Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Tellurium Dioxide

EC Number: 231-193-1
CAS Number: 7446-07-3

CLH-O-0000006811-75-01/F

Adopted

11 June 2020

11 June 2020

CLH-O-0000006811-75-01/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 an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Tellurium Dioxide**

EC Number: **231-193-1**

CAS Number: **7446-07-3**

The proposal was submitted by **the Netherlands** and received by RAC on **1 May 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted 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 **17 June 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **16 August 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Annemarie Losert**

Co-Rapporteur, appointed by RAC: **Ralf Stahlmann**

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 RAC opinion on the proposed harmonised classification and labelling was adopted on **11 June 2020** by **consensus**.

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	Tellurium Dioxide	231-193-1	7446-07-3	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
RAC opinion	TBD	Tellurium Dioxide	231-193-1	7446-07-3	Repr. 1B Lact.	H360Df H362	GHS08 Dgr	H360Df H362			
Resulting Annex VI entry if agreed by COM	TBD	Tellurium Dioxide	231-193-1	7446-07-3	Repr. 1B Lact.	H360Df H362	GHS08 Dgr	H360Df H362			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The CLH dossier states that tellurium dioxide can exist in different crystalline structures. Under normal conditions, a yellow orthorhombic (tellurite) and a colourless tetragonal form (paratellurite or alpha-TeO2) can exist (Champarnaud-Mesjard *et al.*, 2000; Thomas, 1988; Wells, 1995). In addition, amorphous forms (Dewan *et al.*, 2008) as well as nano-tellurium dioxide are known (Arab *et al.*, 2017). The REACH registration dossier does not specify the crystalline structure of the substance covered by the dossier. Crystalline structures of the test items used in the different toxicological studies were not provided either. Based on granulometry information (median particle size of about 18 µm), the dossier submitter (DS) concluded that nano tellurium dioxide is not covered by the data building the basis of the CLH dossier. As there is no information on possible toxicological differences between the different crystalline forms of tellurium dioxide, RAC, in line with the DS, assumes that the effects observed in the available toxicological studies are representative for tellurium dioxide, irrespective of the crystalline structure.

Toxicokinetic information

The DS presented the data of eight studies and reviews investigating the toxicokinetics of tellurium and tellurium dioxide. These data include information on absorption, distribution, metabolism and elimination in animals and humans, as well as an *in vitro* study on the inhibiting effect of tellurium on squalene epoxidase. In addition, the solubility of tellurium and tellurium dioxide in artificial alveolar and gastrointestinal fluid has been investigated. In summary, there is no guideline toxicokinetic study available, and some of the information is limited but the following information can be extracted from the available studies.

Absorption of tellurium after oral exposure is low, up to 25% in humans (no clear differentiation between the different tellurium compounds, studies have been performed with elemental tellurium but also with tetra- and hexavalent tellurium salts). In rats and rabbits oral absorption was in the range of 10 – 40%. Tellurium can also enter the organism via the lungs (MAK, 2006). No information on dermal uptake was identified.

Tellurium is reduced to telluride after its uptake into the body, which is then stepwise methylated to mono- di- or trimethylated tellurium. These methylated forms are then excreted via urine, faeces or air. Dimethylated tellurium is the compound responsible for the typical garlic odour after intake of tellurium or tellurium dioxide. As tellurium dioxide or its reaction product with water, tellurous acid with the corresponding tellurites, is stepwise reduced in the body to telluride (Te^{2-}), the typical form of reduced tellurium in the body, it can be concluded that tellurium and tellurium dioxide are metabolised in an equivalent manner and resulting in identical metabolites. This is the basis for the read-across as can be seen in the section below.

Tellurium is eliminated as dimethyl telluride in urine, sweat and expired air, but trimethyl telluride is the predominant form in urine. Biphasic elimination was observed in rats after i.p. administration of radioactive substance. Most of orally administered tellurium was unabsorbed and appeared in the faeces. Excretion in man was about 83% via the urine, 15,6% via the faeces and 1,6% in exhaled air. In rats excretion is mainly via faeces (about 70%).

Read-across

The DS proposed a read-across with the source substance being metallic tellurium (CAS: 13494-80-9), and tellurium dioxide (CAS: 7446-07-3) as the target substance.

A read-across for the human health endpoints genotoxicity, carcinogenicity and reproductive toxicity was proposed based on the fact that the source and target substance are metabolised by the same route and because the source and target substance have very similar physico-chemical properties. On this basis, the DS hypothesised that human health toxicity, especially genotoxicity, carcinogenicity and reproductive toxicity, of source and target substance will be similar.

The DS identified Scenario 1 of the read-across assessment framework (RAAF) (ECHA, 2017a) as the most adequate scenario to perform the read-across, as the source and target substances are transformed to common compounds.

Tellurium dioxide is the oxidised form of the source substance, the metalloid tellurium. Tellurium dioxide is transformed to tellurite in the body, which is then further reduced to telluride (Te^{2-}). When tellurium enters the body it is also reduced to telluride (Te^{2-}) which is then stepwise methylated.

Ogra (2009) proposed a metabolic pathway of tellurium compounds, which was slightly adapted by the DS:

Tellurite ($(TeO_3)^{2-}$) => telluride (Te^{2-}) ➔ CH_3TeH ➔ $(CH_3)_2Te$ ➔ $(CH_3)_3Te^+$

=> reduction

➔ methylation

Since tellurium and tellurium dioxide are metabolised in a comparable way resulting in identical metabolites it is assumed that tellurium dioxide is a suitable read-across substance for tellurium.

Physico-chemical properties of both substances are very similar and both compounds are considered to have a rather low water solubility, which is also the case for tellurous acid (H_2TeO_3), the reaction product of tellurium dioxide with water. In contrast, other tellurium compounds like tellurates (TeO_4^{2-}) and tellurites (TeO_3^{2-}) have a higher water solubility.

