MUT/2015/04

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)

CHROMIUM VI – IS THERE A THRESHOLD FOR MUTAGENICITY AND CARCINOGENICITY?

1) The Environment Agency has asked for an opinion on a paper by Thompson et al which asserts that there is a threshold for mutagenic and carcinogenic effect for Chromium VI following oral exposure. We wish to know whether we should still regard Chromium VI as potentially mutagenic and carcinogenic for the oral route of exposure with no assumed threshold (our current UK view), or whether there is sufficient evidence for a threshold mode of genotoxic action?

2) Members are asked to evaluate the attached overview document and key papers, and to provide comments. A number of questions are presented for consideration.

Secretariat/PHE Toxicology Unit

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MUT/2015/04

Chromium VI – is there a threshold for mutagenicity and carcinogenicity?

Context:

1. The Environment Agency has asked for an opinion on papers by Thompson et al. (2013 a,b – ANNEX 1) 'A chronic reference dose for hexavalent chromium-induced intestinal cancer'. This contends that the mode of action for intestinal neoplasms following oral exposure to Cr(VI) involves cytotoxicity and not mutagenicity. Therefore a threshold for carcinogenicity is claimed, and that it is appropriate to derive an oral reference dose (RfD/TDI) from intestinal tumour data. This is contrary to the current UK position that affirms the mutagenic potential of Cr(VI) via oral exposure and therefore, that there is no threshold for carcinogenicity. Thus, potentially there are implications for public health risk assessments.

Background:

2. Hexavalent chromium (Cr(VI)) has been extensively evaluated for mutagenicity and carcinogenicity including an NTP carcinogenicity study in rats and mice (Stout et al 2009) and a number of substantial reports from a variety of regulatory bodies and organisations are available (ATSDR 2012, IARC 2012, IPCS CICAD 2013). Cr(VI) is known to generate a variety of different DNA lesions, including adducts, cross links, strand breaks, chromosome aberrations and several oxidative DNA base lesions and is classified as carcinogenic to humans by IARC (group 1) although it is noted that this classification relates to lung tumours via inhalation route.

3. A recent EFSA evaluation (EFSA 2014) concludes there is clear evidence for the mutagenic potential of Cr(VI) both *in vitro* and *in vivo*. (section 7.2.2.4 – see also Table H7-8 p219-222 – ANNEX 2). However, following oral exposure the results are not clear cut. There are a number of positive *in vivo* studies albeit with some unconventional protocols ((ie p(un) reversion assay in mice) but mainly negative results in bone marrow and peripheral blood cells. It is noted that the inconsistent results may be due to the reductive capacity of the gastrointestinal tract limiting or preventing Cr(VI) uptake. Carcinogenicity assessment following oral exposure is based principally on the NTP 2008 study (Stout et al 2009) which reported squamous cell neoplastic lesions in the oral cavity and epithelial neoplastic lesions in the small intestine of treated F344 rats and B6C3F1 mice respectively.

4. The EFSA report also provides a detailed mode of action (MOA) evaluation including how the oxidation state of chromium influences its bioavailability, cellular uptake and genotoxicity (section 7.4 page 102-112). It is contended that the genotoxic activity of Cr(VI) is attributable to intracellular reduction via CrV to CrIII and that the reduction is also a key factor in the bioavailability of Cr(VI) following oral exposure. It was also suggested that reactive oxygen species (ROS) generation during reduction reactions contribute to the genotoxic mode of action of Cr(VI). The overall conclusion is that Cr(VI) should be considered as both mutagenic and carcinogenic and that the margin of exposure (MOE) approach to risk assessment is applicable.

5. Chromium was the subject of a US EPA case-study for the mode of action framework for mutagenic carcinogens (McCaroll et al 2009). The conclusions were that Cr(VI) acts via a mutagenic MOA and therefore a linear extrapolation for oral risk assessment is recommended.

ATSDR (2012) concluded on genotoxicty as follows:

> 'Chromium (VI) compounds were positive in the majority of tests reported, and their genotoxicity was related to the solubility and, therefore, to the bioavailability to the targets' (see pages 215-238)

They also concluded that Cr(VI) is carcinogenic via the oral route and carcinogenic effect levels (CEL's) were derived from the NTP 2008 data.

6. The IPCS evaluation (IPCS 2013) concluded on carcinogenicity via the oral route as follows:

> "There is significant uncertainty associated with the carcinogenic risk to humans associated with oral exposure to chromium(VI) compounds. This extends to both the interpretation of an epidemiological study of a population exposed to chromium(VI)-contaminated drinking-water and the relevance to human exposures at low concentrations in drinking-water of the long-term animal studies that have been conducted. Owing to these uncertainties, a quantitative assessment of carcinogenic risk to humans from ingested chromium(VI) is not presented".

