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**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
(CPMP)**

**NOTE FOR GUIDANCE ON SPECIFICATION LIMITS FOR RESIDUES
OF METAL CATALYSTS**

This document is no longer valid.

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NOTE FOR GUIDANCE ON SPECIFICATION LIMITS FOR RESIDUES OF METAL CATALYSTS

1. INTRODUCTION

The objective of this guideline is to recommend, for the safety of the patient, maximum acceptable metal* residues arising from the use of catalysts in the synthesis of pharmaceutical substances. Since there is no therapeutic benefit from residual catalysts, specification limits should be applied to those present in pharmaceutical substances, in a manner that is consistent with safety- and quality-based criteria. Since the use of catalysts is restricted to defined chemical reactions in the synthesis of substances, limitation of residues in these substances is sufficient, and there is no need to set limits for metal residues in the final medicinal products containing these substances.

This guideline primarily focuses on metal catalysts that are actually used in the synthesis of pharmaceutical substances. The guideline can be updated to include other sources of metal residues and additional elements in due course. Any interested party can make a request and submit relevant safety data.

2. SCOPE OF THE GUIDELINE

Residual catalysts in active substances and excipients that are used in medicinal products are within the scope of this guideline. Residues of metal catalysts can either be present as the original catalyst or as a form of the metal element altered by downstream chemical processing.

If, for the production of a pharmaceutical substance, a specific metal catalyst is used and the synthetic processes are known or suspected to lead to the presence of residues of this catalyst, element-specific assays should be undertaken to determine the actual amount of residues. If the synthetic processes are validated for the removal of potential residues¹ of this particular catalyst, routine testing may be replaced by skip testing. On the other hand, if the synthetic processes do lead to potential residues, routine testing with a suitable, validated method is necessary. This testing cannot replace the requirements of relevant monographs of the European Pharmacopoeia (Ph. Eur.), which may for instance describe a general test for heavy metals. This general heavy metal test is indicative for the overall quality of production, which includes sources of metal contamination which are outside the scope of this guideline, such as manufacturing equipment and environment. This guideline does not apply to potential new active substances, or excipients, used during the clinical research stages of development of a medicinal product.

The guideline applies to all dosage forms but different limits are applied to oral and parenteral routes of administration. For other routes of administration see section 4.2. This is because many transition element metals have low oral bioavailability due to poor gastrointestinal absorption.

¹ A catalyst can be considered adequately removed if, in an appropriate number of production scaled batches of the final substance or an intermediate less than 10 % of the option 1 limit could be found.

* The term "metal" is used in preference to "heavy metal" since the latter term has been given such a wide range of meanings by different authors that it is effectively meaningless (Duffus, 2001).

3. BASIS OF PROPOSED SPECIFICATION LIMITS

For the purposes of this guideline, permitted daily exposure (PDE) has been employed as the key indicator of the maximum safe intake limit for individual elements.

PDE is defined as the maximum patient exposure (expressed in $\mu\text{g}/\text{kg}/\text{day}$) to an element, possibly on a chronic basis, that is unlikely to produce any adverse health effects.

In order to derive safe metal limits an evaluation of data in the toxicological literature is required. Problematic aspects, which are encountered while setting safety limits for metals, are discussed in Appendix 1.

For each of the 14 elements included in this guideline, considerations for setting the PDEs are given in a separate monograph. The monographs are listed in Appendix 2 to this guideline.

The resulting estimated PDEs are shown in Table 1.

Table 1: Recommended Oral and Parenteral PDEs for 14 elements

| Element | Oral PDE ($\mu\text{g}/\text{kg}/\text{day}$) | Parenteral PDE ($\mu\text{g}/\text{kg}/\text{day}$) |
|------------------------|---|---|
| Pt, Pd, Ir, Rh, Ru, Os | 2.6 (group) | 0.25 (group) |
| Mo | 5 | 2.5 |
| V | 10 | 0.5 |
| Cu | 50 | 10 |
| Ni | 20 | 2 |
| Cr | 25 | 2.5 |
| Mn | 100 | 5 |
| Zn | 300 | 30 |
| Fe | 250 | 25 |

Oral PDEs are derived as indicated in the element monographs. Parenteral PDEs (apart from that for Pt) are obtained by multiplying the oral PDE by the estimated oral bioavailability of the particular element (ranging from 5% to 50%).

The following oral bioavailabilities are used:

5% for V and Mn

10% for Ni, Fe, Zn, and Cr

20% for Cu

50% for Mo

For the platinum elements Pd, Ir, Rh, Ru, and Os, no specific PDEs can be recommended, due to lack of toxicity data. For these elements the PDEs set for Pt should be used. This PDE applies to the whole group; if more platinum elements are present, the PDE is allocated to the total amount.

