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RAC/35/2015/09

35TH MEETING OF THE COMMITTEE FOR RISK ASSESSMENT

24-27 NOVEMBER _ 1-4 DECEMBER 2015

HELSINKI, FINLAND

Concerns:	**Amendment of the RAC note "Application for Authorisation: Establishing a reference dose-response relationship for carcinogenicity of hexavalent chromium" to include the intrinsic property "Toxic to reproduction" of the Cr(VI) compounds** (RAC/27/2013/06 Rev.1) agreed on 4 December 2013 at RAC-27).
Agenda Point:	**9.1.c**
Action requested:	**For discussion and agreement**

This document introduces an Amendment (in the form of Appendix 1 below) to the RAC note "Application for Authorisation: Establishing a reference dose-response relationship for carcinogenicity of hexavalent chromium" **to include the intrinsic property "Toxic to reproduction"** of the Cr(VI) compounds" (RAC/27/2013/06 Rev.1) agreed on 4 December 2013 at RAC-27.

APPENDIX 1

APPLICATION FOR AUTHORISATION: ADRESSING THE INTRINSIC PROPERTY "TOXIC FOR REPRODUCTION" OF THE Cr(VI) COMPOUNDS LISTED IN ANNEX XIV EXCEPT FOR LEAD CHROMATE

Background

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

RAC noted that it is important to establish risk estimates for this endpoint. While RAC considers that the carcinogenic effects of Cr(VI) are much more potent than its reprotoxic effects, it is nevertheless important to know if there are exposure levels during use at which considerations of reproductive toxicity could make a significant contribution to the assessment of the overall Cr(VI) health risk. 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.

Lead chromates
Where Cr(VI) occurs in combination with lead, i.e. in a small number of lead-chromate substances listed on Annex XIV of REACH. RAC considered that the developmental neurotoxicity of lead should be addressed. In such cases, it is recommended that the dose-response relationship established by EFSA (EFSA, 2013) for the developmental neurotoxicity of lead should be used for this purpose.

RAC in this note has therefore assessed only the relevant reproductive toxicity (fertility and development) data on the Cr(VI) ion.

Reproductive and developmental toxicity

Similarly to the original methodology on carcinogenicity of Cr(VI) (ECHA/2011/01-SR-11), 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 identified. These were the EU RAR (2005), the Draft USEPA (2010) and the ATSDR (2012).

From these three reviews, the relevant reproductive toxicity studies were extracted and tabulated in Table 1.3 of the main report (HSE (2015). ECHA 2015/248: Assess the risk estimate for the intrinsic property "Toxic for reproduction" of the Cr(VI) compounds listed in

Annex XIV except for lead chromate. Final report). Given the harmonised classification of these four chromates, the hazard identification aspects of each study were not re-appraised critically - the main findings, including information on dose-response, NOAEL and LOAEL values were simply reproduced.

All the available experimental studies evaluating the potential reproductive effects of hexavalent chromium used the water-soluble compounds potassium dichromate, sodium dichromate or chromium trioxide (chromic acid) 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.

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. Unfortunately, a substantial proportion of the available studies were relatively old, not very well reported and not in line with current standards.

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.

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

In contrast to all of these "positive" results, no adverse effects on reproduction were 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.

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. Acknowledging this oddity, nevertheless there is an overwhelming majority of studies which have shown adverse effects on fertility and development. These studies are consistent with the current harmonised classification of the 4 soluble Cr(VI) substances as described below in Table 1 of this note.

A key step in the analysis would normally be the identification of an appropriate NOAEL from a reliable study. Unfortunately, from the overall data available, such a reliable NOAEL value is not apparent; therefore an approach based on reliable LOAEL values has been used.

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).

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).

From an analysis of all the NOAELs/LOAELs obtained from the better quality studies, no other NOAEL or LOAEL values chosen as the point of departure would result in lower DNELs than the DNELs derived from the two LOAEL values specified above.

Critical studies

Fertility

Li et al., 2001

Groups of 8–11 male Wistar rats (60 days old) were administered Cr(VI) trioxide 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) trioxide for 6 days; a NOAEL was not identified, as effects were seen at the lowest dose administered.

Development

Elsaieed and Nada, 2002

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 days 6 to 15 of gestation. 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 (40%) less in treated dams, compared with controls ($p < 0.05$). 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 anomalies 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 Wistar rats to drinking water containing 50 mg Cr(VI)/L as potassium dichromate (approximately 7.9 mg Cr(VI)/kg bw/d) on days 6–15 of gestation 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 selection of critical studies

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.

The only lower NOAEL and LOAEL values (around 1-2 mg Cr(VI)/kg bw/d) 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. Hence the chosen starting point is the most conservative of the more reliable values available.