Source and target substance have also been compared using the OECD QSAR Toolbox, and a data matrix containing information on physico-chemical as well as toxicological properties is included in the CLH dossier. The DS concluded that relevant, but limited, information was obtained by the application of the QSAR Toolbox. The results are supportive of the similarities between target and source substance.

The known toxicological properties of both substances further point towards a high degree of concordance. Both substances have low acute toxicity, are neither skin nor eye irritant, but both give positive results in the local lymph node assay. Also the available genotoxicity and reproductive toxicity studies presented in the CLH dossier further support the similarity of source and target substance.

Read-across is performed for genotoxicity and reproductive toxicity, including effects on /via lactation and these endpoints are assessed in the CLH dossier. Carcinogenicity is not covered in the CLH dossier, as no carcinogenicity study is available, neither for tellurium nor for tellurium dioxide. Also repeated dose toxicity is not covered in the dossier, as only a 28-day and a 90-day study with tellurium dioxide are available for this endpoint.

RAC supports the analysis carried out by the DS and the applied read across.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH dossier provides three *in vitro* studies with tellurium: a bacterial gene mutation study (OECD TG 471, GLP; Anonymous, 2012b), a chromosome aberration study in mammalian cells (OECD TG 473, GLP; Anonymous, 2013a) and a gene mutation study in mammalian cells (OECD TG 476, GLP; Anonymous, 2013b) as well as one bacterial gene mutation study (OECD TG 471, GLP; Anonymous, 2012a) with the read-across substance tellurium.

No *in vivo* studies are available.

The OECD TG 471 (Anonymous, 2012b) study with tellurium dioxide was clearly negative in all strains, both in the plate incorporation assay and the pre-incubation assay in the presence and absence of metabolic activation. In addition, tellurium gave negative results in a comparable study (OECD TG 471, Anonymous, 2012a).

Clearly negative results were also obtained in an *in vitro* chromosome aberration assay with tellurium dioxide (OCDE 473, Anonymous, 2013a).

In a mouse lymphoma assay with tellurium dioxide (OECD TG 476, GLP; Anonymous, 2013b) L5178Y cells were exposed with and without metabolic activation for 3 hours and for 24 hours without metabolic activation. Excessive cytotoxicity was seen at doses ≥ 20µg/mL, so that only low concentrations could be evaluated. After 3 hours with metabolic activation (Assay 1) statistically significant increases in the mutation frequency were observed at the four concentrations evaluated (10, 7.5, 5, 2.5, 1.25 µg/mL). However, the difference between the mutation frequency of the test item treated sample and the corresponding vehicle control value did not exceed the Global Evaluation Factor (GEF, a validity criterion recommended in the guideline to assess the biological relevance of statistical significant increases) in case of the 7.5, 5 and 2.5 µg/mL concentrations. Thus, they were considered as biologically non-relevant increases. In case of the 10 and 1.25 µg/mL concentrations, the values were above the limit of the biological relevance (the difference was higher than the GEF), but the results did not follow a clear dose response and the increases were not reproduced in Assay 2 (repetition under identical conditions: 3 hours treatment with metabolic activation).

Overall, the DS concluded that tellurium dioxide and the read across substance tellurium were clearly negative in the available *in vitro* tests.

Comments received during public consultation

One Member State Competent Authority (MSCA), one trade organisation and one company supported no classification for germ cell mutagenicity based on the *in vitro* genotoxicity studies presented in the dossier.

Assessment and comparison with the classification criteria

RAC agrees with the DS's analysis and interpretation of the *in vitro* mutagenicity studies presented in the CLH dossier. No indication for a mutagenic potential of neither tellurium dioxide nor tellurium can be derived from the presented *in vitro* studies.

However, it is noted that the available studies have some limitations. For the bacterial reverse mutation assay (OECD TG 471, Anonymous, 2012b) it is reported that cytotoxicity was observed in the two or three highest concentrations of the Initial Mutation Test, where the plate incubation technique was used. Stronger cytotoxicity (no further detail) was seen in the Confirmatory and Complementary Confirmatory Mutation Tests, where the pre-incubation test was used, leaving doubts regarding which doses cytotoxicity was actually seen. As for tellurium, water solubility is also rather low for tellurium dioxide, but no formation on precipitates is reported, although concentrations up to 5000 µg/plate were tested.

In contrast, in the *in vitro* mammalian gene mutation test (OECD TG 476, Anonymous, 2013b), in which the same vehicle (methyl cellulose) was used, it is described that for some concentrations insolubility was detected in the final treatment medium at the end of treatment. No further details on the exact concentrations where insolubility occurred is presented. Due to the excessive cytotoxicity only rather low doses could be tested (Assay 1, with metabolic activation: ≤ 10 µg/mL, Assay 1, without metabolic activation: ≤ 20 µg/mL, Assay 2, with metabolic activation: ≤ 5 µg/mL, Assay 2, without metabolic activation: ≤ 8 µg/mL).

Also for the *in vitro* mammalian chromosome aberration test (OECD TG 473, Anonymous, 2013a) inconsistencies were observed with regard to concentrations at which cytotoxicity and precipitation occurred. While in Assay 2 without metabolic activation cytotoxicity was seen as low as 10 µg/mL, in the other assays cytotoxicity only started at 75 or 100 µg/mL. "Minimum" solubility was reported in three of the four assays at 100 and 200 µg/mL.

Also in the bacterial reverse mutation assay with tellurium (OECD TG 471, GLP; Anonymous, 2012a), no information on the formation of precipitates is presented. However, as the substance has a rather low water solubility it can be assumed that at the tested doses some precipitation may have occurred.

