HSE

Health and Safety Executive

ECHA 2015/248:

Assess the risk estimate for the intrinsic property “Toxic to reproduction” of the Cr(VI) compounds listed in Annex XIV except for lead chromate

Final Report

Report prepared by the UK
Health and Safety Executive’s
Chemicals Regulation Directorate

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CONTENTS

INTRODUCTION

1. In 2013, the contractor performed a review of the carcinogenic dose response of 14 hexavalent chromium (Cr(VI)) compounds included in Annex XIV of REACH (Final report Nov 2013). The 14 compounds are listed in table 1.1 below.

Table 1.1 Hexavalent chromium substances included in Annex XIV, the 4th recommendation for inclusion in Annex XIV and the candidate list. The reproductive toxicity Cat 1B classification of some compound is highlighted in bold.

SUBSTANCE NAME	EC Number	Intrinsic properties specified in Annex XIV/recommendation
Ammonium dichromate	231-143-1	Carcinogenic cat 1B Mutagenic cat 1B **Toxic for reproduction cat 1B**
Potassium chromate	232-140-5	Carcinogenic cat 1B Mutagenic cat 1B
Acids generated from chromium trioxide and their oligomers. Names of the acids and their oligomers: Chromic acid, Dichromic acid, Oligomers of chromic acid and dichromic acid	231-801-5; 236-881-5	Carcinogenic cat 1B
Chromium trioxide	215-607-8	Carcinogenic cat 1A Mutagenic cat 1B
Potassium dichromate	231-906-6	Carcinogenic cat 1B Mutagenic cat 1B **Toxic for reproduction cat 1B**
Sodium chromate	231-889-5	Carcinogenic cat 1B Mutagenic cat 1B **Toxic for reproduction cat 1B**
Sodium dichromate	234-190-3	Carcinogenic cat 1B Mutagenic cat 1B **Toxic for reproduction cat 1B**
Lead sulfochromate yellow (C.I. Pigment Yellow 34)	215-693-7	Carcinogenic cat 1B **Toxic for reproduction cat 1B**
Lead chromate molybdate sulphate red (C.I. Pigment Red 104)	235-759-9	Carcinogenic cat 1B **Toxic for reproduction cat 1B**
Lead chromate	231-846-0	Carcinogenic cat 1B **Toxic for reproduction cat 1B**
Dichromium tris(chromate)	246-356-2	Carcinogenic cat 1B
Strontium chromate	232-142-6	Carcinogenic cat 1B
Pentazinc chromate octahydroxide	256-418-0	Carcinogenic cat 1A
Potassium hydroxyocataoxodizincatedichromate	234-329-8	Carcinogenic cat 1A

2. As intended, the focus of the 2013 contract report was exclusively carcinogenicity. However, as shown by the table, some of these chromates (four) are also classified for reproductive toxicity in category 1B (this effect also being produced by the Cr(VI) ion). In addition, three lead chromate compounds are listed in the table; lead has well-recognised developmental neurotoxicity properties leading to a harmonised classification for reproductive toxicity in category 1B.

3. During discussions at RAC (Risk Assessment Committee) of the output of the original 2013 project on carcinogenic risk, the committee considered that in the Authorisation process for Cr(VI) compounds, the possible risk of reproductive effects ought to be understood.

4. For the four chromates classified for reproductive toxicity category 1B (ammonium dichromate, sodium dichromate, potassium dichromate and sodium chromate), as a consequence of the action of the Cr(VI) ion, RAC noted that it was important to establish dose-response relationships for this endpoint. RAC's opinion was that, although it is most likely that the carcinogenic risk would drive the risk-benefits analyses for authorisation purposes, it would be important to know if there are exposure levels during use at which reprotoxicity considerations could make a significant contribution to the assessment of the total ill-health risk arising from Cr(VI) exposure. RAC also noted that for (local) carcinogenicity, no dermal DNEL had been set, and therefore it would be important to address the dermal risks arising from reproductive toxicity. Furthermore, RAC considered that the developmental neurotoxicity of lead in the three lead chromate substances should be addressed. It was suggested that the dose-response relationship established by EFSA (EFSA, 2013) for lead developmental neurotoxicity should be used for this purpose.

5. Given the agreed approach for the lead-containing chromates, this sub-contract was established to retrieve and assess the relevant reproductive toxicity (fertility and development) data on the Cr(VI) ion. The requirement was to identify the dose-response relationships for these endpoints and to propose appropriate DNELs (Derived No Effect Levels).

6. Beyond that, the project then needed to reflect on the situation for other Cr(VI) compounds in Table 1.1. Of these, it is unclear why the readily water-soluble substance potassium chromate is not classified at all for reproductive toxicity, when one would predict very similar properties to those of potassium dichromate and/or sodium chromate (and dichromate). Similar considerations apply to the acids generated from chromium trioxide and their oiligomers), when viewed against the position for chromium trioxide.

7. For the insoluble and sparingly soluble chromates among the 14 substances (pentazinc chromate octahydroxide, dichromium tris(chromate), potassium hydroxyocataoxodizincatedichromate and strontium chromate), it is possible that the lack of solubility results in the reproductive toxicity of Cr(VI) not being expressed.

REPRODUCTIVE TOXICITY HAZARD IDENTIFICATION AND CHARACTERISATION

8. Similarly to the original project, existing detailed, good-quality reviews of the toxicology (including reproductive toxicity data) of Cr(VI), published in the scientific literature or by particular authorities around the world since the year 2000 were obtained. These are outlined in table1.2 below.

Table 1.2: Outline of reviews of Cr(VI) compounds used as the basis of this sub-project

Reference/year	Title	Organisation	Content/aim of publication
ESR (Existing Substance Regulation) Risk Assessment Report, 2005	EU Risk Assessment Report (RAR) for chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate and potassium dichromate	EU	Hazard and risk assessment of 5 Cr(VI) substances for the purposes of subsequent risk management
Draft USEPA, 2010	Toxicology review of hexavalent chromium. In support of summary information on IRIS	Environmental Protection Agency, USA	Hazard and risk assessment of Cr(VI) compounds for the purpose of establishing oral standards for the general population
ATSDR, 2012	Toxicological profile for chromium	Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services	Hazard assessment of Cr(VI) compounds aimed at health care providers

9. The registration dossiers and associated CSRs (Chemical Safety Reports) for the four chromate substances classified for Cat 1B reproductive toxicity were also retrieved. The contractor noted that these registration dossiers refer only to the data evaluated in the EU RAR, without consideration of any new data that might have become available since around 2000. The contractor also noted that despite using the reproductive toxicity data described in the EU RAR, the toxicological starting points for risk assessment chosen by the registrants differ from those used in the EU RAR.

