Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

potassium permanganate

EC Number: 231-760-3
CAS Number: 7722-64-7

CLH-O-0000001412-86-134/F

Adopted

9 December 2016

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

9 December 2016

CLH-O-0000001412-86-134/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: **potassium permanganate**

EC Number: **231-760-3**

CAS Number: **7722-64-7**

The proposal was submitted by **France** and received by RAC on **13 November 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

France 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 February 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 April 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Anna Bíró**

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 **9 December 2016** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	025-002-00-9	potassium permanganate	231-760-3	7722-64-7	Ox. Sol. 2 Acute Tox. 4 * Aquatic Acute 1 Aquatic Chronic 1	H272 H302 H400 H410	GHS03 GHS07 GHS09 Dgr	H272 H302 H410	**-**	-	-
Dossier submitters proposal	025-002-00-9	potassium permanganate	231-760-3	7722-64-7	**Add** Repr. 1B	**Add** H360Df	**Add** GHS08	**Add** H360Df	**-**	**-**	**-**
RAC opinion	025-002-00-9	potassium permanganate	231-760-3	7722-64-7	**Add** Repr. 2	**Add** H361d	**Add** GHS08	**Add** H361d	**-**	**-**	**-**
Resulting Annex VI entry if agreed by COM	025-002-00-9	potassium permanganate	231-760-3	7722-64-7	Ox. Sol. 2 Repr. 2 Acute Tox. 4 * Aquatic Acute 1 Aquatic Chronic 1	H272 H361d H302 H400 H410	GHS03 GHS08 GHS07 GHS09 Dgr	H272 H361d H302 H410	-	-	-

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The Dossier Submitter (DS) proposed a harmonised classification and labelling of potassium permanganate as Repr. 2 for effects on sexual function and fertility.

A one-generation reproductive toxicity study in rats (Plodicová, 2008, OECD TG 415 and GLP compliant) and two 28-day studies, one with oral and one with dermal exposure to potassium permanganate (OECD TG 408, GLP), were included in the CLH report by the DS for the assessment of effects on sexual function and fertility.

In the one-generation study Wistar rats were exposed to 0, 20, 80 and 320 mg/kg bw/d of potassium permanganate. Some reproductive parameters were impaired. These included a markedly lower number of pregnant females and number of dams bearing live pups at the highest dose level. Decreased fertility index, conception index and gestation index was also reported at 320 mg/kg bw/d. Other reproductive parameters such as average duration of pregnancy, post-implantation losses and pup viability index were not adversely affected. Effects reported in the reproductive organs in the one-generation study were a significant decreased weight of prostate gland and various damages of spermatogenesis. There were no significant pathological findings which indicated damage to female reproductive organs.

Parental toxicity included a reduced body weight and body weight gain in both sexes with a significant effect in males. This was associated with reduced food consumption. The primary target organ was the digestive tract with inflammation, erosion, ulceration or haemorrhage. However, in 5 of the 9 females in the high dose group that were not pregnant, no effects on the digestive tract were reported.

In the 28-day oral study rats were exposed to 0, 40, 100 and 250 mg/kg bw/d of potassium permanganate. Two satellite groups were also included and exposed to 0 or 250 mg/kg bw/d of potassium permanganate for 28 days with a recovery period of 14 days. In the male reproductive organs an increased relative weight of testes and epididymis was reported at 100 and 250 mg/kg bw/d. These effects were not reported in the satellite groups. Histopathological effects in the male reproductive tract were only sporadic and not dose-related.
In females an increase in absolute and relative weight of uterus was reported in all dose groups including the satellite treated groups. Histopathological effects in the female reproductive tract were only sporadic and not dose-related.

Systemic toxicity in the 28-day study included a slight decrease in body weight at all doses in males and at the highest dose in females. Decreased body weight gain was also reported at all doses in males and at the two highest doses in females. These effects were associated with a decrease in food consumption. The water consumption was also decreased at 250 mg/kg bw/d in males and females. Variations in haematology, biochemistry and urinalysis were reported, in

some animals from 100 mg/kg bw/d. The relative weight of the liver was increased in all dose groups in males. The spleen weight (relative and absolute) was increased in males and females in the high dose group. In females the kidney weight (relative and absolute) was also increased in the high dose group. Microscopic examination showed effects in the liver and stomach in both sexes. In 6 females eosinophil infiltration and oedema of mucosa were reported in the stomach in the high dose group; however, similar effects were not reported in the other groups. Only sporadic changes were reported in males in the liver and stomach.