4. PROCEDURE FOR DETERMINING LIMITS OF CATALYST RESIDUES

4.1 Options for Setting Limits

Two options are available when setting limits for metal residues. Both options take into account only a percentage of the PDEs. The reason to use variable proportions of the PDEs for the different elements, is that dietary intake of the different elements also varies. For the platinoids the dietary intake is virtually zero and so a high proportion of the PDE can be allocated to pharmaceutical use. For the essential minerals, a significant proportion of the oral PDE is accounted for by dietary intake, for example in the case of Zn dietary intake represents 61% of the oral PDE. Information on dietary intake for each of the elements can be found in Appendix 1.

In Table 2 the percentage of PDE to be used is stated for each of the 14 elements. These percentages compensate for dietary intake as well as other sources of exposure, such as polypharmacy.

Table 2: % of PDE Employed in Calculation of Concentration Limits

| Elements | % Oral PDE | % Parenteral PDE |
|------------------------|------------|------------------|
| Pt, Pd, Ir, Rh, Ru, Os | 32 | 33 |
| Mo | 33 | 7 |
| V | 17 | 12 |
| Cu | 5 | 2.5 |
| Ni | 8 | 8 |
| Cr | 7 | 7 |
| Mn | 2.5 | 5 |
| Zn | 1 | 1 |
| Fe | 1 | 1 |

Option 1

Option 1 limits normally apply. They are stated in ppm in Table 3. They were calculated using Equation 1, assuming a daily intake of 10 g of any active ingredient (or excipient).

Equation 1:

$$\text{Concentration (ppm)} = \frac{y/100 \times \text{PDE} \times \text{Body weight}}{\text{Dose}}$$

y is the variable % as stated in Table 2

PDE is given in Table 1 in terms of $\mu\text{g}/\text{kg}/\text{day}$

Dose is the daily intake of the substance containing the metal residue given in g/day.

In the equation, 60 kg is used as average body weight.

Table 3: Option 1 Concentration Limits

| Elements | Oral Concentration Limit (ppm) | Parenteral Concentration Limit (ppm) |
|------------------------|--------------------------------|--------------------------------------|
| Pt, Pd, Ir, Rh, Ru, Os | 5 (group) | 0.5 (group) |
| Mo, V, Ni, Cr | 10 | 1.0 |
| Cu, Mn | 15 | 1.5 |
| Zn, Fe | 20 | 2.0 |

If two or more elements derived from metal catalysts are present, the total oral concentration limit should not exceed 20 ppm; total parenteral concentration limit should not exceed 2 ppm

For intakes > 10 g/day, the Option 1 concentration limits are reduced *pro rata*. For example, for an active given to patients at 15 g/day, the concentration limits would be 10/15 of those tabulated above.

A group limit is applied to the platinoid elements; if two or more such elements are present, the group concentration limit is applied to the total amount.

Option 2

If batch manufacturing data indicate that it is not feasible to achieve the Option 1 concentration limits, and optimising the production process for removal of metal residues is not successful either, an Option 2 limit based on the *actual* daily intake of the particular active ingredient or excipient may be applied. The Option 2 limits can also be calculated by using Equation 1.

For the calculation of an Option 2 limit, a minimal daily intake of the substance containing the metal residues of 1 g per day should be used. So, under Option 2, the Option 1 limits may be increased by a factor 10 at most. These are limits that are regarded as feasible in routine practice.

However, as specification level, the lowest limit that is reasonably achievable should be used, with the Option 2 limit as maximum.

4.2 Other Administration Routes

If a substance is administered by a route other than oral or parenteral, either the oral PDE or the parenteral PDE should be used. The choice should depend on the expected absorption by that particular administration route. Parenteral limits should be used, unless it is clear that the absorption by a particular administration route will never exceed the absorption following oral administration. In this last case oral limits should be applied.

For example, for cutaneous administration oral concentration limits should be applied and for substances that are administered by inhalation, parenteral concentration limits should be applied.

If a substance (active substance or excipient) can be administered by several routes, the lowest applicable PDE should be used.

NB For cutaneous administration, attention should be paid to local skin reactions as well².

² A concentration of < 5 ppm of any one of the listed elements in the final product is unlikely to cause contact allergy even in highly sensitive patients. (Uter et al, Contact Dermatitis, 1995, 32, 135-142)
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4.3 Active substances Used for Short-term Only

As the PDEs mentioned in this guideline are based on chronic use, higher concentration limits may be acceptable in cases of short-term use (30 days or less). This may be the case if neither an Option 1 nor an Option 2 limit is feasible in practice for a contrasting agent, an antidote, or products for diagnostic use.

If the concerning substance will never be used in an other way than in a single dose or for a very limited duration, it may be toxicologically justified to set a limit higher than the Option 1 or the calculated Option 2 limit.

Justifications should be made on a case by case basis.

5. TEST METHODS

If an active substance (or excipient) may contain residues of metal elements, used as catalyst(s) in the synthesis, for each of these residual elements a specification should be set. For the determination of each of the specified elements an appropriate and validated method should be used in relation to the limit to be applied. Attention should be paid to the fact that the residual element may be present in a different form than the form of the element in the original catalyst.