For developmental effects, after considering all the most adequate of the 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.

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

Bioavailability for derivation of reference DNELs

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) have been used. No other relevant absorption values for the inhalation and dermal routes have been identified in the other reviews or publications.

For the oral route, the kinetic section of the EU RAR mentions a range of 2-9% of the dose being absorbed; 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%

Derivation of reference DNELs

Reference DNELs have been 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).

For workers, only inhalation and dermal DNELs have been established. For the general public, inhalation, dermal and oral DNELs have been set. These are summarised in Table 1 for fertility effects and in Table 2 for developmental effects of this Appendix.

It can be seen that the fertility DNELs are all lower than the corresponding DNELs for developmental toxicity but are nevertheless in the same range. 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. Overview of derivation of reference DNELs for workers and general population for **fertility effects** for the four soluble Cr(VI) compounds in Annex XIV classified for toxicity to reproduction in category 1B by the inhalation, oral and dermal route (please refer to the chapter "Derivation of DNELs" of HSE, 2015 for further details)

Point of departure for DNEL derivation by all routes for Cr(VI) compounds in relation to fertility effects (Li et al., 2001)		
Rat 6-day oral study (testicular toxicity) LOAEL Oral absorption percentage	**5.2 mg Cr(VI)/kg bw/d** 5 %	
Derivation of Reference DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors[$]		
Interspecies, Allometric scaling	1 or 4*	1 or 4*
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	1[£]	1[£]
LOAEL to NAEL extrapolation	3	3
Hours/day	8	24
INHALATION		
Absorption percentage	30%	30%
Standard respiratory volume in m^3/kg bw/day	0.384	1.15
Breathing rate for workers light activity vs rest	6.7/10	-
LAEC (corrected) in mg Cr(VI)/m^3	1.6	0.8
Reference DNEL INHALATION in µg Cr(VI)/m³	**43**	**11**
DERMAL		
Absorption percentage	4%	4%
LAEL (corrected) in mg Cr(VI)/kg bw/d	6.5	6.5
Reference DNEL DERMAL in µg Cr(VI)/kg bw/d	**43**	**22**
ORAL		
LOAEL (mg Cr(VI)/kg bw/d)	5.2	5.2
Reference DNEL ORAL in µg Cr(VI)/kg bw/dy	**-**	**17**

*An allometric scaling factor of 4 (for the rat) is used for the oral and dermal DNELs, but not for the derivation of the inhalation DNELs;

[$] Justification for selection of assessment factors is given in the main report;

[£] In the Li et al (2001) study, 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 RAC 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.

Table 2. Overview of derivation of reference DNELs for workers and general population for **developmental toxicity** for the four soluble Cr(VI) compounds in Annex XIV classified for toxicity to reproduction in category 1B by the inhalation, oral and dermal route (please refer to the chapter "Derivation of DNELs" of HSE, 2015 for further details)

Point of departure for DNEL derivation by all routes for Cr(VI) compounds in relation to developmental toxicity (Elsaieed and Nada, 2002)		
Rat oral developmental toxicity study (GD 6-15) (several foetal effects in the absence of significant maternal toxicity)		
LOAEL	**7.9 mg Cr(VI)/kg bw/d**	
Oral absorption percentage	5 %	
Derivation of Reference DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors[$]		
Interspecies, Allometric scaling	1 or 4*	1 or 4*
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Subacute to chronic	1	1
LOAEL to NAEL extrapolation	3	3
Hours/day	8	24
INHALATION		
Absorption percentage	30%	30%
Standard respiratory volume in m^3/kg bw/day	0.384	1.15
Breathing rate for workers light activity vs rest	6.7/10	-
Correction for duration of treatment (7d/wk in animals vs 5d/wk in workers)	x7/5	x7/7
LAEC (corrected) in mg Cr(VI)/m^3	3.2	1.1
Reference DNEL INHALATION in µg Cr(VI)/m^3	**85**	**15**
DERMAL		
Absorption percentage	4%	4%
Correction for duration of treatment (7d/wk in animals vs 5d/wk in workers)	x7/5	x7/7
LAEL (corrected) in mg Cr(VI)/kg bw/d	14	10
Reference DNEL DERMAL in µg Cr(VI)/kg bw/d	**93**	**34**
ORAL		
LOAEL (mg Cr(VI)/kg bw/d)	7.9	7.9
Reference DNEL ORAL in µg Cr(VI)/kg bw/dy		**26**

*An allometric scaling factor of 4 (for the rat) is used for the oral and dermal DNELs, but not for the derivation of the inhalation DNELs;

[$] Justification for selection of assessment factors is given in the main report;

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