10. From these three reviews, the relevant reproductive toxicity studies were extracted and tabulated below. Given the harmonised classification of these five chromates, the hazard identification aspects of each study were not re-appraised critically, but the main findings,

including information on dose-response, NOAEL and LOAEL values were simply reproduced in the table. It should be noted that all the available experimental animal studies were conducted by the oral route and used potassium dichromate, sodium dichromate or chromic acid.

***Table 1.3: Relevant reproductive toxicity studies extracted from the 3 reviews, with* associated NOAEL and LOAEL values (as identified by the review)**

EU RAR, 2005						
Endpoint	**Study description**	**Repro findings**	**NOAEL (mg CrVI/kg bw/d)**	**LOAEL (mg CrVI/kg bw/d)**	**Reliability according to review**	**Reference**
Fertility and reproductive performance	Swiss mice; Potassium dichromate; Drinking water; Exposure for 12 weeks (353 -1,765 mg Cr(VI)/L), then mating;	Effects on implantations, resorptions and viable foetuses at relatively high dose levels of Cr(VI)	Cannot be set due to inadequacies in reporting	Cannot be set due to inadequacies in reporting	Reliable but inadequacies in reporting	Elbetieha and Al-Hammond, 1997
Fertility and reproductive performance	Balb/c mice (24 male and 48 females per group); Potassium dichromate; Diet; Exposure for 9 weeks (1- 32 mg Cr(VI)/kg bw/d, then mating	No effects on fertility and reproductive performance up to 32 mg Cr(VI)/kg bw/d	32 (highest dose tested)	>32	Reliable	NTP, 1996a
Fertility and reproductive performance	Sprague-Dawley rats (24 males and 48 females); Potassium dichromate;	No effects on fertility and reproductive performance up to 48 mg Cr(VI)/kg bw/d	8 (highest dose tested)	>48	Reliable	NTP, 1996b

	Diet Exposure (1 – 48 mg Cr(VI)/kg bw/d) for 9 weeks, then mating					
Fertility and reproductive performance	Balb/c mice (20 males and females); Potassium dichromate; Dietary (7/20 – 30/86 mg Cr(VI)/kg bw/d in f/m); Continuous breeding study; GLP	No effects on fertility and reproductive performance up to 30 mg Cr(VI)/kg bw/d	30 (highest dose tested)	>30(f)	Reliable	NTP, 1997 (Wolfe, 1997)
Male reproductive organs	Male rats; Sodium dichromate; Gavage; Exposure (7, 14, 21 mg Cr(VI)/kg bw/d) for 90 days	Degeneration of testis at 14 and 21 mg Cr(VI)/kg bw/d	7	14	Sufficiently reliable	Chowdhury and Mitra, 1995
Fertility (in females)	Female Swiis mice (15/group); Potassium dichromate; Drinking water; Exposure (20, 40, 60 mg Cr(VI)/kg bw/d) before mating and pregnancy	↓ no corpora lutea; ↑pre-implantation loss; Fetotoxicity at 40 mg Cr(VI)/kg bw/d	20	40	Sufficiently reliable	Junaid et al., 1996b
Developmental toxicity	Pregnant female Swiss mice (10/group);	↑implantation loss; ↓ossification	70 (fetotoxicity)	125 (fetotoxiciy)	Reliable	Junaid et al., 1996a

	Potassium dichromate; Drinking water; Exposure 35, 70, 125 mg Cr(VI)/kg bw/d) during gestation (GD 6-14)	at doses without significant maternal toxicity				
Draft USEPA, 2010						
Male reproductive organs	Male monkeys (3/group); Potassium dichromate; Drinking water; Exposure for 6 months (1, 2.1, 4.1, 8.3 mg Cr(VI)/kg bw/d) + 6 month recovery	Testis degeneration from 2.1 mg Cr(VI)/kg bw/d; Effects reversible after 6 months recovery	No reliable NOAEL can be set due to small group sizes (3 animals)	No reliable LOAEL can be set due to small group sizes	Studies have limitations	Aruldhras et al., 2004; 2005; 2006
Male reproductive organs	Male monkeys (3/group); Potassium dichromate; Drinking water; Exposure (1, 2, 4, 8 mg Cr(VI)/kg bw/d) for 6 months + 6 month recovery	Effects on sperm from 2 mg Cr(VI)/kg bw/d; Effects reversible after 6 months	No reliable NOAEL can be set due to small group sizes	No reliable LOAEL can be set due to small group sizes	Study has limitations	Subramanian, 2006
Effects on mating in males	Male Sprague-Dawley rats (1213/group); Potassium dichromate;	↓mating Aggressive behaviour at a relatively high dose	No reliable NOAEL can be set due to reporting inadequacies	No reliable LOAEL can be set due to reporting inadequacies	Study has limitations	Bataineh et al., 1997

	Exposure (353 mg Cr(VI)/L)for some weeks before mating					
Male reproductive organs	Male Wistar rats (8-11/group); Chromium trioxide; Gavage; Exposure 5.2, 10.4 mg Cr(VI)/kg bw /d for 6 days	Various effects on testes from lowest dose tested	< 5.2	**5.2**	Reliable	**Li et al., 2001**
Male reproductive organs	Weanling male Swiss mice; Potassium dichromate; Diet; Exposure (6.4, 12.7, 25.5 mg Cr(VI)/kg bw/d) for 35 days	Effects on testes from the lowest dose tested of 6.4 mg Cr(VI)/kg bw/d	No reliable NOAEL can be set due to methodological deficiencies	No reliable LOAEL can be set due to methodological deficiencies	Unreliable (according to NTP)	Zahid et al., 1990
Female reproductive organs	Female Swiss mice (30/group); Potassium dichromate; Drinking water; Exposure (0.01 – 5 mg CrVI)/kg bw/d) for 20 or 90 days at very low doses	No effects reported	No reliable NOAEL can be set due to methodological deficiencies	No reliable LOAEL can be set due to methodological deficiencies	Unreliable	Murthy et al., 1996