General semi-quantitative heavy metal limit tests based on the precipitation at pH 3.5 of coloured metal sulphides are described in several publications (e.g. Ph. Eur.). Such a test is not suitable to quantitatively determine the actual levels of a specific metal residue in an active substance or excipient. If adjusted (e.g. by using standard addition methods) and properly validated (including cross-validation with an element-specific test), a test based on the principle of sulphide precipitation, may be suitable for routine testing in some cases.

Validation of methods to determine metal residues should conform to the ICH-guidelines “Validation of analytical procedures: definition and terminology” and “Validation of analytical procedures: methodology.”

GLOSSARY

| | |
|------------------------|--|
| WHO | World Health Organization |
| RDA | Recommended daily allowance for adults |
| ESADDI | Estimated safe and adequate daily intake. |
| LOEL | lowest-observed effect level |
| NOEL | no-observed effect level |
| <i>iv</i> | intravenous |
| <i>ip</i> | intraperitoneal |
| <i>po</i> | per os (oral) |
| LD₅₀ | median lethal dose |
| US EPA | United States Environmental Protection Agency |
| RfD | Reference Dose |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| PMTDI | Provisional Maximum Tolerable Daily Intake |
| MRL | Minimal Risk Level |

APPENDIX 1: SETTING PDEs FOR RESIDUAL METALCATALYSTS: ISSUES AND UNCERTAINTIES

A variety of metals, mostly transition elements, are employed as such and/or as complexes or salts in organic chemical synthesis to act as catalysts particularly for reactions such as alkene hydrogenation. Any pharmaceutical active ingredient (or excipient) whose synthesis involves the use of one or more metal catalysts thus may contain residual metal(s) in the form of the original catalyst(s) or as derivatives produced by downstream chemical processing.

There are a number of problematic aspects associated with providing recommendations on safe limits for metal catalyst residues in pharmaceuticals, the main ones being:

- **Speciation and Form:** Transition elements have the potential to form numerous molecular species mainly dependent on oxidation state, co-ordinating ligands and solvation. This property leads to uncertainty over the likely form of metal catalyst residues in biological systems. In addition, toxicity can vary greatly depending on the aqueous solubility of the particular metal salt employed for toxicological evaluation. Consequently, PDEs have been derived based on the worst-case assumption of high water solubility.
- **Balance of nutritional and toxic effects:** Although all metals have inherent toxic properties, some elements such as Fe, Zn, Cr, Mn, Cu and Mo (and possibly V) are important in human nutrition.
- **Route of administration:** Many metals are poorly absorbed from the gastrointestinal tract and so are likely to show differential toxicity between oral and parenteral routes of administration.
- **Duration of/age at exposure:** Some elements (eg Pb, Cd) are cumulative toxins while others, particularly the essential elements, are excreted efficiently. Infants and young children may be particularly sensitive to toxic effects of metals because they tend to absorb a higher fraction of an oral dose, and developing body systems (particularly the nervous system) may be more sensitive than mature systems. Fortunately, on the basis of the available data none of the 14 elements under consideration appears to have a significant propensity to accumulation following oral administration. Infants and young children are likely to receive proportionately lower doses of pharmaceutical products and, although the proposed limits apply principally to adults, they have been set at a sufficiently low level to be applicable to younger age groups.
- **Data availability:** Toxicological (animal and/or human) information on most metals is restricted to a limited number of test types using just the oral route and is available on only a few representative compounds. Except for Pt the toxicological database on the platinum group metals, which appear to have broadly similar chemical and biological properties, is extremely limited. Consequently, sufficient data were available on which to base a PDE only in the case of Pt, and this value has been applied as a group PDE to all six platinum group elements.
- **Extrapolation of toxicological data:** PDEs are derived in general by applying an appropriate “safety” or “uncertainty” factor to the designated lowest-observed effect level (LOEL) or no-observed effect level (NOEL). Owing to wide variability of the nature, quality and quantity of toxicological data amongst the metal elements of interest, it is not possible to employ a totally consistent approach. Human data and previous published evaluations by regulatory bodies have been utilised wherever possible. Published evaluations are usually in the context of continuous chronic or lifetime intake, whereas exposure to pharmaceutical products is expected to be intermittent in most cases. Consequently, for some elements (eg V, Cr) recommendations relating to subchronic rather than chronic exposure have been followed. As there are very limited non-oral data, oral bioavailability data is used to estimate the parenteral PDEs.
- **Interactions:** Competition for absorption sites, nutritional status and other factors can lead to interactions amongst the metal elements, particularly Mo, Zn, Fe and Cu. However, this is unlikely to be a major issue in the case of metal catalyst residues given their anticipated minor contribution to metal intakes.

Note on References: In the interests of brevity, monographs on the individual metal elements in Appendix 2 are not extensively referenced. Detailed bibliographies are available elsewhere, eg Merrill et al , 2001 and US EPA, 2001.