Regarding cytotoxicity it is stated that it was seen only in the top dose of 5000 µg/plate in the Initial Mutation Test, but at clearly lower doses in the Confirmatory and the Complementary Confirmatory Mutation Test: in the test strain E. coli WP2 uvrA at concentrations of 5000, 1581 & 500 µg/plate and in the test strains TA100, TA1535 & TA1537 at 158.1 and 50 µg/plate (it is unclear if in these strains cytotoxicity was not seen at higher doses). These results are on the one hand conflicting and, on the other hand, leave only few lower dose concentrations for the assessment of possible genotoxic potential of the test material.

Overall, there are minor limitations in the presented *in vitro* mutagenicity studies, but no indication for a mutagenic potential can be derived.

RAC noted that in a review by MAK (2006) further *in vitro* genotoxicity studies with several tellurium compounds are described. Several of these studies, including a DNA repair study in bacterial cells with the read across substance tellurium dioxide (Yagi & Nishioka, 1977), gave positive results (see section in the Background Document). It is, however, noted that the study did not follow generally accepted guidelines and the information presented on the test system is rather scarce.

It can be concluded that the well-conducted *in vitro* genotoxicity studies presented in the CLH dossier have some minor deficiencies with regard to solubility and cytotoxicity and reporting thereof and it is not clear whether these issues interfered with the tests ability to detect any mutagenic potential, but gave negative results. A non-guideline study by Yagi & Nishioka, 1977 gave a positive result.

On this basis and in the absence of any *in vivo* study, **no classification for germ cell mutagenicity is proposed**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

According to the DS there are no studies in humans addressing adverse effects on sexual function and fertility. The DS presented two recent studies in animals dealing with the impairment of fertility or mammalian development after exposure to TeO_2, one Reproduction/Developmental toxicity screening study in rats (OECD TG 421, Anonymous, 2013c) and a sub-chronic study in rats (OECD TG 408, Anonymous, 2017). According to the DS, relevant and severe effects on sexual function and fertility have been observed in an OECD TG 421 screening study at doses, which elicited severe general toxicity, up to mortality, but also at doses without marked systemic toxicity. Therefore, the DS was of the opinion that classification as Category 1B for adverse effects on sexual function and fertility is justified for tellurium dioxide.

In the Reproduction/Developmental toxicity screening study from 2013, according to GLP and OECD TG 421 (Anonymous, 2013c), twelve Wistar rats/sex/dose were exposed via gavage to tellurium dioxide at 0, 25, 120 and 600 mg/kg bw/d 7 days/week. Males were exposed for 28 days (14 days pre-mating and 14 days post-mating) and were sacrificed afterwards. Females were dosed 14 days pre-mating, up to 14 days of mating, throughout gestation and up to day 4 of lactation. In the P0 generation systemic effects occurred at 120 (mid dose, MD) and 600 (high dose, HD) mg/kg bw/d. In HD females pronounced mortality was observed. Five females died between days 14 and 45, one female died due to a gavage accident, one female died on day 13 of the mating period. In HD animals, clinical signs like decreased activity, liquid faeces, hunched back, laboured respiration, lethargy, piloerection and red liquid from mouth and vulva were noted. In MD and HD animals, bw, bw gain and food consumption were decreased in males and females, resulting in terminal bw in males about 7 and 14% lower than controls, and in females about 5 and 11% lower (on day 14 of exposure). The NOAEL for systemic effects is 25 mg/kg bw/d.

In HD females, the mating and fertility indices were decreased to about 73% and 63%. In MD and HD, females the gestation index was decreased (92, 100, 67, 0% in ctrl, LD, MD, HD, respectively). Four out of six HD females were non-pregnant with reduced or no corpora lutea and no implantation sites. Oestrus cycle of HD females was characterised by dioestrums. The gestation period in MD females was statistically significantly prolonged by 0.7 days (Control: 22.73 days, MD: 23.42). The two females of the HD group that delivered had a mean duration of pregnancy of 24 days (+1.3 days).

Histopathological observations detected atrophy of ovary, uterus and vagina in 4/5 dead HD females, a moderate vacuolation of corpora lutea and pigment deposits in the right ovary of 1/5 females. In other HD females, also histopathologic changes of reproductive organs as well as on kidney, liver and thymus (histopathology only in control and HD animals) were observed. No adverse effects on male reproductive organs were observed.

The DS mentioned mechanistic studies (Harry *et al.*, 1989, Wagner *et al.*, 1995), which were conducted to clarify the mechanism of tellurium-induced neuropathy. In these studies, it was demonstrated that tellurium interferes with the squalene epoxidase, the enzyme catalysing the first and rate limiting step of cholesterol synthesis. As cholesterol is a precursor for steroidal hormone synthesis, the DS indicated that there is some suspicion that tellurium could interfere with the endocrine system, which might explain the observed disturbance of hormonally regulated processes (oestrus cycle, duration of gestation). However, experimental verification is missing.

In a subchronic oral toxicity study in male Wistar rats according to GLP and OECD TG 408 from 2017, the animals were exposed via gavage to 0, 10, 30 and 100 mg/kg bw/d. No treatment related effects were observed at any dose group.

The DS mentioned that also in a rat 28-day study, which is also part of the registration dossier (Anonymous, 2013), effects on female reproductive organs were noted at 600 mg/kg bw/d; in 2 of 4 females, moderate diffuse epithelial atrophy of the vagina was noted. No further details on this study are presented in the CLH dossier.

Development

There are no studies addressing adverse effects of tellurium or tellurium dioxide on human development. The DS evaluated 9 developmental toxicity studies in rats and one study in rabbits. Two studies are performed with tellurium dioxide and eight with tellurium.