In the 28-day dermal repeated dose toxicity study, rats were exposed to 0, 150, 300 and 600 mg/kg bw/d. Two satellite groups were also included exposed to 0 or 600 mg/kg bw/d of potassium permanganate for 28 days with a recovery period of 14 days. No marked effects were reported in male and female reproductive organs. Systemic toxicity was evident as a slight decrease in body weight with a more marked decrease in body weight gain at all dose levels in both sexes. This was associated with no or low reduction in food consumption. Some variations in urinanalysis, haematology and biochemistry were also reported in both sexes. No statistically significant changes in organ weight were reported, and the main histopathological effects included inflammation of skin with parakeratosis or hyperkeratosis in both sexes in the mid- and high dose group.

Overall, the DS concluded that the adverse effects to the male reproductive organs were slight to moderate and that the data does not establish a link between the effects on the male reproductive organs and the decrease in fertility index. Further, the decreased fertility index was only observed in the high dose group which also caused systemic toxicity. The evidence was therefore not sufficiently convincing to place potassium permanganate in Repr. 1B for effects on sexual function and fertility. However, a classification for reproductive toxicity in Repr. 2 was proposed.

Developmental toxicity

The DS proposed a harmonised classification and labelling of potassium permanganate as Repr. 1B for effects on development.

A prenatal developmental toxicity study performed according to OECD TG 414 and GLP compliant (Plodíková, 2009) and the one-generation reproduction toxicity study (Plodíková, 2008) in rats were included by the DS to assess the developmental toxicity of potassium permanganate.

In the developmental toxicity study, Wistar rats were exposed to 0, 20, 100 and 500 mg/kg bw/d of potassium permanganate from gestation day (GD) 5-19 (Plodíková, 2009). In this study the body weight of the female pups were statistically significantly decreased at 500 mg/kg bw/d. This dose also induced a 3-fold increase in post-implantation loss and an increase in total resorptions. A decreased number of ossification sites in sternum and incomplete ossification of cervical vertebrae were also observed in all treated groups. Maternal toxicity was noted as decreased body weight in the high dose group, and several clinical signs and microscopic changes in the stomach in the mid- and high dose groups.

In the one-generation reproductive toxicity study Wistar rats were exposed to 0, 20, 80 and 320 mg/kg bw/d of potassium permanganate. In this study the gestation index was 68.8% in the high dose group compared to 90.5% in the controls. In the pups the target organ was the brain with an increased weight at 320 mg/kg bw/d and marked vacuolisation of cell nuclei in cortex and/or hippocampus in all treated groups. Other effects included a slight decrease of viability index at 320 mg/kg bw/d and late opening of eyes from 80 mg/kg bw/d. Maternal toxicity was

noted as microscopic changes in the stomach. However, in 5 of the 9 females in the high dose group that were not pregnant no effects on the digestive tract were reported.

Overall, the DS considered that based on the low gestation index (68.8%) and high incidence of post-implantation losses (42%) a classification for developmental toxicity as Repr. 1B; H360D was justified. The DS also noted that these effects were only reported in the high dose in the one-generation study (320 mg/kg bw/d) and in the developmental toxicity study (500 mg/kg bw/d). However, since these doses were not associated with excessive parental toxicity, the effects reported at these dose levels were considered relevant for classification. The DS considered the effects as severe and not as a non-specific consequence of maternal toxicity. Other developmental effects of less severity (late opening of eye, skeletal variations and effects on pup brain) were also reported and occurred at doses not associated with maternal toxicity. The evidence was therefore considered sufficiently convincing and the DS proposed a classification of potassium permanganate for developmental toxicity as Repr. 1B; H360Df.

Comments received during public consultation

Three Member States Competent Authorities (MSCAs) and one Company-Manufacture commented on the CLH report during public consultation.

One MSCA supported the classification proposed by the DS as Repr. 1B; H360Df. One MSCA questioned the classification as Repr. 1B for developmental effects and thought that maybe a classification as Repr. 2 for both fertility and development was more appropriate based on the available data. One MSCA agreed with the DS's proposal for a classification as Repr. 1B for developmental toxicity but suggested also a classification for fertility in category 1B based on the important effects on the fertility parameters.