Table 4: Dietary intakes of 14 elements

| Element | Dietary Intake ,mg/day (µg/kg/day) | |
|---------|------------------------------------|-----------------|
| | Mean (Adult) | 97.5 Percentile |
| Ir | 0.002 (0.031) | 0.003 (0.046) |
| Pd | 0.001 (0.015) | 0.002 (0.046) |
| Rh | 0.0002 (0.0031) | 0.0004 (0.0062) |
| Ru | 0.004 (0.061) | 0.006 (0.092) |
| Os | <0.001 (,0.015) | - |
| Pt | 0.0002 (0.0031) | 0.0003 (0.0046) |
| Mo | 0.12 (1.85) | 0.21 (3.23) |
| V | 0.013 (0.20) | - |
| Cu | 1.4 (21.5) | 3.2 (49.2) |
| Ni | 0.12 (1.85) | 0.21 (3.23) |
| Cr | 0.10 (1.54) | 0.17 (2.62) |
| Mn | 4.5 (69.2) | 8.2 (126) |
| Zn | 11 (169) | 20 (308) |
| Fe | 15 (231) | 26 (400) |

Dietary intake data from Ysart et al 1999, and Ysart et al, 2000, except for V (Evans et al, 1985) and Os (estimate).

IRIDIUM (Ir)

Introduction

Ir is a Group VIII platinoid element of the third transition series. The chemistry of Ir and its complexes is quite similar to that of Rh. Ir exhibits four main oxidation states I-IV, though only Ir II and Ir IV appear to be relevant to aqueous environments.

Dietary Intake

Mean UK intake: 2 µg/day; 97.5 percentile intake 3 µg/day (Ysart *et al*, 1999).

Toxicological Data

There are very few published animal data, and those available are of uncertain reliability. No data have been reported from studies in humans.

Conclusion

Insufficient toxicological data are available on which to base a reliable assessment.

PALLADIUM (Pd)

Introduction

Pd is a Group VIII platinoid element of the second transition series. Its principal oxidation states are II and IV. Unlike Pt, Pd co-ordination complexes have not been shown to be present in biological systems.

Dietary Intake

Mean daily UK intake: 1 µg; 97.5 percentile intake 2 µg/day (Ysart *et al*, 1999)

Toxicological Data

Few useful animal toxicity data are available. After a single oral dose of ¹⁰³Pd (as ¹⁰³PdCl₂) in rats, absorption was poor, only 0.5% of dose being excreted subsequently in urine. In comparison, ¹⁰³Pd was detected in all major tissues after *iv* administration, though no significant placental transfer of ¹⁰³Pd was noted using this route. Approximate oral rat LD₅₀ values for PdCl₂ are 170 (*po*), 5 (*iv*) and 70 (*ip*) in mg Pd/kg.

No good quality data from repeated-dose toxicity studies or human studies appear to be available. PdCl₂ and several other Pd compounds were non-genotoxic in an in-vitro micronucleus assay.

No occupational poisoning has been shown via the inhalation or dermal routes. There are a number of case reports of Pd allergy connected with exposure through the use of dental alloys.

Conclusion

Due to the extremely limited database on Pd and its compounds, no risk assessment is possible.

RHODIUM (Rh)

Introduction

Rh is a platinum group VIII element of the second transition series. Its principal oxidation states are I, II and III, though Rh III is the most common state, especially in terms of aqua ion formation. Rh catalysts (Rh-Pt metal alloy; Rh CO complexes) are widely used.

Dietary Intake

Mean UK intake: 0.2 µg/day; 97.5 percentile intake 0.4 µg/day (Ysart *et al*, 1999).

Toxicological Data

Oral uptake is reported to be very low.

Single-dose rat toxicity data on RhCl_3 indicate that the *iv*/oral LD_{50} values are *ca* 200 and > 500 mg/kg respectively.

Simple Rh compounds such as RhCl_3 have been reported as genotoxic, e.g. in *Salmonella typhimurium* TA98, and others as cytotoxic. No repeated-dose toxicity data were located.

Conclusion

Insufficient animal/human toxicity data are available for risk evaluation purposes. Rh compounds appear to be less toxic than their Pt counterparts.

RUTHENIUM (Ru)

Introduction

Ru is a platinum group metal of the second transition series. It exhibits a wide range of oxidation states, the most common being II, III and IV.

Ruthenium tetroxide, RuO_4 , is a more volatile and vigorous oxidant than OsO_4 .

$\text{Ru}(\text{OH})_2$, RuCl_4 and RuO_2 are stable and water soluble; generally Ru III salts are insoluble in water. Ru is employed as a hardener in Pt/Ru alloys which are used in electrical contacts.

Dietary Intake

Mean UK intake: 4 $\mu\text{g}/\text{day}$; 97.5 percentile intake, 6 $\mu\text{g}/\text{day}$. (Ysart *et al*, 1999).

Toxicological Data

Oral absorption of Ru is low (up to 3.5% in rodents).