Based on the severe effects consistently seen in a pre-natal developmental toxicity study in rats (Perez-D'Gregorio & Miller, 1988) and a reproductive screening study in rats (Anonymous, 2013c) with tellurium dioxide and several prenatal developmental toxicity studies conducted with the read-across substance tellurium (Johnson *et al.*, 1988, Agnew & Curry, 1972, Agnew *et al.*, 1968, Garro & Pentschew, 1964, Duckett, 1971a, b, Duckett, 1970) in rats and rabbits (Johnson *et al.*, 1988), including doses with only slight or absent maternal toxicity, the DS proposed to classify tellurium dioxide as Repr. 1B; H360D.

Several studies with the read-across substance tellurium investigated different exposure durations and identified a time window, which is relevant for the induction of the main malformation, i.e. hydrocephalus. The relevant exposure period is from gestation day (GD) 9 to 15. Even single doses, if high enough, administered within this period resulted in the induction of hydrocephalus.

The studies with tellurium dioxide are summarised in the following table (modified):

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal Developmental Toxicity Study Similar to OECD TG 414 GLP: no information Female Wistar rats (10 animals/dose group) K2	Tellurium dioxide (Purity: 99.99%) 0, 10, 100, 500, 1000 µmol TeO_2/kg bw/d in olive oil (corresponding to 0, 1.6, 16, 80, 160 mg TeO_2/kg bw/d) from GD 15 to GD 19 GD 20: caesarean section Subcutaneous application	<u>Dams:</u> NOAEL: 16 mg TeO_2/kg bw/d (100 µmol/kg bw/d: effects at the highest dose group were weight loss, centrilobular fatty changes in the liver and 40% lethality). <u>Foetuses:</u> NOAEL: 1.6 mg TeO_2/kg bw/d (10 µmol/kg bw/d); most prominent effects were hydrocephalus (100% at 16 mg/kg bw/d), additionally oedema, exophthalmia, ocular haemorrhage, umbilical hernia, undescended testis and small kidneys followed by increased foetal mortality in the two highest doses (11% and 81% at 500 and 1000 µmol TeO_2/kg bw/d, respectively) Remarks: In parallel a pair fed study was performed. All tellurium dioxide exposed foetuses revealed malformations, however, no such effects were observed in pair-fed control animals, indicating that reduced food consumption was not responsible for the effects.	(Perez-D'Gregorio and Miller, 1988) also reported in (ECHA Dissemination, 2018)

Reproduction / Developmental Toxicity Screening Test OECD TG 421 Deviations: no GLP: yes Male/female Wistar rats 12 animals per sex and dose group K1	Tellurium dioxide 0, 25, 120 and 600 mg TeO_2/kg bw/d Exposure: Males were dosed for 28 days (14 days pre-mating and 14 days mating/post-mating). They were sacrificed afterwards. Females were exposed 14 days pre-mating, for up to 14 days of mating (1:1), throughout gestation and up to day 4 of lactation, which was the day before necropsy. Daily application via gavage Vehicle: 1 % (v/v) methyl cellulose solution	<u>Offspring:</u> LOAEL: 25 mg TeO_2 /kg bw/d (increased pup mortality at all dose groups) 600 mg TiO_2 mg/kg bw/d: no live pups 120 mg TeO_2/kg bw/d: 33/137 stillborn pups, 28/137 pups found dead but born alive (positive floating test), 39 cannibalised pups, and 65 were not nursed, more male than female pups died between PND 0 and PND 4 25 mg TeO_2/kg bw/d: no effect on number of live born pups (148/173), 33 pups did not suckle, on PND4 the survival index was 75 % and therefore below the normal control range Total litter weights were below the normal control range in the MD group on post-natal days (PND) 0 and 4, and in the LD group at PND 0. The observed differences are probably a consequence of pup mortality, without an effect on the growth of survivors.	Anonymous, 2013c reported from (ECHA Dissemination, 2018)

K: Klimisch

The DS compiled maternal and foetal findings from the key developmental study with tellurium dioxide in the following table (Perez-D'Gregorio and Miller, 1988):

Dose level (mg/kg bw/d)	**0**	**1.6**	**16**	**80**	**160**
Maternal mortality	0/10	0/10	0/10	0/10	4/10
Early/late resorptions	5/1	5/0	3/1	4/1	4/1
No of live/dead foetuses	120/0	112/0	114/0	120/15	12/51
Undescended testis#	2/54	2/51	18/52*	36/51*	29/33*
Hydrocephalus#	0/120	0/120	114/114	135/135	63/63
Oedema#	0/120	0/120	114/114	135/135	63/63

* Significantly different from control group ($p \leq 0.01$); # information taken from Figure 4 of the publication

In a reproductive toxicity screening test from 2013 (Anonymous, 2013c), Wistar rats were exposed to Tellurium dioxide at dosages of 0, 25, 120 or 600 mg/kg bw/d via gavage. The test was performed according to OECD TG 421 (males exposed for 28 days before and after mating; females before gestation, and during gestation until PND4). The most prominent effect in pups was the increased mortality in all dose groups, reaching 100% in the top dose group. In the top dose group 5 out of 12 dams died. Fertility index was 63 %.

The most relevant effects are summarised in the table below.

Dose level (mg/kg bw/d)	0	25	120	600
Maternal mortality	0/12	0/12	0/12	5/12
Fertility index	100%	100%	100%	63%
Gestation index	92%	100%	67%	0%
No of dead/total pups on PND 0	13/160	25/173	33/137	19/19
Number of pups alive on PND 4	147	127	53	0
Absence of cranium, small brain/examined pups	0/12	0/30	0/45	4/16
Whole body subcutaneous gelatinous material/examined pups	0/12	0/30	0/45	16/16

Adverse effects on or via lactation

Effects of tellurium dioxide on lactation have not been studied. In a read across approach, the DS provided a non-guideline study with tellurium for the effects on lactation in rats (Jackson *et al.*, 1989). Female Wistar dams (2 per dose group) were exposed to 0 or 625 mg/kg bw/d from PND 0 to PND 7, 14, 21 or 28. Light and electron microscopic investigations of spinal cord, optic nerve and sciatic nerve; including measurement of myelin density, myelin sheath thickness and myelinated axon diameter were performed in pups.