Two of the MSCAs asked for more clarification regarding the parental toxicity and the influence of the parental toxicity on the effects reported in the Plodíková, 2008 and 2009 studies.

Another MSCA asked for more data on the toxicokinetic properties of KMnO4 for a possible read-across from other tested manganese-oxidated forms. Given the strong oxidating potential, it is expected that a reduced form of Mn will be formed in the stomach. A clarification regarding the statistical significance of the effects in the Plodíková, 2008 and 2009 studies was also requested.

The Company-Manufacturer commented and did not support the classification proposed by the DS. They commented that many studies are available on manganese including inorganic compounds which were not assessed by the DS and which showed no effects on reproduction. The only two reproductive toxicity studies included by the DS were considered by the Company-Manufacturer to lack relevant parameters such as statistical analysis and information on historical control data. Furthermore the studies were also conducted at very high dose levels, thereby questioning their reliability. Therefore, as a precautionary approach they proposed a self-classification as STOT RE 2 based on the general toxicity reported in the reproductive toxicity studies and in the 28-day repeated dose toxicity studies included in the CLH report. They also proposed a harmonised classification as Repr. 2; H361d as there is some evidence of developmental toxicity, although not conclusive, and no classification for sexual function and fertility with the argument that effects on fertility occurred at high doses and as a secondary consequence of general toxicity.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

For the assessment of effects on sexual function and fertility following exposure to potassium permanganate a one-generation study in rats performed according to OECD TG 415 and GLP was included in the CLH report. Two 28-day repeated dose toxicity studies in rats, one with oral exposure and one with dermal exposure to potassium permanganate were also included by the DS to assess the effects on male and female reproductive organs. No information regarding historical control data for the various parameters measured in the studies was included in the CLH report. Due to limited information on effects on fertility and sexual function following exposure to potassium permanganate, information from other manganese compounds (as requested during public consultation) was also included in the RAC assessment of effects on fertility and sexual function following exposure to potassium permanganate. This information is included in a separate section.

In the one-generation study, Wistar Han rats were exposed to 0, 20, 80 and 320 mg/kg bw/d potassium permanganate by oral gavage (10 males and 25 females/dose group). No mortality was found except for one non-pregnant female in the high dose group that died in the first week after mating. All females in all dose groups were mated, measured as the number of females mated/number of females paired x 100. In the highest dose group some reproductive parameters were affected including a decrease in the fertility index (number of pregnant females/no of females paired x 100; the pregnancy was determined by the presence of spermatozoa in vaginal smears), in dams bearing live pups (gestation index), as well as the number of born pups, see table below. As regards the effect reported on gestation index, it is not clear from the data reported if this effect is related to an effect on fertility or development. No clear effects were reported on the viability and weaning index. The data show a decreased ability of rats to achieve or maintain pregnancy following exposure to potassium permanganate at the highest dose. Limited statistical analysis was included in the CLH report on the reported findings.

Table: Reproduction data

Observation parameters	**Dose level (mg/kg bw/d)**			
	0	**20**	**80**	**320**
Males/females paired	10/25	10/25	10/25	10/25
Fertility index %*	84	84	80	64
Gestation index %**	90.5	81	85	68.8
Dams with live pups	19	17	17	11
Number of born pups	207	164	163	99
Viability index	98.6	99.4	98.8	96.0
Weaning index	100	100	100	98.0

*number of pregnant females/number of paired females x 100

**number of females bearing live pups/number of pregnant females x 100

Systemic toxicity in males was evident as a decrease in body weight during the 13-week exposure period in the highest dose group reaching a 18.7% decrease (statistically significant) in week 13 of exposure. The decrease in body weight was associated with a decrease in food consumption starting from week 3 and was considered as marked from week 8. The health condition of males was affected in the two highest dose groups, with more pronounced effects in the high dose group including dyspnea, decreased activity, red secretion around nose or eyes, rigidity, piloerection and salivation. In females, no statistically significant changes in body weight were reported before mating, during gestation and lactation in all three dose groups. The food consumption was decreased during the pre-mating period in the high dose group and was associated with a decreased body weight gain. The health condition of females was affected in

the high dose with dyspnea in 3 females the 1st week of exposure, in 2 females in the 2nd week, in one animal in the 4th week, and in two animals in the 7th week.