Oral and *ip* LD_{50} values have been determined in rodent species for several Ru compounds:

| Compound | Oral LD_{50} (mg/kg) | <i>ip</i> LD_{50} (mg/kg) | Species |
|-----------------------------|----------------------------------|---------------------------------------|---------|
| Ru Chloride hydroxide | 463 | 225 | Mouse |
| Ru Oxide (RuO_2) | 5570 | 3050 | Mouse |
| RuO_2 | 4580 | - | Rat |

Several Ru complexes with potential medical application have been reported to cause genotoxic responses *in vitro* (e.g. in *Salmonella typhimurium* strains TA98 and TA100), although the magnitude of the effects was much less than in the case of *cis*-platin.

No data from repeated-dose toxicity studies could be located.

Conclusion

Based on data from acute toxicity studies on simple Ru compounds, and on the genetic toxicity of some complexes containing nitrogenous ligands, Ru compounds/complexes appear likely to be less toxic than their Pt counterparts. However, insufficient data are available on which to base a reliable assessment.

OSMIUM (Os)

Introduction

Os is one of the six platinum group metals, the six heaviest elements in Group VIII. Os belongs to the third transition series. Os complexes exhibit a wide range of oxidation states, the most common being Os III, IV and VI.

Osmium tetroxide, OsO₄ is a volatile, toxic and powerful oxidant used in chemical synthesis and in dilute aqueous solution as a biological stain for adipose tissue, being readily reduced by organic matter to a black oxide. OsO₄ is a severe irritant of the eyes and respiratory tract.

There are strong analogies between Os and Ru in terms of chemical reactivity.

Dietary Intake

No information on dietary intake could be found. However, given the rare occurrence of Os, human dietary intakes are expected to be extremely low (< 1 µg/day) except perhaps in areas close to metal smelters.

Toxicological Data

No data appear to be available apart from several reports on the effects of OsO₄ on synovial membranes in connection with the use of 1% OsO₄ solutions for chemical synovectomy of arthritic joints. Most of the injected Os is excreted in urine with no evidence for accumulation in the contralateral knee, the regional lymph nodes, the liver or the heart.

Conclusion

Insufficient toxicological data are available on which to base a reliable assessment.

PLATINUM (Pt)

Introduction

Pt is a Group VIII element of the third transition series. It is the most important of the six heaviest of the group VIII elements, collectively called the “platinum group metals” or “platinoids”.

Pt and Pd are more chemically reactive than the other platinoids. Metallic Pt has been shown to catalyse many oxidation-reduction and decomposition reactions and the major industrial use of Pt is as a catalyst.

Pt complexes exhibiting a range of oxidation states are known, although the principal valences are Pt II and IV. Pt II forms a tetra-coordinate aqua ion [Pt (H₂O)₄]²⁺. A variety of amine complexes such as the anti-tumour compound *cis*-PtCl₂(NH₃)₂ (*cis*-platin) are also known. The most important Pt IV compounds are salts of the red hexachloroplatinate ion PtCl₆²⁻.

Dietary Intake

Mean UK dietary intake 0.2 µg/day; 97.5 percentile intake 0.3 µg/day (Ysart *et al*, 1999).

Toxicological Data

Gastrointestinal absorption of Pt salts is extremely low (<5% of oral dose). Excretion of most of the absorbed fraction is normally via the faeces.

The acute toxicity of Pt salts is dependent on water solubility (the more soluble salts being more toxic) and speciation. A range of Pt IV salts are reported to have rodent oral LD₅₀ values from 10 to >1100 mg/kg.

Repeated-dose toxicity studies have been undertaken in the rat using Pt salts in the drinking water for periods of up to 30 days. Only PtCl₄ and Pt(SO₄)₂.4H₂O produced initial transient effects on growth rate. The NOEL for administration of PtCl₄ for 30 days is 13 mg Pt/kg/day.

Some soluble platinum salts have been reported to be mutagenic *in vitro*.

Cis-platin and carboplatin, widely employed in cancer chemotherapy, are thought to act mainly by adduct formation with both nuclear and mitochondrial DNA. As well as being generally cytotoxic, they exhibit a range of other animal and human toxicities. In animals and/or humans *cis*-platin has been reported to show myelotoxicity, nephrotoxicity and ototoxicity. In clinical use a variety of dosing regimens are used for *cis*-platin, employing for example daily administration for 5 days at *ca* 0.25 mg Pt/kg/day *iv*, possibly with additional doses at weekly or 2-weekly intervals. The human 13-day LOEL in terms of toxicity is reported as 0.3 mg Pt/kg *iv* (Lewis, 1996).

Occupational exposure to platinum compounds, particularly those containing reactive chlorine, is associated with skin hypersensitivity reactions.

Discussion

No regulatory assessments appear to be available for Pt except for TLVs (Threshold Limit Values) applied to occupational exposure. A TLV of 0.002 mg/m³ for soluble Pt salts, which protects against worker sensitisation, has been adopted. The TLV for Pt metal is 500 times higher (Merrill *et al*, 2001).