Offspring of dams being exposed via diet showed clinical signs of toxicity already a few days after start of exposure via breast milk. The microscopic examination of nerve tissues of the offspring revealed typical effects of tellurium intoxication like hypomyelination, myelin degeneration and Schwann cell degeneration. Depending on the nerve tissue investigated, these effects were detectable at all time points or at the three last time points of investigation with PND 7 being the first time point. The transmission of tellurium to the offspring was demonstrated by the presence of garlic odour within 2-3 days and skin discolouration. Garlic odour and greyish discolouration of the skin was also seen in dams, but no other toxic signs were reported in dams.

The DS did not propose to classify for lactation due to doubts about the validity of the study. It was mentioned that only one rather high dose was tested with no information on purity of the test material, only two pups were tested at the indicated time points for the different effects and no analytical measurement of tellurium in milk was conducted. The DS further questioned the validity of the study because no toxicity was reported in the dams, despite the rather high dose applied (625 Te mg/kg bw/d), whereas in another dietary study (Johnson *et al.*, 1988) toxic effects were already seen at 18 mg/kg bw/d and in the reproductive screening study using gavage application at 120 mg/kg bw/d. The DS classified the study as Klimisch 3.

Comments received during public consultation

One MSCA supported the read-across approach between tellurium dioxide and tellurium for reproductive toxicity.

One MSCA did not support classification as Repr. 1B; H360F because of increased mortality, which indicates severe toxicity of the substance and proposed to classify as Repr. 2 for fertility instead. Repr. 1B; H360D however was supported.

One company manufacturer and one industry association supported the classification as Repr. 1B; H360D, but without classification for fertility as the fertility observed effects might be secondary effects due to general toxicity.

No comments on lactation were submitted.

Assessment and comparison with the classification criteria

Sexual function and fertility

At the top dose of 600 mg TeO_2/kg 5 of 12 rats died, body weight was reduced by 11%, and fertility index was reduced to 63% (5 of 8 rats). Due to the high mortality at this dose level, effects on reproductive parameters cannot be properly evaluated. Changes in the gestation index and gestation length were observed at doses, which reduced body weight of the females by 5% and did not cause mortality (MD, 120 mg/kg). Histologically effects were noted at the lethal dose level, but corresponding data for the MD are missing (only control and HD animals were histopathologically examined in case no macroscopic findings were observed). RAC agrees with the DS that the known interference of tellurium squalene epoxidase and the resulting inhibition of cholesterol synthesis might lead to hormonal imbalance due to the dependence of steroid hormone synthesis on cholesterol. However, additional information on clinical chemistry, e.g. measurements on hormone levels, which might provide information on endocrine imbalances, is missing. It is further noted that the study authors did not consider the observed effects in the female reproductive organs as secondary to the observed general toxicity.

In order to allow for a better interpretation of the results an overview of the maternal toxicity is presented below.

No clinical signs were seen in control and LD, but at MD and HD, all animals had dark faeces. At the HD, considerable clinical signs like reduced activity, hunched back or piloerection was seen in females. The effects on female body weight and body weight gain are summarised in the following table.

***Table**: Female body weights in the OECD TG 421 study (Anonymous, 2013c).*

Body weight (%, compared to control)	**LD (n = 12)**	**MD (n = 12)**	**HD (n = 2)**
Day 14	- 4.3	- 4.7	- 11 *
GD 0	- 5.1	- 6.1	- 15.2 **
GD 7	- 5.3 *	- 7.8 **	- 14 **
GD 14	- 6.5 *	- 10 **	- 16.1 **
GD 20	- 6.9 *	- 13.3 **	- 19.8 **
PND 0	- 7.1 *	- 14.4 **	- 29.2 **
PND 4	- 7.8 *	- 18.3 **	- 30.2 **
Body weight gain (%, compared to control)	**LD (n = 12)**	**MD (n = 12)**	**HD (n = 2)**
Day 14 – GD 0	- 45.3	- 70.4	- 206.5
G 0 – GD 14	- 6.6	-18.9	-6.0
GD 7 – G 14	- 14.5 *	-25.1 **	-30.8 *
GD 14 – GD 20	- 8.4	- 25.3 **	-32.9 *
GD 0 – GD 20	- 9.5	- 23.8 **	- 26.5 **
GD 20 – PND 0	- 6.4	- 9.8	+11.9
PND 0 – PND 4	- 26.5	- 115.8	- 56.3

Statistically significant *: $p \leq 0.05$; ** $p \leq 0.01$
It is stated that changes in the LD were within the historical control range (historical control data not presented).

In summary, effects on fertility and histological changes of female reproductive organs were observed in the HD, which induced pronounced maternal lethality. As no histopathological examination of the MD female reproductive organs was performed it is not possible to exclude

similar changes in the MD. In the original study summary, it is concluded that the observed atrophies were substance related and not secondary to the observed general toxicity.

It is further noted that a screening study is not equivalent to a generation study, as it uses fewer animals and the pre-mating exposure duration is only for two weeks. In addition, there is a rather large space between the MD of 120 mg/kg bw/d, where only minor maternal toxicity was observed and the HD of 600 mg/kg bw/d, which induced severe maternal toxicity and death. Relevant effects might have been observed if doses between MD and HD would have been tested. Furthermore, the test guideline recommends to histologically assess the organs of all animals affected by toxicity in the HD, but also the organs from animals in low and mid dose. Although the uterus, the ovaries and the vagina were affected in HD animals, no detailed histopathological analyses of these organs was performed in LD or MD animals.