Macroscopic examination showed that all males at the highest dose level showed marked changes in the stomach with blood erosions of mucosa. Microscopic examination in males also showed marked changes in the stomach at this dose level, including erosion, ulceration and inflammation. This is in accordance with the fact that if manganese is in the (VII) oxidation state in potassium permanganate, then ingestion may lead to severe corrosion at the point of contact (ATSDR Report 2012). In the testes a slight damage of spermiogenesis in the high dose group including atrophy of germinal epithelium and atrophy or decreased number of Leydig cells were reported. However, no statistical analysis was included in the CLH report on these findings. Information from other experimental animal studies have also reported harmful effects following exposure to other manganese compounds on male fertility, see separate section on information from other manganese compounds below.

Table: Microscopic findings in males

Observation parameters	**Dose level (mg/kg bw/d)**			
	0	**20**	**80**	**320**
Testes: insignificant damage of spermiogeneis	1	2	0	0
Testes: slight damage of spermiogeneis	0	0	0	5
Stomach: erosion and ulceration	0	0	1	8
Stomach: inflammation	0	0	1	10

Microscopic examination in females included marked changes in the stomach including erosion, ulceration and inflammation in the highest dose group. Effects were also reported in the female reproductive system including cysts and cellular hyperplasia in the ovaries; and cellular hyperplasia of endometrium, hydrometra and degenerative changes in the uterus. However, the effects in the uterus and ovaries were not dose-related.

In the oral 28-day study male and female Wistar rats were exposed to 0, 40, 100 and 250 mg/kg bw/d potassium permanganate by gavage. Two satellite groups were also included exposed to 0 or 250 mg/kg bw/d of potassium permanganate for 28 days with a recovery period of 14 days. No information was included in the CLH report regarding the statistical significance of the effects reported.

Regarding the general toxicity, a slight decreased body weight in all doses in males and in the high dose females were reported. However, this was associated with decreased food intake. The reduction in body weight was reversible during the recovery period, but not in males in the high dose group. The water intake was also decreased in males and females in the high dose group.

Variations in haematology, biochemistry, and urinalysis were reported, some from 100 mg/kg bw/d. An increase in the relative liver weight was reported in males in all dose groups. Further, an increase in absolute and relative spleen weight in males and females, and in absolute and relative kidney weight in females in the high dose group, was reported.

Microscopic analysis showed effects in the liver and stomach in both sexes. In females eosinophil infiltration and oedema of mucosa was reported in the stomach in the high dose group. However, only sporadic findings in the liver or stomach were reported in males.

As regards effects in the reproductive organs an increase in relative weight of testes and epididymis was reported at 100 and 250 mg/kg bw/d. These effects were not reported in the satellite group. In females an increase in the absolute and relative weight of uterus was reported in all dose groups and in the satellite group.

Histopathological effects in the male and female reproductive organs included no significant effects in testes and epididymis in males nor in the uterus of females.

In the dermal 28-day study, male and female Wistar rats were exposed to 0, 150, 300 and 600 mg/kg bw/d potassium permanganate. The study included two satellite groups exposed to 0 or 600 mg/kg bw/d for 28 days with a recovery period of 14 days. No information was included in the CLH report regarding the statistical significance of the effects reported.

Regarding the general toxicity a slight decrease in body weight and more marked decrease in body weight gain was reported at all doses in both sexes. Some variations in urinanalysis, haematology and biochemistry were reported in both sexes. No statistically significant changes in organ weights were reported. The main histopathological effects included inflammation of skin with parakeratosis or hyperkeratosis in both sexes in the mid- and high dose group.

As regards effects in the reproductive organs, no significant changes were reported in male and female reproductive organ weights and there were no histopathological changes.

Summary

In the one-generation study in rats a decrease in the fertility index, in dams bearing live pups, as well as the number of born pups were reported in the highest dose group. However, at this dose level severe systemic toxicity was observed in males, including body weight loss, dyspnea, decreased activity, red secretion around nose or eyes, rigidity, piloerection and salivation. Macroscopic and microscopic examination also showed marked changes in the stomach, such as ulceration, erosion and inflammation. In pregnant dams effects including erosions, ulceration and haemorrhage were reported on the digestive tract, but there was no apparent correlation between the stomach effects in females and the ability to achieve pregnancy.