Pt and its compounds have a wide spectrum of toxicity ranging from relatively low toxicity of Pt metal to genotoxic/cytotoxic effects (e.g. *cis*-platin) and sensitisation reactions associated with some Pt salts and complexes. Consequently, a conservative approach to the assessment of an appropriate PDE has been adopted.

Conclusion

Oral PDE: rat NOEL of 13 mg Pt/kg/day for PtCl₄ and safety factor of 5000 yields a PDE of 2.6 µg Pt/kg/day.

Parental PDE: human *iv* 13-day LOEL for *cis*-platin of 300 µg Pt/kg, equivalent to *ca* 25 µg Pt/kg/day. Applying a safety factor of 100 (human data but LOEL not NOEL) yields a PDE of 0.25 µg Pt/kg/day.

MOLYBDENUM (Mo)

Introduction

Mo is a Group VIB element of the second transition series. Its main oxidation states are IV and VI, the most common forms of which are oxyanions. The predominant form of Mo occurring in soils and natural waters is the molybdate ion, MoO₄²⁻ which forms soluble compounds with a variety of cations including K⁺, NH₄⁺ and Ca²⁺. MoO₂ and MoS₂ are insoluble in water. Mo metalloenzymes have a vital role in plants and bacteria particularly in respect of nitrate reductase and nitrogenase. In man, Mo-containing xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine as part of the degradation pathway of purine nucleic acids to uric acid. Mo deficiency, characterised by night blindness, nausea, disorientation and coma and associated with various biochemical abnormalities including high plasma methionine and almost undetectable serum uric acid, has been reported in a patient receiving total parenteral nutrition.

Dietary Intake

Mean UK intake 0.12 mg/day; 97.5 percentile intake 0.21 mg/day (Ysart *et al* 1999). The US ESADDI is based on the current US dietary intake of 75-250 µg/day. A maximum level in drinking water of 0.07 mg/l has been recommended by WHO. Intakes of 10-15 mg/day may be associated with altered nucleotide metabolism and impaired Cu bioavailability. A safe intake for UK adults of 50-400 µg/day has been recommended (Department of Health, 1991). WHO has estimated a daily adult Mo requirement of 100-300 µg.

Toxicological Data

Absorption of Mo VI from the gastrointestinal tract is reported to be good for soluble compounds (40-85% in the rat; 85-93% in man). Absorption and retention of Mo is markedly influenced by interactions with dietary Cu and sulphate. Cu forms insoluble copper thiomolybdate in the digestive

tract and high dietary inorganic sulphate is believed to reduce intestinal absorption by blocking the transport of Mo through the cell membrane.

The acute toxicity of Mo compounds is related to their solubility. MoO_3 , CaMoO_4 and $(\text{NH}_4)_2 \text{MoO}_4$ caused fatalities in rats at oral doses of 1.2-6.0 g Mo/kg, whereas insoluble MoS_2 was essentially non toxic at up to 6.0 g Mo/kg.

The chronic toxicity of Mo has not been extensively studied in laboratory animals. Virtually all of the published studies date back to the 1940s-1970s and focus on Mo toxicity in cattle and other livestock that seem to lack tolerance for Mo. In a 5/7-week dietary study in the rabbit with Na_2MoO_4 , the NOEL is estimated as 7 mg/kg/day.

Regulatory Assessment

US EPA. Oral RfD for Mo = 5 $\mu\text{g}/\text{kg}/\text{day}$, based on an increase in urinary uric acid in humans exposed to 10 mg Mo/day in the diet (uncertainty factor of 30).

Conclusion

Oral PDE: 5 $\mu\text{g Mo}/\text{kg}/\text{day}$ (following US EPA approach – human LOEL of 150 $\mu\text{g Mo}/\text{kg}/\text{day}$ and uncertainty factor of 30). Estimated parenteral PDE: 2.5 $\mu\text{g Mo}/\text{kg}/\text{day}$ (based on a bioavailability of 50%).

VANADIUM (V)

Introduction

V is known to exist in a variety of oxidation states (-1, 0, +2, +3, +4 and +5). The principal species in biological materials are vanadate, VO_3^- , and vanadyl, VO^{2+} . The anionic pentavalent form predominates in extracellular fluids whilst the cationic quadrivalent vanadyl ion is the most common intracellular form. Vanadium is a major trace metal in fossil fuels.

Dietary Intake

UK: 13 $\mu\text{g}/\text{day}$ (Evans *et al*, 1985)

USA: 6.2 - 18.3 $\mu\text{g}/\text{day}$ (FDA Total Diet Study, Pennington and Jones, 1987).

V is considered by some as an essential trace mineral; vanadyl sulphate supplements (at microgram to milligram levels) are marketed to normalise blood glucose (in diabetics) and to promote muscle growth (in body builders), and are not associated with reports of any short-term adverse effects.

Toxicological Data

Most orally ingested vanadium in humans appears to be unabsorbed, only 1-5% of dose being excreted in urine. In the rat however, much higher absorption (>10%) has been reported.

