

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

MUT/03/14

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

MUTAGENICITY OF TRIVALENT CHROMIUM AND CHROMIUM PICOLINATE

Introduction

1. Chromium is a group 6 metal which is ubiquitous in the environment. It is an essential element, being involved in insulin metabolism. It is present in some foods notably processed meats, whole grain cereals and herbs and spices. Chromium picolinate is a widely available food supplement.

2. The adverse effects of chromium were recently reviewed by the Expert Group on Vitamins and Minerals (EVM). The reports of genotoxicity associated with chromium picolinate were noted and chromium picolinate was excluded from their recommendations for an upper safe level. Following the publication of the EVM report, the Food Standards Agency advised that consumers should use other forms of trivalent chromium supplements until more detailed advice is available.

Advice from COM

3. COM are asked to advise on the mutagenicity of trivalent chromium and chromium picolinate.

Background: Public Health Issues

4. Hexavalent chromium is known to be carcinogenic and mutagenic in both humans and laboratory animals (IARC, 1990). It produces a variety of genotoxic damage and produces tumours in laboratory animals and in human subjects that have been occupationally exposed such as tanners and chrome platers. It was considered that there was sufficient evidence for the carcinogenicity of chromium (VI) in both humans and laboratory animals. Overall, chromium (VI) was classified as category 1 a known human carcinogen (IARC, 1990).

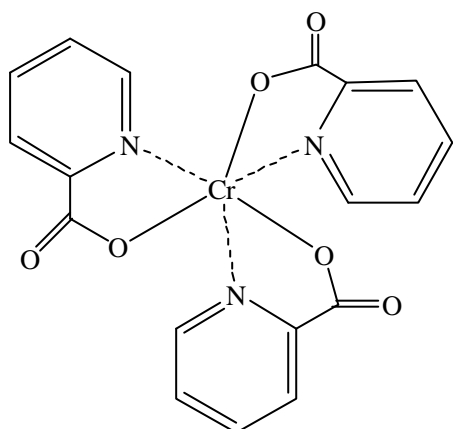
5. In contrast, the situation for trivalent chromium is less clear. Animal carcinogenicity data, where available, for trivalent chromium is negative, mutagenicity studies are generally negative though some positive mutagenic effects are reported at high, cytotoxic, doses or where contamination with Cr(VI) may have occurred. IARC (1990) considered that there was inadequate evidence for the carcinogenicity of chromium (III) compounds in both humans and animals and stated that overall, chromium (III) compounds were not classifiable as to their carcinogenicity in humans and animals (group 3). It is

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

generally considered that the lower toxicity of trivalent compared to hexavalent chromium compounds is due to the low level of absorption and solubility and the fact that unlike hexavalent chromium, trivalent chromium does not cross cell membranes.

6. The trivalent chromium compound chromium picolinate (see Fig 1) is a widely available food supplement, which is considerably more bioavailable than other trivalent forms of chromium. This compound has been reported to cause mutations and chromosomal aberrations in mammalian cells and mutations in *Drosophila*. However it is negative in a number of bacterial mutagenicity assays, and did not cause the induction of micronuclei in mouse bone marrow or rat peripheral lymphocytes *in vivo*.

Fig 1. Structure of chromium picolinate



7. A detailed review of the mutagenicity and carcinogenicity of trivalent chromium and chromium picolinate is attached at Annex 1 and summarised below. The review concentrates on trivalent chromium and includes data on hexavalent chromium where relevant. Key papers related to the mutagenicity of chromium picolinate are attached at Annex 2.

Mutagenicity and carcinogenicity of trivalent chromium

Chemistry

8. Chromium is a metallic element of the transition group which is ubiquitous in the environment. It has a range of oxidation states, with +3 and +6 being the most stable. These form trivalent (chromic) and hexavalent (chromous and chromate) compounds respectively. Hexavalent chromium compounds are strong oxidising agents and thus are readily reduced to trivalent chromium. Consequently, hexavalent chromium does not occur in biological systems.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

9. Trivalent chromium forms stable coordination complexes with water or other ligands. The coordination complexes are stable at acid pH, but as the pH rises the compound coordinates with hydroxo groups rather than water and begins to undergo oligation, which ultimately form large complexes that precipitate out of solution. Other ligands can stabilise chromium in solution and solubilise previously insoluble complexes.

10. Picolinic acid is a minor metabolite of tryptophan metabolism. It is an isomer of nicotinic acid, one of the forms of the B group vitamin niacin.