A reduction in the number of implantations and viable foetuses has also been reported in a study where female mice were exposed to manganese chloride, and a reduction in pregnant females in rats were reported following exposure to Mn_3O_4. However, due to the highly corrosive/oxidizing effect of potassium permanganate, it is not possible to draw conclusions from these studies, as the effects seen with potassium permanganate were seen at the high dose which caused severe toxicity.

In males effects on spermatogenesis were reported in the presence of a decreased body weight and severe irritation of the digestive tract in the high dose group. The effects reported on the testes could have influenced the decreased fertility index. However, the data available does not permit to identify which females were mated with which male, therefore a clear link could not be established. Male mice exposed to other manganese compounds have also shown effects on male reproductive organs, sperm quality and fertility. Further, in male rats a Mn related maturational delay in male reproductive parameters was reported following exposure to Mn_3O_4 at day 100 of exposure. However, from the one-generation study with potassium permanganate it is not clear if the effects on male reproductive organs is responsible for the effects on fertility reported since severe toxicity in males was reported at the same dose levels as testicular toxicity was observed. In the 28-day oral and dermal repeated dose toxicity studies no clear relationship between

exposure to potassium permanganate and effects on male and female reproductive organs could be seen.

Comparison with the CLP criteria

Repr. 1A:

There is no information regarding effects on fertility following exposure to humans, so RAC considers that a classification of potassium permanganate as Repr. 1A is not justified.

Repr. 1B:

According to the CLP criteria, classification of a substance in Category 1B is largely based on data from animal studies. Such data should provide clear evidence of an adverse effect on reproductive toxicity in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction should be considered not to be a secondary non-specific consequence of other toxic effects. RAC concludes that the decrease in fertility index, gestation index, dams with live pups and number of born pups observed in the high dose group in the one-generation study occurred together with severe toxic effects and so are considered to be secondary non-specific consequences of parental toxicity, therefore classification of potassium permanganate as Repr. 1B is not justified.

Repr. 2:

According to the CLP criteria classification of a substance in Category 2 is justified when there is some evidence from humans or experimental animal, possibly supplemented with other information of an adverse effect on sexual function and fertility, 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. RAC concludes that the effects reported in the one-generation study observed in the high dose group in the presence of severe toxicity are considered to be secondary non-specific consequences of parental toxicity. Also taking into account the general poor quality of the study (very limited statistical analysis, no historical control data) RAC concludes that classification of potassium permanganate as Repr. 2 is not justified.

Therefore, **RAC concludes not to classify potassium permanganate for effects on sexual function and fertility**.

Developmental toxicity

For the assessment of developmental toxicity following exposure to potassium permanganate a one-generation study (OECD TG 415 and GLP) and a developmental toxicity study (OECD TG, GLP) was included by the DS. No information regarding historical control data for the various parameters measured was included in the CLH report.

In the one-generation study where Wistar rats were exposed to 0, 20, 80 and 320 mg/kg bw/d potassium permanganate a decreased gestation index was reported at 320 mg/kg bw/d (see relevant table above). This corresponds to a decrease in dams having live pups among the pregnant females. A decrease in the number of viable foetuses was also reported in the study by Elbethieha et al. (2001) following exposure to manganese (II) chloride tetrahydrate (see relevant table above). However, as regards the effect reported on the number of dams having live pups, it is not clear from the data reported if this is related to an effect on fertility or development. Some delay in opening of eyes (until 14 days after birth) was reported at 80 mg/kg bw/d in 2 out of 16 litters and at 320 mg/kg bw/d in 3 out of 10 litters. The delay was not associated with decreased pup body weight. At microscopic examination sporadic pathological findings were

reported in the high dose group and included missing testes and epididymis (one pup) one testis reduced (one pup) and stomach mucous membrane congested and chime with blood (two pups). In the brain, an increased absolute and relative weight was reported that reached statistical significance in the high dose level. Vacuolisation in the cortex and/or hippocampus was also more marked in the treated groups compared to the control group (see relevant table above). Manganese has been shown to easily pass through the placenta (Erikson *et al*., 2007) and has been shown to accumulate in greater amounts in the blood and tissues of pregnant laboratory animals (Cawte, 1985) and accumulate in foetal brain after gestational exposure (Kontur and Fetcher, 1988). Further, Donaldson (1987) and Komura and Sakamoto (1992) reported that elevated levels of manganese can be neurotoxic and produce central nervous system damage.