Reduction of the gestation index in a small number of rats (4/12 animals with stillborns) and a slight increase of gestation length (+0.7 days), but no changes in the fertility index, were observed at the MD. Effects on male reproductive organs were not observed. Although data are not conclusive, they point to effects on female fertility. This is underlined by the observation that tellurium dioxide causes structural changes in female reproductive organs and leads to reduced numbers of corpora lutea.

Histological changes of the vagina were also seen in the top dose in a 28-day study (Anonymous, 2013d), which tested the same doses as the reproductive screening study (Anonymous, 2013c). In 2 out of 4 top dose females, moderate diffuse epithelial atrophy of the vagina was noted. Furthermore, in this study considerable toxicity was seen in the MD and HD, including one death in the HD females.

Human data on fertility and reproductive function are not available.

RAC concurs with the proposal by the DS that classification is justified for TeO_2 for adverse effects on sexual function and fertility. A reduction in gestation index as well as a prolonged gestation period was seen at doses, which did not cause severe general toxicity. However, there is no information on possible effects on reproductive organs at doses without severe general toxicity. Taking this into account, RAC considers that **classification for adverse effects on sexual function and fertility in category 2 is justified**.

Developmental toxicity

As there are no epidemiological data available for humans, classification of tellurium dioxide in Cat. 1A is not justified.

However, developmental toxicity has consistently been observed in all available developmental toxicity studies performed with tellurium dioxide and tellurium. Studies with tellurium dioxide are performed with subcutaneous injection or application via gavage. Even in the lowest dose in the developmental toxicity study, severe effects like hydrocephalus occurred in all foetuses without any effects in dams. The screening study revealed increased pup mortality already at doses, which did not cause toxicity in dams.

These findings are supported in a series of further studies with tellurium. Only two studies, one in rats and one in rabbits are considered reliable. In both studies hydrocephalus and increased pup mortality was observed. Additional studies date back to the 1960ies or 1970ies and details of the findings are often missing (e.g., most of these studies did not report the effects in dams or did not clearly state that there were no effects in dams). However, these studies investigated the time period relevant for the induction of hydrocephalus, one using the intra-muscular route of exposure. It could be demonstrated that even a single dose can induce hydrocephaly, if the dose is high enough and when applied within the relevant time window (GD 9 – 15). Studies with tellurium are summarised in the following table.

Reproductive Toxicity of Tellurium (supporting evidence for Tellurium dioxide)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Prenatal Developmental Toxicity Study Similar to OECD TG 414 Deviations: no GLP: yes Female Crl COBS CD (SD) BR rats (32-33 animals/dose group) K2	Tellurium (Purity: 99.99%) 0, 30, 300, 3000, 15000 ppm Te (corresponding to 0, 1.9, 18, 173, 579.4 mg/kg bw/d) from GD 6 to GD 15 GD 20: 2/3 of the females underwent caesarean section, 1 out of 2 of the foetuses were examined for soft tissue anomalies and ½ for osseous skeletal status; 1/3 of the dams delivered naturally; foetuses and dams were sacrificed on post-natal day (PND) 7. Application via diet	Dams: NOEL: 1.9 mg Te/kg bw/d (reduced feed consumption, reduced body weight gain during exposure period which recovered after cessation of exposure, no maternal death) Offspring: NOAEL: 18 mg Te/kg bw/d (skeletal and soft tissue malformations, primarily hydrocephalus at 173 and 579.4 mg Te/kg bw/d, number of pups surviving 7 days reduced at highest dose) No. (%) of foetuses with dilated lateral ventricles: 1 (0.7); 0 (0); 1 (0.7); 11 (8.3); 67 (54.9) at 0, 1.9, 18, 173, 579.4 mg/kg bw/d, respectively); No. (%) of litters with dilated lateral ventricles: 1 (4.6); 0 (0); 1 (4.8); 3 (14.3); 17 (85) at 0, 1.9, 18, 173, 579.4 mg/kg bw/d, respectively) % litters/foetuses with variations: 18.2 (2.1); 35.0 (2.9); 28.6 (3.2); 57.1(10.6); 100 (40.6) at 0, 1.9, 18, 173, 579.4 mg/kg bw/d, respectively)	(Johnson *et al.*, 1988) also reported in (ECHA Dissemination, 2018)
Prenatal Developmental Toxicity Study Similar to OECD TG 414 Deviations: no GLP: yes Female New Zealand white rabbits (17 animals/dose group) K2	Tellurium (Purity: 99.99%) 0, 17.5, 175, 1750, 5250 ppm Te (corresponding to 0, 0.7, 7, 70, 210 mg/kg bw/d) from GD 6 to GD 18 GD 29: all females underwent caesarean section, all foetuses were examined for soft tissue anomalies and thereafter for skeletal variations Application via diet	Dams: NOAEL: 7 mg Te/kg bw/d (reduced feed consumption, reduced body weight gain during exposure period which recovered after cessation of exposure, soft or liquid faeces, alopecia, thin appearance, and/or decreased motor activity, no maternal death up to highest dose) Offspring: NOAEL: 70 mg Te/kg bw/d (HD: decreased foetal body weights, increased incidence of foetuses or litters with variations, malformations including hydrocephalus and with reversible delays in ossification, no individual numbers provided. It was stated that 'There were low incidences of hydrocephalus, enlarged and/or irregularly shaped anterior fontanelle, incomplete ossification of, or small holes in, the frontals and parietals; frontals with thickened ossification; umbilical hernia; fused pulmonary artery and aorta; asymmetric and/or irregularly shaped and/or fused	(Johnson *et al.*, 1988) also reported in (ECHA Dissemination, 2018)