A variety of toxicity studies have been undertaken in animals, and the principal target organs are the digestive system, kidneys and blood. In lifetime studies rats and mice exhibited no adverse effects when exposed to 5 ppm V (as VOSO_4) in drinking water (Schroeder *et al* 1970, Schroeder and Mitchener 1975), NOELs being 0.7 and 0.9 mg V/kg/day for rats and mice respectively.

Sodium metavanadate (NaVO_3) is considerably more toxic when given parenterally. Oral LD_{50} values were 41 and 31 mg V/kg in rats and mice respectively, whereas *ip* LD_{50} s are reported as *ca* 0.1 mg V/kg in these species. NaVO_3 given to rats in drinking water for 3 months produced impaired kidney function at 50 ppm and the NOEL was considered to be 10 ppm (1.32 mg/kg/day) (Domingo *et al*, 1985).

In a 6-week human study involving oral doses of 50-125 mg V/day (as vanadyl tartrate), no adverse effects were observed at the lower doses (Dimond *et al*, 1963). The NOEL is conservatively estimated as 0.5 mg V/kg/day.

Regulatory Assessments

US EPA: NOEL 0.7 mg V/kg/day (as VOSO₄)

A range of RfDs have been proposed based on the above NOEL and on a NOEL for NaVO₃ (10 ppm in a 3-month rat drinking-water study). The oral RfDs range from 0.001 mg V/kg/day (chronic RfD based on NOEL of 1.32 mg V/kg/day for NaVO₃ and an uncertainty factor of 1000) to 0.02 mg V/kg/day (subchronic and chronic RfD for VOSO₄).

Conclusion

In consideration of the data on dietary intakes (up to 0.3 µg V/kg/day), the use of VOSO₄ supplements and the US EPA RfDs, an oral PDE of 0.01 mg V/kg/day is proposed (based on a human NOEL of 0.5 mg/kg/day and a safety factor of 50). This recommended PDE is equivalent to the subchronic RfD for NaVO₃ proposed by EPA and 50% of the chronic RfD for VOSO₄.

Estimated parenteral PDE: 0.5 µg V/kg/day (based on a bioavailability of 5%).

COPPER (Cu)

Introduction

Cu is a Group IB element of the first transition series and has two main oxidation states, Cu I and Cu II. Cu is the functional component in a variety of cuproenzymes (e.g. cytochrome c oxidase, asorbic acid oxidase and superoxide dismutase); it plays an important biological role in redox reactions and in the scavenging of radicals.

Dietary Intake

Mean UK intake 1.4 mg/day; 97.5 percentile intake 3.2 mg/day (Ysart *et al*, 2000). Cu is an essential element; the US RDA is 0.9 mg/day in adult men and women aged >19 years. The Joint Expert Committee on Food Additives has recommended a PMTDI of 0.5 mg/kg/day. For the UK (Department of Health, 1991) a dietary reference value of 1.2 mg/day has been proposed for adults aged 18 years and over.

Toxicological Data

Cu is readily absorbed following oral ingestion, absorption being greatest for the most soluble salts.

Virtually all toxicological data relate to oral Cu II, particularly CuSO₄. Few adequate data are available on chronic toxicity. Data are available from a number of acute and sub-chronic studies (generally up to 90 days) mainly using dietary administration. In studies on copper sulphate, chloride, carbonate and cyanide, mainly in rats, NOELs ranged from 1.7 mg Cu/kg/day (increased systolic blood pressure and increases in haemoglobin at higher dose of 9.6 mg Cu/kg/day - CuCO₃ used as dosing material) to 17 mg Cu/kg/day (hyperplasia and hyperkeratosis of forestomach mucosa at ca 30 mg Cu/kg/day - CuSO₄ used as dosing material). At the higher doses employed in subchronic toxicity studies the main target organs were the kidneys and liver. Microcytic anaemia occurred in rats due to depletion of iron stores. In the rat over 90 days using Cu(CN)₂ given by gavage, the NOEL was 5 mg Cu/kg/day.

Discussion

Copper is subject to a number of homeostatic mechanisms *in vivo* following oral ingestion that reduce the likelihood of toxic sequelae if intake exceeds the normal requirements. The mechanisms involved include binding to metallothionein, absence of significant storage, binding to albumin and transcuprein and biliary excretion.

Regulatory Assessments

US EPA : RfD (subchronic) = 0.05 mg Cu/kg/day
(NOEL of 5 mg/kg/day and assessment factor of 100)

$$\text{RfD (chronic)} = 0.005 \text{ mg Cu/kg/day}$$

(same NOEL and assessment factor of 1000).

Conclusion

The subchronic rat oral NOEL of 5 mg/kg/day with a safety factor of 100 yields a PDE of 50 µg Cu/kg/day. This is considered to be suitable for both subchronic and chronic ingestion given that in a 70 kg patient, Cu intake (3.5 mg/day) would be only marginally higher than the UK 97.5 percentile dietary intake, and significantly less than the PMTDI of 0.5 mg Cu/kg/day.