Maternal toxicity in the one-generation study was evident as marked changes in the stomach including erosion, ulceration and inflammation in the high dose group. No statistically significant changes in maternal body weight were reported before mating, during gestation and lactation in all three dose groups.

Table: Effects in the brain in pups

Observation parameters	**Dose level (mg/kg bw/d)**			
	0	**20**	**80**	**320**
Number of examined pups	20	20	20	20
Absolute weight of brain (g)	1.28	1.32	1.30	1.35*
Relative weight of brain (g)	3.34	3.35	3.50	3.68*
Microscopic findings (no. of pups with changes)				
Without changes	17	1	2	2
Vacuolisation of cell nuclei (mild)	3	7	3	2
Vacuolisation of cell nuclei (more marked)	0	12	15	16

*Statistically significant on probability level 0.05 (ANOVA test)

In the developmental toxicity study Wistar rats were exposed to 0, 20, 100 and 500 mg/kg bw/d of potassium permanganate from GD 5 to 19 (Plodíková, 2009). No maternal mortality was reported.

In the high dose group, an increased number of resorptions was reported (implantations without recognisable embryo/foetuses or dead embryo foetuses with external degenerative changes). A dose-depended increase in post-implantation losses (resorptions/implantations x 100) were also reported, however, no statistical analysis was performed on these finding. But when the DS performed a Kruskal Wallis test on the increase in post-implantation losses, statistical significance was reached in the high dose. It should be noted that in the high dose group the post-implantation losses included 8 dams with full litter resorptions which is considered to explain the absence of effects reported on the litter size. At this dose there was also severe toxicity in the maternal animals. A decrease in the number of implantations was also reported in the study by Elbethieha *et al*., 2001 following exposure to manganese (II) chloride tetrahydrate (see relevant table above), however, there was no information regarding whether these were post- or pre-implantation losses. The increased number of resorptions is also considered to be consistent with the decrease in gestation index reported in the one-generation study at 320 mg/kg bw/d. No effect on pre-implantation losses (corpora lutea minus implantations/corpora lutea x 100) were reported in the developmental toxicity study by the DS, see table below. However, in a developmental study performed according to OECD TG 414 with exposure starting on GD 5 pre-

implantation losses occurred before the exposure to potassium permanganate started. Therefore, the number of pre-implantation losses reported in the developmental toxicity study is considered to have no impact on the assessment of developmental toxicity since it was induced before the exposure to potassium permanganate started.

Table: Parameters of reproduction in the developmental toxicity study

Observation parameters	**Dose level (mg/kg bw/d)**			
	0	**20**	**100**	**500**
Implantations	9.13	8.95	8.19	8.57
Resorptions	0.61	1.59	1.38	2.83
Corpora Lutea	11.57	12.18	11.24	11.43
Pre- and post-implantation losses (% per female, average)				
Pre-implantation loss[a]	22.84	25.61	28.39	25.49
Post-implantation loss[b]	14.18	22.80	24.65	42.25*
No of foetuses/litter	9.80	9.16	8.41	8.80

*statistically significant with Kruskal Wallis test

[a] intra uterine death, early

[b] intra uterine death, late

A decrease in foetal body weight that reached statistical significance in the highest dose level in female foetuses was reported (3.45, 3.13, 3.12 and 2.78g* at 0, 20, 100 and 500 mg/kg bw/d, respectively).

From the internal examination of variations and malformations a dose dependent increase in skeletal variations was reported for "sternum – decreased no. of ossification sites" and "vertebra, incomplete ossification of cervical vertebrae" both on the foetal and litter level, see table below. The only alterations described as a malformation by the DS was the absence of supraoccipital bone that was seen in one foetus/one litter in the high dose group.

Table: Skeletal alterations (no. of affected foetuses/no. of affected litters)

Alterations	**Dose level (mg/kg bw/d)**			
	0	**20**	**100**	**500**
Total no. of examined foetuses/litters	101/20	91/19	78/17	67/14
Cranium – absence of supraoccipital bone	0/0	0/0	0/0	1/1
Cranium – unossified supraoccipital bone	0/0	1/1	0/0	0/0
Cranium – incomplete ossification of parietal bone	3/2	0/0	8/5	1/1
Cranium - incomplete ossification of frontal bone	0/0	0/0	2/1	0/0
Cranium - incomplete ossification of interparietal bone	1/1	0/0	0/0	0/0
Sternum – decreased no. of ossification sites	40/13 39.6%[a] 65%[b]	49/14 53.8% 73.7%	48/14 61.5% 82.4%	45/12 67.2% 85.7%
Vertebrae - incomplete ossification of cervical vertebrae	0/0 0% [a] 0% [b]	8/2 8.8% 10.5%	8/2 10.3% 11.8%	12/4 17.9% 28.6%
Vertebrae – unossified sacral vertebrae	5/1	18/4	8/2	11/3
Robes – wavy (undulation along the length of a rib)	7/6	3/3	6/4	0/0