Male reproductive organs	Male New Zealand rabbits (6/group); Potassium dichromate; Gavage; Exposure (3.6 mg Cr(VI)/kg bw/d) for 10 weeks	Effects on testes	No reliable NOAEL can be set due to reporting inadequacies	No reliable LOAEL can be set due to reporting inadequacies	Reliable but inadequacies in reporting	Yousef et al., 2006
Fertility and reproductive performance (females)	Female Swiss mice (20/group); Potassium dichromate; Drinking water; Exposure (6.4, 12.2, 15.3 mg Cr(VI)/rat/day) for 20 days prior mating and gestation	↓mating and fertility at high dose (15.3 mg Cr(VI)/mouse/d; Resorptions, post-implantation loss at all doses (from 6.4 mg Cr(VI)/mouse/d); Skeletal abnormalities at high dose	No reliable NOAEL can be set due to reporting inadequacies	No reliable LOAEL can be set due to reporting inadequacies	Reliable but inadequacies in reporting	Kanojia et al., 1996
Fertility and reproductive performance (females)	Druckerey female rats (20/group); Potassium dichromate; Drinking water; Exposure (70, 127, 170 mg Cr(VI)/kg bw/d) for 3 months prior to gestation	↓mating and fertility from lowest dose; Post-implantation loss from lowest dose; Skeletal abnormalities from lowest dose	< 70	70	Sufficiently reliable	Kanojia et al., 1998
Developmental toxicity	Pregnant Wistar rats (10/group); Potassium chromate (one dose); Drinking water;	Post-implantation loss; Resorptions; Dead foetuses; ↓foetal wt; Skeletal and visceral anomalies	< 7.9 (fetotoxicity)	**7.9 (fetotoxicity)**	Reliable, with limitations	**Elsaieed and Nada, 2002**

	Exposure during gestation (GD 6-15)	in the absence of significant maternal toxicity				
Developmental toxicity	Pregnant Sprague-Dawley rats (10/group); Potassium dichromate; Gavage; Exposure (35 mg Cr(VI)/kg bw/d) GD1-3 or 4-6; at relatively high dose	Pre and Post-implantation loss; Resorptions; Dead foetuses	< 35 (fetotoxicity)	35 (fetotoxicity)	Reliable	Bataineh et al., 2007
Developmental toxicity and effects during lactation	Pregnant Swiss mice (25/group); Potassium dichromate; Drinking water Exposure GD 12 – LD 20 at high dose of 2 mg Cr(VI)/mouse/d	↑time to vaginal opening; ↓female fertility in offspring; ↓implantations ↓viable foetuses	No reliable NOAEL can be set due to reporting inadequacies	No reliable LOAEL can be set due to reporting inadequacies	Reliable but inadequacies in reporting	Al-Hamood et al., 1998
Effects during lactational exposure on sexual development of female offspring	Lactating Wistar rats (18/group); Potassium dichromate; Drinking water; Exposure (70.6 mg Cr(VI)/L) LD1-21	↑onset of puberty in offspring; Effects on oestrus cycle in offspring; Delayed follicular development in offspring; ↓sex hormones in offspring	No reliable NOAEL can be set due to reporting inadequacies	No reliable LOAEL can be set due to reporting inadequacies	Reliable but inadequacies in reporting	Banu et al., 2008
Developmental toxicity	Mated female Swiss mice (10/group); Potassium dichromate;	Post-implantation loss and dead foetuses from 101 mg Cr(VI)/kg bw/d;	< 53	53	Sufficiently reliable	Junaid et al., 1995

	Drinking water; Exposure (53, 101, 152 mg Cr(VI)/kg bw/d) in late gestation GD 14-19	↓foetal wt and skeletal abnormalities from lowest dose				
ATSDR, 2012						
Male reproductive organs	F344/N male rats; Sodium dichromate; Drinking water Exposure for 3 months up to 20.9 mg Cr(VI)/kg bw/d	No effects reported	20.9 (highest dose tested)	> 20.9	Reliable	NTP, 2007
Male reproductive organs	B6C3F1 male mice; Sodium dichromate; Drinking water Exposure for 3 months up to 27.9 mg Cr(VI)/kg bw/d	No effects reported	27.9 (highest dose tested)	> 27.9	Reliable	NTP, 2007
Male reproductive organs	F344/N male rats; Sodium dichromate; Drinking water Exposure for 2 years up to 5.9 mg Cr(VI)/kg bw/d	No effects reported	5.9 (highest dose tested)	> 5.9	Reliable	NTP, 2008
Male reproductive organs	B6C3F1 male mice; Sodium dichromate; Drinking water Exposure for 2 years up to 5.9 mg Cr(VI)/kg bw/d	No effects reported	5.9 (highest dose tested)	> 5.9	Reliable	NTP, 2008
Effects on sperm counts	B6C3F1, Balb/c and C57BL/6N male mice;	No effects reported	8.7 (highest dose tested)	> 8.7	Reliable	NTP, 2008