Absorption, distribution, metabolism and excretion

11. Oral absorption of trivalent chromium is poor, with hexavalent chromium being slightly better absorbed, although is largely reduced to trivalent chromium in the gastric environment.

12. Following i.v. and i.p administration, chromium is found in many tissues, particularly the liver, kidney and spleen. It has also been reported in the brain, testes and skeletal tissues. However, it has also been reported that the chromium may be confined to the fatty layers of the bone marrow and may not reach the red cell precursors. The tissue distribution of chromium is altered when administered in a buffered compared to an unbuffered solution. Hexavalent chromium is readily reduced by intracellular components such as glutathione and ascorbate. In the plasma, chromium is largely bound to transferrin.

14. Only hexavalent chromium is able to cross erythrocyte cell membranes where it is readily reduced to trivalent chromium and is then unable to leave the cell. Similarly, hexavalent but not trivalent chromium is able to enter white blood cells. However, insoluble trivalent chromium may be able to enter cells by endocytosis.

15. Chromium is largely excreted in the urine, though some biliary excretion does occur.

Chromium picolinate

16. Chromium picolinate is a more bioavailable form of trivalent chromium. It is thought that it is sufficiently stable to be absorbed into the cell as an intact complex. Labelled chromium picolinate has been found in the liver and kidney of rats. Within the cell, trivalent chromium is generally found in the mitochondria and the nucleus, however chromium picolinate is more commonly found in the cytosol. Cr(III) is released by hepatic activation *in vitro*, *N*-1-methylpicotinamide being the primary organic metabolite. The chromium complex is thought to be broken down within 24 hours *in vivo*.

Cytotoxicity and oxidative damage

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

17. Hexavalent chromium is readily reduced producing reactive oxygen species (ROS) and causing oxidative damage to cell membranes and proteins and to DNA, causing strand breaks. Hexavalent chromium only binds to DNA in the presence of a reducing agent. Low levels of ROS and oxidative damage have been attributed to trivalent chromium.

Chromium picolinate

18. Chromium picolinate has also been reported to produce ROS and lipid peroxidation. It has also been reported to produce oxidised DNA bases, though at lower levels than chromate. It has been suggested that the structure of the chromium picolinate complex makes the Cr(III) centre reducible to Cr (II) which would allow it to enter a Fenton-like cycle producing ROS and thus DNA damage. However other reports have not found this effect. Chromium picolinate treatment of cells results in damage to the mitochondria

Interactions with nucleic acids and protein

Cell free systems

19. Trivalent chromium binds DNA directly, causing cross links within and between strands. It also binds phosphate groups, proteins and forms complexes with glutathione. It has been reported that crosslinks may occur in phosphate but not Tris buffer. Hexavalent chromium induces Cr(III)-DNA adducts generally resulting in mutations at G:C base pairs and resulting in transitions to A:T and transversions to T:A.

20. The binding of chromium to DNA results in polymerase arrest in some studies. This occurs either as a direct reaction of DNA with trivalent chromium or following the reduction of hexavalent chromium. Direct binding of chromium and enzyme has also been reported. *In vitro* treatment of DNA with chromate decreases the fidelity of DNA synthesis.

Chromium picolinate

21. Chromium picolinate has been reported to relax supercoiled DNA by nicking the strands in the presence of reducing agents such as ascorbate.

Intact cells

22. Hexavalent but not trivalent chromium compounds caused crosslinks in intact cells. The cross-link is thought to be an amino acid/glutathione-Cr(III) DNA link. The mutations induced by cross-linking occur preferentially at G:C rich sites. Cross-links were not found in the leukocytes of human volunteers given Cr(III) or Cr(VI).

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

23. Hexavalent but not trivalent chromium compounds induced unscheduled DNA synthesis *in vitro* indicating its ability to produce DNA damage.
24. Chromium picolinate has been reported to induce DNA fragmentation.

In vitro mutagenicity assays

Bacterial cells

25. Hexavalent chromium is mutagenic in a variety of bacterial assays. Trivalent chromium is essentially negative though some positive reports exist (possibly due to contamination with hexavalent chromium). Certain ligand types have been reported to increase the mutagenic activity of trivalent chromium. It has been suggested that the Ames test is not appropriate for assessing trivalent chromium since the phosphate buffer could form complexes reducing entry into the cell.
26. Analysis of the mutations found in bacterial cells, indicates that chromium appears to act at G:C sites resulting in transitions and transversions. It has been suggested that this could disrupt the recognition site for RNA polymerases which are surrounded by GC rich regions. In contrast, it has been reported that hexavalent chromium causes mutations in *Salmonella* strains which have extensive AT regions in the mutation site.

