphoma (thymus, spleen, lymph nodes) was diagnosed as hyperplasia in thymus""

Histiocytic sarcomas in mice

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 number 0426 (mid-dose level, killed after 78-week and already analysed in the main study) no histopathological change was found for haematopoietic system or uterus in the additional histopathological investigation, whereas "*Haematopoietic system: M-sarcoma, histiocytic' and 'Uterus: N sarcoma, histiocytic, metastasis*" was reported in the main study and was 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 (number 0317, originally diagnosed with lymphoma).

The statistical analysis provided by the Applicant in its position paper (Report No TST-0131) contained 50 animals in the low and mid-dose male groups and in the low-dose female groups, whereas actually the number of animals per group was 51. In the up-dated position paper (Report No TST-190) the final tables and statistics are reported. (For transparency in the above tables of carcinogenicity in mice both the DS and the Applicant assessment is reported based on 50 or 51 animals. Anyway, this change (50 or 51 animals) does not affect the overall conclusion.)

DS prepared its own statistical analysis, as shown in the table below:

Table on histiocytic sarcomas in female mice

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[1] 50[2]	51	51
Histiocytic sarcomas	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 are reported in the final assessment
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

The DS analysed all the new material on neoplastic findings in rats provided by the Applicant, and DS found the following uncertainties. In the additional histopathological examination (Report No TST-0176) the author declared that animals killed or dying during the study were not examined (but this was already done in the main study). However, it seemed that data for animals terminated at the scheduled sacrifice were (re-)examined. This re-assessment of scheduled terminated animals included those already examined in the main study where 'abnormalities' were found. This is in contradiction with the statement in the Report No TST-0180: "only tissues from animals terminated at the schedule sacrifice and not examined in VRY0054 (ref. the main carcinogenicity study in rats)". Therefore, for these animals, it seemed that histopathological examination was conducted twice. Sometimes the results of this additional histopathological investigation were not consistent with the results obtained in the main study and in the peer review analysis report.

For example, for male number 0187 (mid-dose level): No histopathological change 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 number 0301 (low-dose level): No histopathological change 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 female numbers 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 DS's doubts on the assessments provided in the different reports.

For each type of tumour considered treatment-related in the CLH report, the tables below, provided by the DS, describe the incidences reported in the different reports (main carcinogenicity study, additional histopathologic examination and peer review).

Malignant lymphomas in rats

In the additional histopathological investigation (Report No TST-0180), one male of the mid-dose group (number 0187) had a malignant lymphoma and this was already reported in the main study. Moreover, in the peer review of histopathological findings (Report No TST-0177) one male of the low dose group (number 0033) and one male of the high dose group (number 132) were diagnosed with lymphoma in the peer review, but not in the main carcinogenicity study. In females, one female from the high dose group (number 429) seems to have been misdiagnosed in the main study. According to the peer review experts, malignant lymphoma was included without any organ affected and they considered that this was probably entered by mistake. The DS considered this assumption uncertain.

DS prepared its own statistical analysis, as shown in the two tables below:

Table on malignant lymphoma in male and female rats

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 numbers 0033 and 0132 changed to lymphoma, LGL-type"
b "In animal number 429, malignant lymphoma was included without any organ noted to be affected. In this case, the tumour 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 showed histiocytic sarcomas.

Following the peer review of histopathological findings, two females of the high dose group (out of three initially diagnosed with malignant schwannoma in the uterus) were considered 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 number 0404 and in the uterine cervix ('cervix' with metastasis in the vagina) for female number 0429.

The DS noted that malignant schwannomas are tumours originated from nerve sheath, which can arise from different organs, and as female reproductive organs are especially affected DS considered appropriate to combine malignant schwannomas from different organs.

DS prepared its own statistical analysis, as shown in the table below:

Table on malignant uterine schwannomas in female rats

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	*0%, 0%, 0%, 0%* *Mean: 0.0%; range: 0-0%*			
[HCD not available for other organs]				
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			
One-tailed Peto pairwise test (applicant)[2]	-	0.0668	0.1170	0.0352
One-tailed Peto trend test (applicant)[2]	0.0869			

[1] DS/RMS assessment
[2] Applicant assessment
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report:
a " Malignant schwannoma in female number 404: Uterus and vagina are not affected. This tumour 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 peer review".
b " Malignant schwannoma in female number 429: Uterus has an endometrial stromal polyp (Figure 14 of Report No TST-0177). 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 tumours in rats:

In the additional histopathological investigation (Report No TST-0180) no animals of the low and mid-dose level killed at the terminal sacrifice were reported to show mammary tumours.