	Sodium dichromate; Drinking water Exposure for 3 months up to 8.7 mg Cr(VI)/kg bw/d					
Effects on ovaries	B6C3F1 female mice and F344/N female rats; Sodium dichromate; Drinking water; Exposure for 3 months up to 27.9 mg Cr(VI)/kg bw/d in mice and up to 20.9 mg Cr(VI)/kg bw/d in rats	No effects reported	27.9 (highest dose tested) in mice; 20.9 (highest dose tested) in rats	> 27.9 in mice; > 20.9 in rats	Reliable	NTP, 2008
Developmental toxicity	Pregnant female mice (10/group); Potassium dichromate (3 dose levels); Drinking water; Exposure during gestation (GD 0-19)	↑implantation loss; ↓litter size; ↓foetal wt and length; ↓cranial ossification at doses without maternal toxicity	< 57 (fetotoxicity)	57 (fetotoxicity)	Reliable with reporting limitations	Trivedi, 1989
Effects on ovaries	B6C3F1 female mice and F344/N female rats; Sodium dichromate; Drinking water; Exposure for 2 years up to 8.6 mg Cr(VI)/kg bw/d in mice and up to 7.0 mg Cr(VI)/kg bw/d in rats	No effects reported	8.6 (highest dose tested) in mice; 7.0 (highest dose tested) in rats;	> 8.6 in mice; > 7.0 in rats	Reliable	NTP, 2008

Developmental toxicity	Female mice Sodium dichromate (up to 4.8 mg Cr(VI)/kg bw/d; Potassium dichromate (up to 2.4 mg Cr(VI)/kg bw/d; Drinking water Exposure GD 0-18	No effects on parameters investigated	No reliable NOAEL can be set due to limited investigations	No reliable LOAEL can be set due to limited investigations	Limited study (foetal wt and no of foetuses/litter investigated only)	De Flora et al., 2006
Mechanistic study during lactational exposure	Female rat pups; Potassium dichromate; Drinking water; Exposure PND 1-21	↓antioxidant enzyme activities and ↑ lipid peroxidation in uterine tissue from 2.9 mg Cr(VI)/kg bw/d	Mechanistic study – not suitable to set NOAEL	Mechanistic study – not suitable to set LOAEL	Sufficiently reliable	Samuel et al., 2011

11. All the available studies evaluating the potential reproductive effects of hexavalent chromium compounds have used water-soluble compounds and the oral exposure route. Standard reproductive toxicity studies have been conducted in several species - monkeys, rats, mice and rabbits. In addition, several studies have specifically evaluated the potential effects of pre-gestational, gestational, or lactational exposure on foetal development in rats and mice. Unfortunately, numerous studies are relatively old, not very well reported and not in line with current standards.

12. In these studies, animals were exposed through the diet, drinking water or by gavage. In general, studies that evaluated developmental effects of hexavalent chromium were conducted at higher exposure levels than those that evaluated fertility effects.

13. Collectively, the available studies provide evidence that oral exposure of laboratory animals to hexavalent chromium compounds can produce adverse reproductive effects, including: histopathological changes to reproductive organs in males and females; alterations in sperm, including decreased count, decreased motility, and abnormal morphology; decreased plasma testosterone levels; increased oestrous cycle length; changes in mating behaviour and decreased fertility in males; and adverse reproductive outcomes, including decreased numbers of live foetuses and implantations, and increased numbers of resorptions and pre- and post-implantation losses.

14. Developmental effects observed have included decreased foetal weight and length; external and skeletal abnormalities and delayed sexual maturation and function in female offspring.

15. In contrast to these results, adverse effects were not observed in dietary and drinking water exposure studies conducted by the NTP that investigated the potential for hexavalent chromium to produce adverse effects on male and female reproductive organs in rats and mice and on reproductive outcomes in a continuous breeding study in mice.

16. It is unclear why the findings of the NTP studies were so different, with no adverse effects on reproduction being seen at similar dose levels to those producing effects in other studies. Accepting this oddity, nevertheless, there is an overwhelming majority of studies which repeatedly show adverse effects on fertility and development. These studies are consistent with the current harmonised classification of the 4 soluble Cr(VI) substances as described above in Table 1.1.

17. In pursuit of the objectives of this project, it would be expected that a key step would be the identification of an appropriate NOAEL from a reliable study. Unfortunately, from the overall data available, suitable

NOAEL values are not apparent; therefore an approach based on LOAEL values has been used.

18. For fertility effects, including effects on reproductive organs, the most sensitive and sufficiently reliable toxicological starting point is the oral **LOAEL of 5.2 mg Cr(VI)/kg bw/d** for effects on the testes in rats treated for 6 days (Li et al., 2001).

19. For developmental effects, the most sensitive and sufficiently reliable toxicological starting point is the oral **LOAEL of 7.9 mg Cr(VI)/kg bw/d** for a number of foetal effects (post-implantation loss, resorptions, dead foetuses, decreased foetal weigh, skeletal and visceral anomalies in the absence of significant maternal toxicity) in rats during gestation (Elsaieed and Nada, 2002).

20. From an analysis of all the NOAELs/LOAELs obtained from the sufficiently reliable studies in Table 1.3, other NOAEL or LOAEL values would not result in lower DNELs than those calculated from the values proposed above.

FURTHER DETAILED DESCRIPTION OF KEY STUDIES AND JUSTIFICATION FOR THEIR SELECTION

Fertility

Li et al., 2001

21. Groups of 8–11 male Wistar rats (60 days old) were administered Cr(VI) oxide by gavage at doses of 0, 10, or 20 mg Cr(VI) oxide/kg bw/d (equivalent to 0, 5.2, or 10.4 mg Cr(VI)/kg bw/d, respectively) for 6 days (Li et al., 2001).. After 6 weeks, rats were sacrificed; testes and epididymis were removed and analyzed for epididymal sperm count and abnormal sperm; and testes were prepared (fixed in formaldehyde, embedded in paraffin, sliced, and stained with H&E) for histological evaluations of morphological abnormalities and diameter of seminiferous tubules.

    Epididymal sperm counts were significantly ($p < 0.05$) decreased by 76 and 80%, and the percentage of abnormal sperm was significantly ($p < 0.01$) increased by 143 and 176% in the 5.2 and 10.4 mg Cr(VI)/kg bw/d groups, respectively. Treatment-related histopathological findings included decreased diameter of seminiferous tubules and disruption of germ cell arrangement within seminiferous tubules in both treatment groups. Based on decreased sperm counts and histopathological changes to the testes, 5.2 mg Cr(VI)/kg bw/d was identified as a LOAEL for male rats exposed to gavage doses of Cr(VI) oxide for 6 days; a NOAEL was not identified because effects were seen at the lowest dose administered.