		sternebrae; and thickened areas in the ribs in foetuses of high-dosage pregnancies.'; No. (%) of foetuses with abnormalities: 3 (6.7); 6 (5.1); 4 (6.0); 2 (1.8); 11 (11.8) at 0, 0.7, 7, 70, 210 mg/kg bw/d, respectively); No. (%) of litters with abnormalities: 2 (22.2); 5 (33.3); 2 (25); 1 (7.1); 6 (46.2) at 0, 0.7, 7, 70, 210 mg/kg bw/d Te, respectively)	
Prenatal Developmental Toxicity Study Not according to guideline Deviations: not applicable GLP: no Female Long Evans rats (5-11 dams per dose group and gestation day; 2-3 dams per control group and gestation day) K4	Tellurium (Purity: not provided) 13 mg Te/kg bw/d Single application on gestation days 7, 8, 9, 10, 11, 12, or 13 Vehicle: olive oil (suspension) Dams were allowed to deliver and offspring observed until PND 10; sacrifice on PND 10 and fixation of offspring in Bouin's solution, examination for hydrocephalus (increased ventricular dilatation was classified as hydrocephalus) and other defects No information on maternal toxicity Application intramuscular	<u>Dams:</u> no NOAEL can be derived since effects on dams were not examined/reported <u>Offspring</u>: LOAEL 13 mg Te/kg bw/d (hydrocephalus in offspring of animals treated on day 9 (14 of 75 (18.6%) offspring) or 10 (10 of 32 (31%) offspring); one offspring with hydrocephalus in the group treated on GD7 (1 of 33 (3%) offspring) and one in an offspring of the control group (1 of 94 (1.1%) offspring), but not in offspring of dams treated on any other day of gestation; no other malformations observed; Foetal resorptions were also observed, but examinations for uterine resorption sites were only performed in animals which failed to deliver by GD 22 (2/10, 3/8, 0/11, 1/6, 1/7, 0/5, 1/5 dams treated on GD 7, 8, 9, 10, 11, 12, 13, respectively)	(Agnew and Curry, 1972) also reported in (ECHA Dissemination, 2018)
Prenatal Developmental Toxicity Study Not according to guideline Deviations: not applicable GLP: no Female Wistar rats (32 dams per dose group, 16 dams in the control group) K4	Tellurium (Purity: not provided) 3300 ppm Te Application throughout gestation Dams (n=10) were allowed to deliver, foetuses were examined for hydrocephalus No information on maternal toxicity Application via diet	<u>Dams:</u> no NOAEL can be derived since effects on dams were not examined/reported <u>Offspring</u>: LOAEL 165 mg Te/kg bw/d (Hydrocephalus were observed in 8/10 litters 4-5 days after birth, 47% (36/77) of all foetuses developed hydrocephalus, with up to 100% of all foetuses of a litter) <u>Remark</u>: No hydrocephalus was observed in preliminary experiments with two groups of four pregnant rats, which received 1250 or 2500 ppm Te.	(Agnew *et al.*, 1968)
Prenatal Developmental	Tellurium (Purity: not	<u>Dams</u>: NOAEL 125 mg Te/kg bw/d (no detailed information provided,	(Garro and Pentschew,

Toxicity Study Not according to guideline Deviations: not applicable GLP: no Female Long Evans rats (> 100 dams, no further information) K4	provided) 500, 1250, 2500 ppm Te (25, 62.5, 125 mg Te/kg bw/d) 'fed during pregnancy', dams of the high dose received normal diet 3-5 days before delivery Dams were allowed to deliver and offspring were examined for the occurrence of hydrocephalus Application via diet	but stated that they behaved normally, tolerated the diet well and delivered on schedule) Offspring: LOAEL 25 mg Te/kg bw/d (100% hydrocephalus in the highest dose group and 60-90% in the mid dose group, at the low dose group only a part of the litters were affected (60% according to Duckett, 1971); hydrocephalus detectable immediately after birth; new-borns appeared smaller than controls; all offspring died within the first month after birth; no detailed examination of the foetuses for other endpoints)	1964) also reported in (ECHA Dissemination, 2018)
Prenatal Developmental Toxicity Study Not according to guideline Deviations: not applicable GLP: no Female Wistar rats (30 dams in treatment group, 20 dams in control group) K4 (according to registration dossier and the authors of this document)	Tellurium (Purity: not provided) 3000 ppm Te (150 mg Te/kg bw/d) fed 'every day of gestation' Dams were allowed to deliver and offspring were examined for the occurrence of hydrocephalus Application via diet	Dams: no NOAEL can be derived since effects on dams were not examined/reported Offspring: LOAEL 150 mg Te/kg bw/d (24 of the female rats fed tellurium gave birth to litters. 20 of the rats gave birth to litters in which all the animals were hydrocephalic, 4 gestating rats gave birth to normal offspring. The hydrocephalus was non-obstructive in type for the first few days, after which obstructions appeared. Most of the animals died by the end of the second week. Only 61 of the 207 hydrocephalic rats born alive were still alive at the age of 10 days and only 44 survived until the age of 1 year.)	(Duckett, 1971) also reported in (ECHA Dissemination, 2018)
Prenatal Developmental Toxicity Study Not according to guideline Deviations: not applicable GLP: no Female Wistar rats (20 dams in treatment group, 20 dams in test group) K4	Tellurium (Purity: not provided) 3000 ppm Te (180 mg/kg bw/d as calculated by the authors of the publication) fed 'every day of gestation' On GD 13 and 15 foetuses (number not specified) were removed via abdominal wall; after closing the wall the dams were allowed to deliver; Only foetuses of Te fed animals who eventually gave birth to hydrocephalic animals, and foetuses of similar age control rats, were examined. Application via diet	Dams: no NOAEL can be derived since effects on dams were not examined/reported Offspring: LOAEL 180 mg Te/kg bw/d (morphological anomalies in the cells in the ependymal layer of the treated foetuses: plasmalemma was without microvilli and the number of mitochondria was 'greatly diminished'; mitochondria 'were often abnormal, smaller and darker than normal and showed distortion of cristae')	(Duckett, 1970) also reported in (ECHA Dissemination, 2018)