However, for female number 0301 (low-dose level, killed after 104-week and analysed in the main study already) no histopathological change 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 (Report No TST-0177). Similarly, female numbers 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 (number 0343 originally diagnosed with fibroadenoma).

DS prepared its own statistical analysis, as shown in the table below:

Table on mammary tumours in female rats

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-w	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 adenoma AND 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
	%	10%	6%	8% Animal number 0487 showed both adenoma and adenocarcinoma	20% Animal number 0402 showed both adenoma and adenocarcinoma
	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
[2] Applicant assessment
WE: Euthanized for welfare reasons
FD: Found dead
Peer review report:
a "Adenoma in mammary gland in female number 343: one fibroadenoma changed to adenoma"

<u>DS's updated overall conclusion on rat and mouse carcinogenicity (after the CLH report submission and information becoming available in the consultations)</u>

The DS considered that the new data provided by the Applicant did not change the DS's initial assessment in the CLH report (DAR Vol 1).

As detailed above, DS noted some inconsistencies in the different documents regarding tumour incidences in the original study report and in the additional histopathological investigation and peer review histopathology reports. Therefore, the re-assessment provided lacks transparency. Disagreement for low and mid doses in the additional histopathology examination raised concerns in DS.

Important discrepancies were noted between the incidences of some tumours reported by the original pathologist in the study report and by the peer reviewer pathologists. Therefore, DS had

doubts in the various assessments provided by the Applicant. DS had concern on the methodology and possible bias followed in the additional peer review. It was unclear to DS why such post hoc peer review was undertaken at late stages in the process if the Applicant had already doubts at the termination of the main study.

Regarding statistics, the DS recalled that statistical analysis is only a line of evidence in an overall weight of evidence approach. Biological significance should still be considered and comparison to the concurrent control group (and to HCD, if appropriate). DS still chose to observe treatment-related trends for some histopathological data based on the additional statistics performed.

DS's 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 (C_{max}) of metyltetraprole and areas under the mean plasma concentration time curves estimated over a 24-hour interval (AUC_{24}) are reported in the tables below.

Table: *Maximum plasma concentration (C_{max}) of metyltetraprole and areas under the mean plasma concentration time curves estimated over a 24-hour interval (AUC_{24})*

Dose Level (ppm)	C_{max} (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)	AUC_{24} (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

Table: *The relationships between the C_{max} of metyltetraprole, AUC_{24} and achieved dietary intake during Week 4, 13, 26 and 52*

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)	C_{max} 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)	AUC_{24} 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

DS drew the following conclusions on toxicokinetics:

- Non-linearity of systemic exposure was obvious after administration of metyltetraprole in rats for 4, 13, 26 and 52 weeks. Indeed, increase of C_{max} and AUC_{24} was not proportional with the dose. The C_{max} and AUC_{24} 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 C_{max} and AUC_{24} values were 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 C_{max} and AUC_{24} 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 were gender differences. The C_{max} and AUC_{24} 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 suggested decrease in absorption rate when theadministered 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 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 (respectively for males/females receiving 2000, 6000 and 20000 ppm).
- The interpretation of the neoplastic findings observed in the carcinogenicity should therefore be considered with care. Since the systemic exposure to metyltetraprole did not increase proportionally with the dose or did even 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 was 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. Analysis was neither performed in the other study available in mice, i.e. the 90-day mouse study. There was however no reason to think that the TK-profile would be different from that observed in rats. 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, was 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 C_{max} and AUC_t values at the highest dose level were 30-46 times higher than those at the low dose level and approximately 96 % lower than those predicted from a linear relationship.

Appendix 2: Summary of Applicant's responses to the information requests (carcinogencity) from EFSA

Metyltetraprole was also under evaluation by EFSA during the procedure for approval of a new active substance in accordance with Regulation (EC) No. 1107/2009 (Pesticides Peer Review Process, PPR). During PPR process, on 25th of September 2023, EFSA requested to the Applicant to provide additional information in accordance with Article 12(3) of Regulation (EC) No 1107/2009. Due to the possible impact of these data on the harmonised classification and labelling in accordance with Regulation (EC) No 1272/2008 and the assessment ongoing by RAC, these responses were also submitted to ECHA.