Development

Elsaieed and Nada, 2002

22. Effects of gestational exposure to Cr(VI) were investigated in Wistar rats (Elsaieed and Nada, 2002). Groups of 10 pregnant rats (mean initial body weight of 170 g) were administered drinking water containing 0 or 50 mg Cr(VI)/L as potassium dichromate on GDs 6 through 15. During the exposure period, dams were evaluated for clinical signs of toxicity, body weights, and food and drinking water consumption. One day before delivery, rats were sacrificed and the following were evaluated: numbers of corpora lutea, pre- and post-implantation losses, resorptions, and live and dead fetuses; fetal weight; and visceral and skeletal anomalies.

    No mortalities or clinical signs of toxicity were observed. Elsaieed and Nada (2002) stated that food and drinking water consumption was comparable between control and treatment groups, although data were not reported. Gestational weight gain was significantly ($p < 0.05$) decreased by 40% in treated dams, compared with controls. Based on an average gestational body weight of 177 g (average calculated using body weights at mating and at the end of gestation) and the allometric equation for drinking water consumption for laboratory mammals ($0.10 \times \text{body weight}^{0.7377}$; U.S. EPA, 1988), a daily dose of 7.9 mg Cr(VI)/kg bw/d was estimated..

    In this study, treatment of rats with Cr(VI) resulted in significant ($p < 0.05$) increases in post-implantation loss/litter (1.5 vs. 0), resorptions/litter (1.2 vs. 0), and dead foetuses/litter (1.2 vs. 0) and decreases in live foetuses/litter (1.5 vs. 6.8 in control) and foetal weight (33% decrease). In the exposed group, increased litters with foetal abnormalities were observed including visceral (renal pelvis dilation: 2.1/litter) and skeletal (incomplete skull ossification: 1.0/litter) changes; no control foetuses showed these changes.

    The results of this study showed that exposure of pregnant Sprague-Dawley rats to drinking water containing 50 mg Cr(VI)/L as potassium dichromate (approximately 7.9 mg Cr(VI)/kg bw/d) on GDs 6–15 produced adverse effects on reproductive outcome and foetal development. Thus, a LOAEL of 7.9 mg Cr(VI)/kg bw/d was identified from this study.

Justification for their selection

23. The database of the potential reproductive effects of soluble Cr(VI) compounds is very large. However, numerous studies are relatively old, not very well reported and not in line with current standards. Some have several limitations and shortcomings.

24. Collectively, these studies provide evidence that oral exposure of laboratory animals to soluble Cr(VI) compounds can produce adverse reproductive effects on fertility and development.

25. In contrast to these results, adverse effects were not observed in dietary and drinking water exposure studies conducted by the NTP that investigated the potential for soluble Cr(VI) compounds to produce adverse effects on male and female reproductive organs in rats and mice and on reproductive outcomes in a continuous breeding study in mice.

26. It is unclear why the findings of the NTP studies are so different, with no adverse effects on reproduction being seen at similar (or even higher) dose levels to those producing effects in other studies. Accepting this oddity, nevertheless, there is an overwhelming majority of studies which repeatedly show adverse effects of soluble Cr(VI) on fertility and development.

27. For fertility effects, including effects on reproductive organs, after considering all the most adequate available studies, the Li et al (2001) study was selected as it provides the most sensitive and sufficiently reliable toxicological starting point (oral **LOAEL of 5.2 mg Cr(VI)/kg bw/d**) for effects on the testes in rats treated for 6 days.

28. The only lower NOAEL and LOAEL values (around 1-2 mg Cr(VI)/kg bw/d) compared to the selected LOAEL for effects on male reproductive organs were identified in the monkey studies. However, due to the very low sample size employed, no reliable conclusions could be drawn from these studies.

29. The NOAEL and LOAEL values from the monkey studies were not so different from the selected LOAEL. In addition, when taking into account that there are several robust NTP studies where no effects on reproduction were seen at much higher doses, the proposed starting point seems appropriate.

30. For developmental effects, after considering all the most adequate available studies, the Elsaieed and Nada (2002) study was selected as it provides the most sensitive and sufficiently reliable toxicological starting point (oral **LOAEL of 7.9 mg Cr(VI)/kg bw/d**) for a number of foetal effects (in the absence of significant maternal toxicity) in rats during gestation.

31. There are a number of uncertainties on how the value of 7.9 mg Cr(VI)/kg bw/d was arrived at. However, when considering that NOAEL and LOAEL values from other developmental toxicity studies are higher, the proposed starting point is the most conservative.

DERIVATION OF DNELs

32. For the derivation of DNELs for the oral, inhalation and dermal routes and the application of route-to-route extrapolation, the following route-specific absorption values for inhalation and dermal specified in the risk characterisation section of the EU RAR and taking into account the available ECHA guidance (lowest absorption value for the starting route and highest absorption value for the end route) will be used. For the oral route, the kinetic section of the EU RAR mentions a range of 2-9%; the risk characterisation section of the EU RAR uses 5% and a value of 10% is mentioned in the original report of the carcinogenicity of chromate (ECHA/2011/01-SR-11). Taking a WoE (weight of evidence) approach, an oral absorption value of 5% would seem to be appropriate.

    Oral: 5%
    Inhalation: 30%
    Dermal : 4%

    The EU RAR also states "*the kinetic behaviour of these substances appear to be similar in those species studied, including humans.*" So the absorption values established above for route-to-route extrapolations are also appropriate for human exposure calculations.

33. DNELs will be derived for both fertility and development for workers and the general public in accordance with the ECHA guidance on chemical safety assessment, chapter R8 (ECHA, 2010).

Workers

34. For workers, only inhalation and dermal DNELs will be derived.

Inhalation

Modification of the starting point

35. For fertility, the starting point is an oral LOAEL of 5.2 mg Cr(VI)/kg bw/d in rats treated for 6 days. For development, the starting point is an oral LOAEL of 7.9 mg Cr(VI)/kg bw/d in rats exposed during gestation.