Chromium picolinate

27. Chromium picolinate is negative in the Ames test.

Eukaryotic cells

28. Hexavalent chromium is mutagenic in mammalian cells, analysis of the mutations indicating damage to A:T base pairs. Trivalent chromium is also positive in some studies; insoluble salts have been suggested to be more mutagenic than soluble ones.
29. Both types of chromium were active in the comet assay in plant cells and human lymphocytes.

Chromium picolinate

30. Chromium picolinate is mutagenic in mammalian cells producing a clear (40 fold) increase in 6-thioguanine resistant mutants in CHO cell compared to controls. Trivalent chromic chloride was also mutagenic producing a 10 fold increase in mutants compared to controls. The equivalent concentration of picolinic acid was cytotoxic, but lower concentrations produced non-significant increases in the number of mutants. There are few conventional mutagenicity data on picolinate.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Clastogenicity

31. Hexavalent chromium causes a variety of clastogenic damage. Some reports of clastogenicity exist for trivalent chromium but this tends to be at cytotoxic concentrations, or where conditions have been altered eg longer exposure to allow increased exposure endocytosis. *In vivo*, hexavalent chromium but not trivalent chromium resulted in the induction of micronuclei in mouse bone marrow.

Chromium picolinate

32. Chromium picolinate has been reported to cause chromosome aberrations *in vitro* in CHO cells, this was attributed to the picolinate ligand which was clastogenic in the absence of chromium. Chromium nicotinate, a structural isomer, was negative in the study, which may reflect the monodentate rather than bidentate ligand structure.

33. Chromium picolinate is negative for the induction of micronuclei in rat bone marrow and in mouse peripheral lymphocytes and chromosome aberrations in rat bone marrow. In human volunteers chromium picolinate did not increase the amounts of the oxidised base 5-hydroxy methyl-2'-deoxyuridine.

Drosophila

34. Hexavalent but not trivalent chromium caused an increase in mutants (wing spots) in conventional *Drosophila* assays; a large proportion of this was due to mitotic recombination.

Chromium picolinate

35. Chromium picolinate but not chromic chloride was active in a multi-generation *Drosophila* study where it was observed to delay pupation and decrease pupal viability. Further analysis indicated that chromium picolinate increased lethal mutations and dominant female sterility. Treatment with picolinic acid alone also increased the numbers of individuals arrested during pupation and reduced larval and adult viability. This was thought to be a coincidence since the metabolism of picolinic acid and chromium picolinate are significantly different. Picolinic acid was not tested for its ability to cause X-linked mutations.

Carcinogenicity

36. Few adequate carcinogenicity studies are available for trivalent chromium; chromic oxide was not carcinogenic in rats.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

37. There are few human epidemiology studies relevant to trivalent chromium because of the problems of multiple exposure. The studies that are available have conflicting results with trivalent chromium being associated with an increase in lung cancer in one study of chromate workers but not in a larger study in a different chromate production plant.

Mechanism of chromium genotoxicity

38. The mechanism of chromium genotoxicity is unclear. It is generally considered that Cr(VI) is the active agent in chromium genotoxicity since it is the only form of chromium that is able to cross cell membranes. Reduction of Cr(VI) to Cr(III) outside the cell or outside the nucleus suppresses its genotoxicity. Exposure to Cr(VI) is known to produce a range of DNA damage including adducts, cross-links strand breaks and oxidised bases. However, it is generally thought that Cr(III) may be the ultimate carcinogen since it is able to react directly with DNA. It is uncertain whether the intracellular reduction of Cr(VI) to Cr(III) with the accompanying production of oxidative damage or structural effects due to the formation of Cr(III) complexes, or both, is the main mechanism for chromium genotoxicity.

39. The intermediates Cr(V) and Cr(IV) may briefly occur during the reduction process and may mediate some of the genotoxic damage associated with chromium.

40. The ready reduction of Cr(VI) in cellular systems may be why the tumours induced by chromate only occur at local sites. This is true for animals (where only inhalation and intratracheal administration result in tumours) and humans where occupational exposure to chromate fumes has only been associated with lung cancer.

41. A number of studies have suggested that chromium preferentially binds to G:C rich regions of DNA as would occur with most electrophilic mutagens. It has been suggested that this could interfere with DNA replication by obstructing the CAAT box recognition site of RNA polymerases which are surrounded by G:C rich regions. However, chromium has also been reported to increase T to C transitions within AT rich regions in *Salmonella* strains TA102 and TA2638.