The following responses to EFSA requests were related to carcinogenicity studies and mode of action and therefore had a potential impact on classification and labelling of the substance: **Q34, Q35, Q36, Q37, Q38, Q48, Q49** and **Q51**.

Moreover, the following three new reports were submitted by the Applicant:

- *Eniola, S., Stewart, J, 2023. VRY0054: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0188.*

This report was focused on the diagnostic differences between the original study **(**Envigo / Labcorp) and the findings of the external peer reviewers and the Expert Panel. The main differences between the two opinions concerned tumour diagnoses of nine animals from different groups of both sexes. A consensus was reached, with the exception of the diagnosis on a male control rat (number 90, subcutaneous tissue analysed). Labcorp classified the tumour as fibrosarcoma while 5 members of the Expert Panel classified it a histiocytic sarcoma but 2 members considered the diagnosis as fibrosarcoma. In the final table of the carcinogenicity in male rats the diagnosis as histiocytic sarcoma was reported. However, it was noted that this difference had no impact on the overall conclusions.

- *Mowat, V., Stewart, J, 2023. VRY0055: Supporting Document to Discuss Findings from External Peer Review and Expert Panel (EP) Labcorp Early Development Laboratories Ltd, Report No TST-0189.*

This report was focused on the diagnostic differences between the original study (Envigo / Labcorp) and the findings of the external peer reviewers and the Expert Panel. In this case the difference of diagnosis regarded proliferative lesions of the lymphoreticular system. The two groups of assessors reached a consensus and the agreed data are shown in the table below (the original data are reported in brackets):

Dose Levels (ppm)	**0**		**700**		**2000**		**7000**	
Number of Animals	**51 M**	**51 F**	**51 M**	**51 F**	**51 M**	**51 F**	**51 M**	**51 F**
Malignant lymphoma	7(5)	9(8)	6	8	8	14 (12)	8	9
Histiocytic sarcoma	0	2(1)	0	0	0	3	0	3

M-males, F-Females

Labcorp agreed to amend the original reports in order to reflect the changes in the histopathological diagnoses and interpretations.

- *The updated position paper of the Applicant, Report No* TST-0190

In this report the responses to the EFSA's questions related to the carcinogenicity were summarised. The approach applied by the Applicant in the revision of carcinogenicity data was clearly described. Moreover, information on the possible MoA was provided.

The Applicant asked the final data available in this updated report to be considered for carcinogenicity assessment. It is noted that RAC assessment, at this stage, was already based on updated data and the tables reported in the document TST-190 are the same as those reported in the above section Additional Key element- Part I.

In this section RAC reports only the conclusion on biological plausibility of the tumours and MoA.

Applicant's conclusion on biological plausibility of tumours:

DS pointed out that some tumours had elevated incidences above concurrent controls across one or more dose groups, but all lacked statistical significance and were without clear dose-relationship. Incidence of all tumours that DS expressed concern for were within the HCD range, except for spontaneously higher incidence of malignant lymphomas in male mice attributed to high background incidence. Also, tumour pathogenesis, including information of hormonal effect and genotoxicity, clearly indicated that all above tumours were spontaneous, which are in line with the lack of pre-neoplastic or related non-neoplastic lesions. Furthermore, information of ADME showed that there was no evidence of significant distribution and accumulation to the possible carcinogenic target organs, which did not support the biological plausibility of these tumours. Taken together, tumours observed in the carcinogenicity studies with metyltetraprole were considered to represent normal variation in the incidence of background findings in aged rodents, and consequently, unrelated to the treatment of metyltetraprole.

Overall, the Applicant determined the strength of evidence for carcinogenicity and concluded that metyltetraprole had no carcinogenic potential in rat and mouse. The Expert Panel also concluded that none of the tumours of DS concern were treatment related ensuring the validity of the Applicant's conclusion. Therefore, metyltetraprole related data on experimental animals did not meet the criteria of sufficient or limited evidence of carcinogenicity as defined in the paragraph 3.6.2.3.1 (ECHA, 2017).

Moreover, the Applicant considered the possible MoA of the tumours.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter. The Background Document (BD) gives the detailed scientific grounds for the opinion. It is the combined Draft Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Records of the targeted public consultation following the submission of additional information on carcinogenicity.