36. The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 5% oral absorption and 30% inhalation absorption, the equivalent inhalation LAELs expressed on a body weight basis would be:

    Fertility: 5.2 x 5%/30% = *0.9 mg Cr(VI)/kg bw/d*

    Development: 7.9 x 5%/30% =1.3*2 mg Cr(VI)/kg bw/d*

37. Taking into account a rat ventilation rate (at rest) for 8 h of 0.38 m^3/kg bw, the following 8h-inhalation rat LAEC values would be calculated:

    Fertility: 0.9/0.38 = *2.4 mg Cr(VI)/m^3/d*

    Development: 1.3/0.38 = *3.4 mg Cr(VI)/m^3/d*

38. In accordance with the ECHA (2010) guidance, an adjustment for the higher ventilation rate (x 0.67) of a worker under light activity (compared to the experimental rat at rest), the corrected inhalation 8h-LAECs can be calculated:

    Fertility: 2.4 x 0.67 = *1.6 mg Cr(VI)/m^3/d (corrected inhalation 8h-LAEC)*

    Development: 3.4 x 0.67 = *2.3 mg Cr(VI)/m^3/d (corrected inhalation 8h-LAEC)*

39. In addition, only for the developmental LOAEL, as the experimental animals were exposed for 7 days/week whilst the workers are assumed to be exposed for 5 days/week, a further adjustment is required as follows:

    Development: 2.3 x 7/5 = *3.2 mg Cr(VI)/m^3/d (corrected inhalation 8h-LAEC for 5 days/wk)*

Application of default assessment factors (AF)

40. To produce a conservative assessment, in a first tier, default AFs will be used. An AF of 3 (from the default 3-10 range) will be applied to extrapolate the LOAEC values to NOAECs. A factor of 3 seems appropriate because there are several studies which provide higher NOAEL and LOAEL values and studies where no effects were reported.

41. In relation to the derivation of the fertility DNEL, the rats were treated for only 6 days. Therefore an AF to extrapolate to a long-term DNEL would seem appropriate. However, given that in a much longer exposure study (the sufficiently reliable 90 day study in rats by Chowdhury and Mitra,1995), a clear NOAEL of 7 mg Cr(VI) /kg bw/d was identified and a much higher LOAEL of 14 mg Cr(VI) /kg bw/d for 90 days was established, the contractor is of the opinion that, taking a WoE approach, an additional factor for duration extrapolation is not necessary. It should also be noted that there are several negative studies, including the reliable NTP studies where no effects were identified up to doses much higher than those at which effects have been reported in other studies.

42. For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

43. For intraspecies differences, the default factor of 5 for workers will be applied. The resulting inhalation (8-hr) DNELs for workers are shown below.

DNEL(worker, inhalation, fertility): $\frac{1.6 \text{ mg Cr(VI)/m}^3\text{/d}}{3 \times 2.5 \times 5}$ = **43 µg Cr(VI)/m³/d**

DNEL(worker, inhalation, development): $\frac{3.2 \text{ mg Cr(VI)/m}^3\text{/d}}{3 \times 2.5 \times 5}$ = **85 µg Cr(VI)/m³/d**

Dermal

Modification of the starting point

44. For fertility, the starting point is an oral LOAEL of 5.2 mg Cr(VI)/kg bw/d in rats treated for 6 days. For development, the starting point is an oral LOAEL of 7.9 mg Cr(VI)/kg bw/d in rats exposed during gestation.

45. The first modification step for the derivation of dermal DNELs is route-to-route extrapolation from oral to dermal by taking into account 5% oral absorption and 4% dermal absorption. This would results in the following equivalent dermal LAELs:

    Fertility: 5.2 x 5%/4% = *6.5 mg Cr(VI)/kg bw/d*

    Development: 7.9 x 5%/4% = *10 mg Cr(VI)/kg bw/d*

46. In addition, only for the developmental LOAEL, as the experimental animals were exposed for 7 days/week whilst the workers are assumed to be exposed for 5 days/week, a further adjustment is required as follows:

    Development: 10 x 7/5 = *14 mg Cr(VI)/kg bw/d*

Application of default assessment factors (AF)

47. To produce a conservative assessment, in a first tier, default AFs will be used. An AF of 3 (from the default 3-10 range) will be applied to extrapolate the LOAEL values to NOAELs. A factor of 3 seems appropriate because there are several studies which provide higher

NOAEL and LOAEL values and studies where no effects were reported.

48. In relation to the derivation of the fertility DNEL, the rats were treated for only 6 days. Therefore an AF to extrapolate to a long-term DNEL would seem appropriate. However, given that in a much longer exposure study (the sufficiently reliable 90 day study in rats by Chowdhury and Mitra,1995), a clear NOAEL of 7 mg Cr(VI) /kg bw/d was identified and a much higher LOAEL of 14 mg Cr(VI) /kg bw/d for 90 days was established, the contractor is of the opinion that, taking a WoE approach, an additional factor for duration extrapolation is not necessary. It should also be noted that there are several negative studies, including the reliable NTP studies where no effects were identified up to doses much higher than those at which effects have been reported in other studies.

49. For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

50. For intraspecies differences, the default factor of 5 for workers will be applied. The resulting dermal DNELs for workers are shown below.

DNEL(worker, dermal, fertility): $\frac{6.5 \text{ mg Cr(VI)/kg bw/d}}{3 \times 4 \times 2.5 \times 5}$ = **43 µg Cr(VI)/kg bw/d**

DNEL(worker, derm, development): $\frac{14 \text{ mg Cr(VI)/kg bw/d}}{3 \times 4 \times 2.5 \times 5}$ = **93 µg Cr(VI)/kg bw/d**

51. The Table below summarises the inhalation and dermal DNELs for workers in relation to Cr(VI)-induced reproductive toxicity. It is proposed that these are applicable to the 4 chromates classified in category 1B reproductive toxicity included in Annex XIV of REACH.