Developmental toxicity study No guideline followed Deviations: not applicable GLP: no data Female rats, strain not provided (20 animals/group) K4	Tellurium (Purity: not provided Diet with 2500 ppm Te (according to authors of the publication rats usually consumed 20 g of diet, i.e. 50 mg tellurium which corresponds to ca. 200 mg/kg bw/d) Group 1: Exposure during day 1-21 of gestation Group 2: exposure of 20 dams from GD 1 to 9 Group 3: exposure of 20 dams from GD 10 to 15 Group 4: exposure of 20 dams from GD 16 to 21 No information on maternal toxicity Application via diet	Dams: no NOAEL can be derived since effects on dams were not examined/reported Offspring: LOAEL 200 mg Te/kg bw/d Group 1: 12/20 dams gave birth to litters with about 8 pups, 6 out of 8 were hydrocephalic Group 2: no foetuses with hydrocephalus Group 3: 12/20 dams gave birth to hydrocephalic animals. Group 4: no foetuses with hydrocephalus Remark: In a second experiment 21 groups of 5 dams received single doses of 200 mg Te/kg bw/d via diet on different days during gestation. Three animals died and 71 gave birth to an average of 8 offspring. None of the offspring had a hydrocephalus. No further details of results provided	(Duckett *et al.*, 1971) also reported in (ECHA Dissemination, 2018)

K: Klimisch

In summary, there is consistent evidence from experimental studies with rats that tellurium dioxide causes developmental toxicity after gestational exposure or exposure to weaning rats at doses which are not, or only slightly, toxic to dams. The most relevant effects caused by tellurium are severe malformations (i.e. hydrocephalus) and pup lethality. Supporting evidence comes from a series of studies with tellurium in rats and rabbits. Elementary tellurium caused hydrocephalus and other major malformations in the two species. Even single exposure to high doses during the relevant time period (GD 9 to 15) resulted in the formation of hydrocephalus.

The highest weight in the assessment is given to those studies which detected developmental toxicity and foetal and pup mortality without any maternal toxicity. These are the pre-natal developmental toxicity study in rats with dietary tellurium exposure (Garro & Pentschew, 1964), the pre-natal developmental toxicity study in rats with sub-cutaneous tellurium dioxide exposure (Perez-D'Gregorio & Miller, 1988) and reproductive screening study in rats with gavage exposure to tellurium dioxide (Anonymous, 2013c). The remaining studies can be regarded as supportive.

In conclusion, RAC concurs with the proposal of the DS that **classification for adverse effects on development of the offspring in Category 1B is justified**.

Adverse effects on or via lactation

No human data are available to assess effects via lactation. Data from experiments with tellurium dioxide are not available. One study in 2 lactating rats with tellurium exposure via diet containing 1.25% Te in comparison to 2 lactating controls was published. A daily dose of 625 mg/kg bw/d was derived, no information on purity of the test substance was provided. Only one dose group was used. A total of 40 offspring were studied, five tellurium exposed and five control pups were investigated at the indicated time points (PND 7, 14, 21, and 28) for toxic effects. Light and electron microscopy was performed in two pups from each group. It can be assumed that only until day 7 the tellurium exposure of the pups was only via milk. After that exposure to tellurium

containing diet might also have occurred. No analysis of Te concentration in the milk was performed. The DS classified the study reliability as Klimisch 3.

No toxicity was reported in dams at the dose of 625 mg Te (equivalent to 782 mg tellurium dioxide/kg bw/d). In another dietary study, the prenatal developmental toxicity study in rats (Johnson, *et al.*, 1988), maternal toxicity was seen at doses ≥ 18 mg Te /kg bw/d.

Offspring showed clinical signs, grey coloured skin and the typical odour of garlic. By 2 weeks of age, all the tellurium exposed pups were lethargic compared to the controls, and showed evidence of hind limb paresis. The tellurium exposed pups gained weight more slowly than the control pups and appeared small for age, an effect that was strongest after 14 days of age. It is noted that in the second week of life, exposure via food cannot be excluded, but this is a time where a big contribution of the pup's food still comes from milk. Light and electron microscopy of nervous tissue from rat offspring exposed via lactation showed typical alterations, starting on day 7 of exposure, where exposure was solely via milk. These observations must be interpreted as induced by tellurium via milk.

In addition, the optic nerves were investigated, but demyelination was not evident before 14 days of age. However, at 7 days of age no myelination was present in either control or treated pups; therefore, it was not possible to detect any demyelination. Clear effects on the optic nerve were seen after 14 days.

It is acknowledged that the study has considerable limitations (low number of animals per group, no toxicity in dams, despite high exposure) but there is a clear causality between general exposure indication (greyish discolouration of the skin, garlic odour in dams and pups) and histopathological changes in the pups, typical for tellurium intoxication starting on day 7 of exposure (sciatic nerves) and on day 14 of exposure (optic nerve). Further evidence for transfer of tellurium to milk comes from a study by Nishimura *et al.* (2003) who could demonstrate that 2% and 3.9% of ^{123m}Te after single i.v. administration to the dams were transferred to the pups on PND1 and PND7, respectively, which was demonstrated by the whole body retention method. On PND14 5% of the administered dose was detected in the pups.

Therefore, despite the deficiencies of the study, RAC concludes that **classification for 'Adverse effects on or via lactation' (Lact.; H362) is justified**.

Additional references

MAK (2006). Tellurium and its inorganic compounds. The MAK-Collection Part I: MAK Value Documentations, Vol. 22. DFG Deutsche Forschungsgemeinschaft, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. https://onlinelibrary.wiley.com/doi/pdf/10.1002/3527600418.mb1349480vere0022

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 evaluation performed by RAC is contained in 'RAC boxes'.

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