42. When a series of trivalent chromium complexes were synthesised to investigate the influence of different ligands on the mutagenicity of trivalent chromium it was only the aromatic bidentate ligands which were active. The same mutagenic complexes were able to relax supercoiled plasmid DNA presumably by the induction of strand breaks. These mutagenic Cr(III) complexes displayed characteristics of reversibility and positive shifts of the Cr(III)/Cr(II) redox couple consistent with the ability of these complexes to serve as cyclical electron donors in a Fenton-like reaction generating superoxide anion and hydroxyl radicals which have DNA damaging potential.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

43. Chromium may be able to reduce the fidelity of DNA replication by interacting directly with DNA polymerases as well as by damaging or interacting with DNA directly.

Chromium picolinate

44. Chromium picolinate has been reported to have a variety of effects. These include causing mutations in mammalian cells *in vitro* and in *Drosophila*. However, other workers have argued that experiments using chromium levels closer to those found physiologically were negative.

45. It has been argued that chromium picolinate may have a chemistry that is different from that of chromium chloride or hexavalent chromium, with the possibility of Fenton chemistry could occur, with oxidised bases being a more significant cause of DNA lesions than single strand breaks.

46. It has been suggested that the rapid degradation of chromium picolinate *in vivo* is reassuring with regard to its safety since the chronic ions generated would be treated in the same way as other chromic ions ie bound to transferrin and thus not subject to damaging redox chemistry. However, chromium picolinate is thought to be sufficiently stable to enter the cell intact, releasing trivalent chromium intracellularly.

47. Picolinic acid in phosphate buffer has been reported to enhance the Fenton reaction

Discussion/Conclusions

48. Hexavalent chromium is a well known genotoxin causing DNA damage by a variety of means; it is thought to be the active agent in chromium genotoxicity since it is able to cross cell membranes. Trivalent chromium is unable to cross cell membranes and is generally negative in conventional mutagenicity assays, except where very high doses have been used, or the conditions altered to increase the entry of trivalent chromium into the cell. However, within the cell Cr(III) may be the ultimate genotoxin since it is able to bind DNA directly. It is unclear to what extent the intracellular reduction of Cr(VI) to Cr(III) with the consequent production of reactive oxygen species or the direct effects of Cr(III) or both, are responsible for chromate genotoxicity.

49. Solubility is key to interpreting and extrapolating the results of *in vitro* tests to *in vivo* situations. Soluble hexavalent chromium compounds are readily detected in conventional mutagenicity assays whereas insoluble compounds may not be due to the short exposure time involved. However, *in vivo*, very long exposures may allow the completion of very slow processes such as endocytosis; this needs to be considered when assessing the potential risks of the compound.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

50. The significance of endocytosis in the genotoxicity of chromium is uncertain. Some authors have reported that the formation of insoluble precipitates reduce mutagenic activity, whereas others argue that endocytosis followed by activation of the compound by lysosomal contents increase the genotoxic risk.

51. Although Cr(III) has been noted to react directly with DNA in simplified, cell-free systems, this is often at concentrations which would not occur in the cell nucleus.

52. Chromium picolinate may be able to cross cell membranes since it is a more stable complex than other forms of trivalent chromium. This could enhance its mutagenic potential. Some reports suggest that chromium picolinate is mutagenic *in vitro* and in *Drosophila*. However *in vivo* studies in rat bone marrow and mouse peripheral lymphocytes are negative. Labelling studies suggest that although trivalent chromium is found in bone after i.p. or i.v. administration, it is possible that it may not have penetrated the fatty layer surrounding the bone marrow. Similarly the inability of trivalent chromium to cross cell membranes suggests that it may not be taken up by erythrocytes. It should be noted however that chromium picolinate is a more stable complex than other forms of trivalent chromium and may be able to pass cell membranes.

53. There are few conventional data on picolinic acid, however, it is known to cause cell cycle arrest. This could increase the mutagenic potential of chromium picolinate by increasing the length of time DNA is exposed to chromium. Picolinic acid is generally more cytotoxic than the equivalent concentration of chromium picolinate.

Advice required from COM

54. The COM are asked to advise on the following points:

- i) Can any conclusions be drawn on the genotoxicity of chromium picolinate *in vitro* and *in vivo*?
- ii) If chromium picolinate is genotoxic, is there likely to be a threshold effect?
- iii) An NTP bioassay of chromium picolinate is currently underway. What further work, if any, would help to determine the genotoxicity of chromium picolinate ?

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

Annexes

Annex 1. Detailed review of the mutagenicity of trivalent chromium and chromium picolinate.

Annex 2. Mutagenicity of chromium picolinate- key literature papers.