Table 1.4: Inhalation and dermal DNELs for workers for fertility and development effects for the 4 soluble Cr(VI) substances classified for category 1B reproductive toxicity on the Annex XIV list

Endpoint	Inhalation (8-hr)	Dermal
Fertility	**43 µg Cr(VI)/m^3/d**	**43 µg Cr(VI)/kg bw/d**
Development	**85 µg Cr(VI)/m^3/d**	**93 µg Cr(VI)/kg bw/d**

General public

52. For the general public, inhalation, dermal and oral DNELs will be derived.

Inhalation

Modification of the starting point

53. For fertility, the starting point is an oral LOAEL of 5.2 mg Cr(VI)/kg bw/d in rats treated for 6 days. For development, the starting point is an oral LOAEL of 7.9 mg Cr(VI)/kg bw/d in rats exposed during gestation.

54. The first modification step is route-to-route extrapolation from oral to inhalation. By taking into account 5% oral absorption and 30% inhalation absorption, the equivalent inhalation LAELs expressed on a body weight basis would be:

    Fertility: 5.2 x 5%/30% = *0.9 mg Cr(VI)/kg bw/d*

    Development: 7.9 x 5%/30% = *1.3 mg Cr(VI)/kg bw/d*

55. Taking into account a rat ventilation rate for 24 h of 1.15 m^3/kg bw, the following 24h-inhalation rat LAEC values would be calculated:

    Fertility: 0.9/1.15 = *0.8 mg Cr(VI)/m^3/d (corrected inhalation 24-hr LAEC)*

    Development: 1.3/1.15 = *1.1 mg Cr(VI)/m^3/d (corrected inhalation 24-hr LAEC)*

Application of default assessment factors (AF)

56. To produce a conservative assessment, in a first tier, default AFs will be used. An AF of 3 (from the default 3-10 range) will be applied to extrapolate the LOAEC values to NOAECs. A factor of 3 seems appropriate because there are several studies which provide higher NOAEL and LOAEL values and studies where no effects were reported.

57. In relation to the derivation of the fertility DNEL, the rats were treated for only 6 days. Therefore an AF to extrapolate to a long-term DNEL would seem appropriate. However, given that in a much longer exposure study (the sufficiently reliable 90 day study in rats by Chowdhury and Mitra,1995), a clear NOAEL of 7 mg Cr(VI) /kg bw/d was identified and a much higher LOAEL of 14 mg Cr(VI) /kg bw/d for 90 days was established, the contractor is of the opinion that, taking a WoE approach, an additional factor for duration extrapolation is not necessary. It should also be noted that there are several negative studies, including the reliable NTP studies where no effects were identified up to doses much higher than those at which effects have been reported in other studies.

58. For interspecies differences, the allometric scaling factor for rat-human of 4 is not necessary as it is implicitly taken into account in the rat ventilation rate. Therefore, only the factor of 2.5 for remaining uncertainties will be applied.

59. For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting inhalation (24-hr) DNELs for the general public are shown below.

DNEL(public, inhalation, fertility): $\frac{0.8 \text{ mg Cr(VI)/m}^3\text{/d}}{3 \times 2.5 \times 10}$ = **11 µg Cr(VI)/m³/d**

DNEL(public, inhalation, development): $\frac{1.1 \text{ mg Cr(VI)/m}^3\text{/d}}{3 \times 2.5 \times 10}$ = **15 µg Cr(VI)/m³/d**

Dermal

Modification of the starting point

60. For fertility, the starting point is an oral LOAEL of 5.2 mg Cr(VI)/kg bw/d in rats treated for 6 days. For development, the starting point is an oral LOAEL of 7.9 mg Cr(VI)/kg bw/d in rats exposed during gestation.

61. The first and only modification step for the derivation of dermal DNELs is route-to-route extrapolation from oral to dermal by taking into account 5% oral absorption and 4% dermal absorption. This would results in the following equivalent dermal LAELs:

    Fertility: 5.2 x 5%/4% = *6.5 mg Cr(VI)/kg bw/d*

    Development: 7.9 x 5%/4% = *10 mg Cr(VI)/kg bw/d*

Application of default assessment factors (AF)

62. To produce a conservative assessment, in a first tier, default AFs will be used. An AF of 3 (from the default 3-10 range) will be applied to extrapolate the LOAEL values to NOAELs. A factor of 3 seems appropriate because there are several studies which provide higher NOAEL and LOAEL values and studies where no effects were reported.

63. In relation to the derivation of the fertility DNEL, the rats were treated for only 6 days. Therefore an AF to extrapolate to a long-term DNEL would seem appropriate. However, given that in a much longer exposure study (the sufficiently reliable 90 day study in rats by Chowdhury and Mitra,1995), a clear NOAEL of 7 mg Cr(VI) /kg bw/d was identified and a much higher LOAEL of 14 mg Cr(VI) /kg bw/d for

90 days was established, the contractor is of the opinion that, taking a WoE approach, an additional factor for duration extrapolation is not necessary. It should also be noted that there are several negative studies, including the reliable NTP studies where no effects were identified up to doses much higher than those at which effects have been reported in other studies.

64. For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

65. For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting dermal DNELs for the general public are shown below.

DNEL(public, dermal, fertility): $\frac{\text{6.5 mg Cr(VI)/kg bw/d}}{\text{3 x 4 x 2.5 x 10}}$ = **22 µg Cr(VI)/kg bw/d**

DNEL(public, dermal, development): $\frac{\text{10 mg Cr(VI)/kg bw/d}}{\text{3 x 4 x 2.5 x 10}}$ = **34 µg Cr(VI)/kg bw/d**

Oral

Modification of the starting point

66. For fertility, the starting point is an oral LOAEL of 5.2 mg Cr(VI)/kg bw/d in rats treated for 6 days. For development, the starting point is an oral LOAEL of 7.9 mg Cr(VI)/kg bw/d in rats exposed during gestation.

67. Modification of the starting point to derive oral DNELs is not necessary as the starting points are oral doses.

Application of default assessment factors (AF)

68. To produce a conservative assessment, in a first tier, default AFs will be used. An AF of 3 (from the default 3-10 range) will be applied to extrapolate the LOAEL values to NOAELs. A factor of 3 seems appropriate because there are several studies which provide higher NOAEL and LOAEL values and studies where no effects were reported.