[a] Percent of affected foetuses

[b] Percent of affected litters

Maternal toxicity was evident as a statistically significant decrease in body weight during gestation reaching the highest reduction of 14% on GD 8. This was associated with a reduction in food consumption from GD 8 to 14 with more marked reduction in the high dose group. However, the corrected body weight gain (corrected for uterus weight) was only slightly lower (4.3%) compared to the control group. Maternal clinical signs were sporadically reported in the

two highest dose groups and included red secreta around nostrils or eyes, piloerection, hoarse breath or dyspnea. In the high dose group cough, gibbous pose, anaemia, apathy and cachexia were also noted. Difficulties with the application of potassium permanganate was also reported in the high dose group and included emesis, return of the test substance into oesophagus and excited behaviour immediately after application of potassium permanganate. No clear correlation could be found between the five dams showing cachexia in the high dose group and the percentages of post-implantation losses.

During necropsy no effects were reported in the control and low dose groups. At 100 mg/kg bw/d erosion of stomach mucosa was reported in 3 females. In the high dose group more frequent occurrence of effects in the stomach were reported (in 18 out of 25 dams) and included erosion, blood in content, ulceration, thickened stomach, oedematous, haemorrhage and congested mucosa.

Summary

In the one-generation study a decrease in gestation index and a slight decrease in viability index was reported at 320 mg/kg bw/d. However, as regards the effect reported on gestation index, it is not clear from the data reported if this is related to an effect on fertility or development. Exposure to other manganese compounds has also shown effects on implantations and the number of viable foetuses. Late opening of eyes from 80 mg/kg bw/d was also reported. In pups it was evident that the main target organ was the brain with increased weight at 320 mg/kg bw/d and marked vacuolisation of cell nuclei (indicating degenerative processes) in the cortex and/or hippocampus in all treated groups with increased severity with increasing dose. Maternal toxicity included severe microscopic changes in the stomach in the high dose group, however, in the lower dose-groups without severe maternal toxicity marked vacuolisation of cell nuclei was reported. Other studies have also reported that exposure to other manganese compounds can induce neurotoxicity and produce central nervous system damage.

In the developmental toxicity study a three times increase in post-implantation losses and an increase in total resorptions were reported compared to control animals in the high dose group. Decreased number of ossification sites in sternum and incomplete ossification of cervical vertebra was reported in all treated groups. The female pup body weight was also statistically significantly reduced in the high dose group. The maternal toxicity included a statistically significant decrease in body weight, however, only marginal decrease in corrected body weight gain, and several clinical signs and severe microscopic changes in the stomach in the high dose group were observed.

Comparison with the CLP criteria

Repr. 1A:

There is no information regarding effects on fertility following exposure to humans, and thus RAC considers that a classification of potassium permanganate as Repr. 1A is not justified.

Repr. 1B:

According to the CLP criteria, a 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 development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects. RAC concludes that the main effect could be found in the pups, where the target organ was the brain with an increased weight at 320 mg/kg bw/d and marked vacuolisation of cell nuclei (indicating degenerative processes) in the cortex and/or hippocampus in all treated groups with increased severity with increased doses, i.e., also at doses that did not cause

maternal toxicity, indicating severe effects on development following exposure to potassium permanganate. However, due to the limitations of the study (lack of statistical analysis, no historical control) and no available developmental neurotoxicology study, RAC considers that the data is not sufficient to justify a classification in Repr. 1B.

Repr. 2:

According to the CLP criteria, a classification of a substance in Category 2 is justified when there is some evidence from humans or experimental animals, possibly supplemented with other information of an adverse effect 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.

RAC concludes that the effects reported in the developmental toxicity study on the histopathological changes in pup brain at doses not causing maternal toxicity is considered as some evidence of developmental toxicity and a **classification in Repr. 2 for development** is considered justified.

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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).