Hypothesis for a threshold effect

7. A substantial body of work has been conducted to investigate the possibility of a threshold mechanism for the carcinogenicity of Cr(VI) to the small intestine by the oral route; much of this is based on the observation of cytotoxicity and subsequent regenerative cell proliferation in the villi and crypts of the small intestine of exposed animals. The studies by the Thompson group (ToxStrategies Inc) are listed at the end of the paper. The overall conclusion reached was that, following oral exposure, there is a threshold for Cr(VI) mutagenicity and therefore carcinogenicity. With regard to the COM opinion, it will be important to evaluate the data that has enabled Thompson et al to discount the known mutagenicity of Cr(VI).

8. A study pivotal for this conclusion is O'Brien et al (2013), which assessed *K-Ras* mutation frequency and micronuclei in the duodenal crypt and villus enterocytes of mice treated with Cr(VI) for 90 days (ANNEX 1). In this study B6C3F$_1$ mice received Cr(VI) (as sodium dichromate dehydrate, SDD) via drinking water at 0.3-520 mg/L for 7 or 90 days. The duodenal crypt area was examined histologically and the number of micronuclei (MN), karyorrhectic nuclei (KN), mitotic figures, apoptotic nuclei and cytotoxicty recorded. The K-ras gene was amplified and allele-competitive blocker PCR was used to investigate K-ras codon 12 GGT to GAT mutations. The authors proclaim that this technique/endpoint is a sensitive marker of early, dose related increases in mutations relevant to the carcinogenic process and that it is of particular relevance to the development of intestinal carcinogenesis.

9. No increases in MN, KN, mitotic and apoptotic indices were found in intact crypts at any dose level. Increases in duodenal villi KN and MN were seen at doses at or above 170 mg/L. There

were no positive controls in this study. There was a high spontaneous rate of K-ras mutations (mutant fraction: 2.43 x 10^{-3}) and no increases in the Cr(VI) treated groups.

10. The derivation of the RfD by Thompson et al (2013a) refers principally to the mouse intestinal tumours seen in the NTP 2008 study and is based on the assumption of a threshold for carcinogenic effect. The argument for the threshold nature of Cr(VI) tumourigenesis after oral exposure is based on the systematic evaluation of data using the US EPA MOA framework (USEPA 2007). A review article describes the stepwise mode of action process and presents data and discussion for each of their proposed key-events (Thompson et al 2013b –ANNEX 1).

11. Of particular note in this report is a philosophical discussion of what constitutes a 'mutagenic MOA' as a preamble to the first critical question in the MOA framework - 'Is mutation an early key event in the chemical's induction of cancer' (page 245). A comprehensive table of the Cr(VI) *in vivo* genotoxicty studies is presented on p248. The importance of these [largely negative] studies is stressed, including those that directly compared oral and i.p. administration (DeFlora 2006).

12. The review describes in detail the derivation of the key events in Cr(VI) induced carcinogenicity as follows:

1) absorption from the lumen; 2) villous cytotoxicty; 3) crypt cell hyperplasia; 4) crypt cell mutagenesis.

Their argument is based on an assumption that mutagenesis is a late stage phenomenon in the development of the mouse intestinal tumours as there are no early preneoplastic lesions (p260).

13. They claim that their application of the MOA framework provides a substantially stronger and more scientifically robust basis for assessing the carcinogenic response using the target specific mechanistic data provided. From this conclusion they went on to derive the RfD as described in Thompson et al 2013a.

Questions for Members?

- Do Members agree that GGT to GAT mutation at K-Ras codon 12 is a sensitive marker of genotoxic carcinogens, as proposed by Thomson et al.
- What are Members opinions of the genotoxicity data for Cr(VI) presented by O'Brien et al (K-ras mutations, micronuclei)?
- Do Members consider the threshold MOA for Cr(VI) carcinogenesis, presented by Thompson et al to be convincing?
- Do Members wish a more detailed examination of the available data before reaching a conclusion?

References :

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Thompson CM, Proctor DM, Suh M, Haws LC, Kirman CR, Harris MA. (2013b). Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans. Critical Reviews in Toxicology. 43(3): 244-274.

USEPA (2007) **Framework for Determining a Mutagenic Mode of Action for Carcinogenicity:** Using EPA's *2005 Cancer Guidelines* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. EPA 120/R-07/002-A External Peer Review Draft September 2007 http://www.epa.gov/osainter/mmoaframework/pdfs/MMOA-ERD-FINAL-83007.pdf*

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ANNEX: 1

Thompson CM, Proctor DM, Suh M, Haws LC, Kirman CR, Harris MA. (2013b). Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans. Critical Reviews in Toxicology. 43(3): 244-274.

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ANNEX 2

Tables H7 and H8 from EFSA 2014

Summary of in vivo genotoxicity of Chromium VI – oral route

Summary of in vivo genotoxicity of Chromium VI – non-oral route