69. In relation to the derivation of the fertility DNEL, the rats were treated for only 6 days. Therefore an AF to extrapolate to a long-term DNEL would seem appropriate. However, given that in a much longer exposure study (the sufficiently reliable 90 day study in rats by Chowdhury and Mitra,1995), a clear NOAEL of 7 mg Cr(VI) /kg bw/d was identified and a much higher LOAEL of 14 mg Cr(VI) /kg bw/d for 90 days was established, the contractor is of the opinion that, taking a WoE approach, an additional factor for duration extrapolation is not

necessary. It should also be noted that there are several negative studies, including the reliable NTP studies where no effects were identified up to doses much higher than those at which effects have been reported in other studies.

70. For interspecies differences, the allometric scaling factor for rat-human of 4 and the factor of 2.5 for remaining uncertainties will be applied.

71. For intraspecies differences, the default factor of 10 for the general public will be applied. The resulting oral DNELs for the general public are shown below.

DNEL$_{(public, oral, fertility)}$: $\frac{5.2 \text{ mg Cr(VI)/kg bw/d}}{3 \times 4 \times 2.5 \times 10}$ = **17 µg Cr(VI)/kg bw/d**

DNEL$_{(public, oral, development)}$: $\frac{7.9 \text{ mg Cr(VI)/kg bw/d}}{3 \times 4 \times 2.5 \times 10}$ = **26 µg Cr(VI)/kg bw/d**

72. The Table below summarises the inhalation, dermal and oral DNELs for the general public in relation to Cr(VI)-induced reproductive toxicity. It is proposed that these are applicable to the 4 chromates classified in category 1B reproductive toxicity included in Annex XIV of REACH.

Table 1.5: Inhalation, dermal and oral DNELs for the general public for fertility and development effects for the 4 soluble Cr(VI) substances classified for category 1B reproductive toxicity on the Annex XIV list

Endpoint	Inhalation (24-hr)	Dermal	Oral
Fertility	**11 µg Cr(VI)/m^3/d**	**22 µg Cr(VI)/kg bw/d**	**17 µg Cr(VI)/kg bw/d**
Development	**15 µg Cr(VI)/m^3/d**	**34 µg Cr(VI)/kg bw/d**	**26 µg Cr(VI)/kg bw/d**

SUMMARY

73. Among the 14 chromate substances listed in Annex XIV of REACH due to their carcinogenic properties, there are four substances (ammonium dichromate, sodium dichromate, sodium chromate and potassium dichromate) which are also classified for reproductive toxicity in category 1B, such effects also arising as a consequence of the Cr(VI) ion.

74. ECHA RAC noted that it was important to establish dose-response relationships for this endpoint. RAC's opinion was that, although it is

most likely that carcinogenic risk would drive the risk-benefits analyses for authorisation purposes, it would be important to know if there are exposure levels during use at which reprotoxicity considerations could make a significant contribution to the assessment of the total ill-health risk arising from Cr(VI) exposure. RAC also noted that for (local) carcinogenicity, no dermal DNEL had been set, and therefore it would be important to address the dermal risks arising from reproductive toxicity. Furthermore, RAC considered that the developmental neurotoxicity of lead in the three lead chromate substances should be addressed. It was suggested that the dose-response relationship established by EFSA (EFSA, 2013) for lead developmental neurotoxicity should be used for this purpose.

75. Given the agreed approach for the lead-containing chromates, this sub-contract was established to retrieve and assess the relevant reproductive toxicity (fertility and development) data on the Cr(VI) ion. The requirement was to identify the dose-response relationships for these endpoints and to propose appropriate DNELs (Derived No Effect Levels).

76. The available studies show that oral exposure of laboratory animals to hexavalent chromium compounds can produce adverse effects on male and female reproductive organs, reproductive performance, fertility and development of the offspring.

77. From these studies, the contractor established that the most sensitive and sufficiently reliable toxicological starting point for fertility is the oral **LOAEL of 5.2 mg Cr(VI)/kg bw/d** for effects on the testes in rats treated for 6 days (Li et al., 2001) and that the most sensitive and sufficiently reliable toxicological starting point for developmental effects is the oral **LOAEL of 7.9 mg Cr(VI)/kg bw/d** for a number of foetal effects in rats exposed during gestation (Elsaieed and Nada, 2002).

78. Using these starting points, DNELs for workers and the general public for relevant routes of exposure were derived.

79. Table 1.6 below summarises the inhalation and dermal DNELs for workers and Table 1.7 summarises the inhalation, dermal and oral DNELs for the general public in relation to Cr(VI)-induced reproductive toxicity. These are applicable to the 4 chromates classified in category 1B reproductive toxicity included in Annex XIV of REACH For the sparingly/poorly soluble compounds 11-14 in Table 1.1, it is quite possible that their poor solubility creates insufficient bioavailable, systemic Cr(VI) to produce adverse effects on reproductive function; i.e. the hazard does not arise.

80. It is noted that the fertility DNELs are lower than the developmental DNELs. It is also noted that when comparing these reproductive toxicity DNELs with the carcinogenic dose-response of Cr(VI), it is clearly

apparent that the carcinogenic effects of Cr(VI) are much more potent than its reprotoxic effects.

Table 1.6: Inhalation and dermal DNELs for <u>workers</u> for fertility and development effects for the 4 soluble Cr(VI) substances classified for category 1B reproductive toxicity on the Annex XIV list

Endpoint	Inhalation (8-hr)	Dermal
Fertility	**43 µg Cr(VI)/m^3/d**	**43 µg Cr(VI)/kg bw/d**
Development	**85 µg Cr(VI)/m^3/d**	**93 µg Cr(VI)/kg bw/d**

Table 1.7: Inhalation, dermal and oral DNELs for the <u>general public</u> for fertility and development effects for the 4 soluble Cr(VI) substances classified for category 1B reproductive toxicity on the Annex XIV list

Endpoint	Inhalation (24-hr)	Dermal	Oral
Fertility	**11 µg Cr(VI)/m^3/d**	**22 µg Cr(VI)/kg bw/d**	**17 µg Cr(VI)/kg bw/d**
Development	**15 µg Cr(VI)/m^3/d**	**34 µg Cr(VI)/kg bw/d**	**26 µg Cr(VI)/kg bw/d**

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