Estimated parenteral PDE: 10 µg Cu/kg/day (based on a bioavailability of 20%).

NICKEL (Ni)

Introduction

Ni is a Group VIII B element of the first transition series. Although it can exhibit valences of 0, I, II and III, its main oxidation state is +2.

Dietary Intake

Mean UK intake: 0.12 mg/day; 97.5 percentile 0.21 mg/day (Ysart *et al*, 2000). Ni appears to be an essential micronutrient in animals, and so it is likely to be essential for humans, but its precise function in humans is unknown. Estimates of the presumed human Ni requirement range from 5-50 µg/day (Department of Health, 1991).

Toxicological Data

In general, more soluble Ni compounds such as Ni(NO₃)₂ are more absorbable than insoluble ones. In the fasted state up to 50% can be absorbed from the gastrointestinal tract, but the extent of absorption can be reduced dramatically in the presence of many food constituents such as ascorbic acid, tannins and phosphates. Thus, most Ni in food remains unabsorbed and *ca* 10% of Ni *with* food is absorbed. Ni from drinking water can be up to 25% absorbed, although this is not a major source since Ni levels are normally low.

The rodent LD₅₀ for NiSO₄ is reported as *ca* 300 mg/kg, whereas that for the almost insoluble NiO is > 5000 mg/kg.

A number of sub-chronic/chronic toxicity studies on Ni compounds with durations ranging from 3 weeks to 2 years using dietary, oral gavage or drinking water administration have been reported in the literature. A two year rat study (Ambrose *et al*, 1976) involving dietary administration of NiSO₄ over 2 years has been used extensively as a basis for regulatory assessments. The NOEL from this study is 5 mg Ni/kg/day. The same NOEL was obtained from a 90-day rat study on NiCl₂ (decreased red blood cell parameters and alterations to serum enzyme activity). A two-year dog study on NiSO₄ (Ambrose *et al*, 1976) revealed lung and bone-marrow lesions at the highest dose; the NOEL from this study is assessed as 29 mg Ni/kg/day.

Although some in-vitro positive findings in genotoxicity assays (particularly for clastogenicity) have been reported, there is no evidence suggesting that Ni compounds are carcinogenic by the oral route. Ni sensitivity and allergic contact dermatitis is well documented, particularly in women (possibly owing to the wearing of Ni-containing earrings in pierced ears).

Regulatory Assessments

Several bodies have made recommendations on safe levels, generally using a NOEL of 5 mg Ni/kg/day. WHO derived a Tolerable Daily intake of 5 µg/kg/day through use of an uncertainty factor of 1000 (to compensate for the absence of reliable chronic toxicity/carcinogenicity/reproductive toxicity data). EPA set an RfD of 20 µg Ni/kg/day after employing an uncertainty factor of 300.

Conclusion

An oral PDE of 20 µg/kg/day is recommended based on a NOEL of 5 mg Ni/kg/day and an uncertainty factor of 300.

Estimated parenteral PDE: 2 µg Ni/kg/day (based on a bioavailability of 10%).

CHROMIUM (Cr)

Introduction

Cr is a Group VIII element of the first transition series. A variety of oxidation states are known, but the most important are Cr II, III and VI. Cr II is readily oxidised and is used as a reducing agent in chemical synthesis. Cr VI is a powerful oxidant, chromate, CrO_4^{2-} , and dichromate, $\text{Cr}_2\text{O}_7^{2-}$, being the best known oxyanions. Cr III, the most abundant environmental form, is an essential element that plays a role in glucose metabolism.

Dietary Intake

Mean UK intake: 0.10 mg/day; 97.5 percentile intake 0.17 mg/day (Ysart *et al*, 2000). An earlier publication (Ysart *et al*, 1999) reported higher intakes of 0.3 and 0.52 mg/day respectively. In the UK it has been recommended that dietary intakes should exceed 0.025 mg/day for adults (Department of Health, 1991). The ESADDI established by the National Research Council in the USA is 50-200 µg Cr/day for adults.

Toxicological Data

Intestinal absorption of Cr III is low (up to 2%) in both humans and animals. Most dietary Cr is not absorbed and is excreted via the faeces. Small amounts of assimilated Cr (up to 0.5 µg/day) are excreted via urine.

Soluble Cr III compounds are moderately toxic, rodent LD_{50} values being 100-400 mg/kg. Rats fed diets containing up to 5% Cr_2O_3 for a lifetime showed no adverse effects. EPA considered that the highest dose (equivalent to 1468 mg Cr/kg/day) to be the NOEL. In a more recent dietary rat study (Anderson *et al*, 1997), no adverse effects were detected at 15 mg Cr III/kg/day.

The Cr VI oxyanions in aqueous solution are in pH-dependent equilibrium. At pH >6 (ie physiological pH), Cr VI forms the tetrahedral yellow chromate ion, CrO_4^{2-} , which is structurally similar to phosphate and sulphate and readily enters all cells via the general anion channel protein. Cr VI is readily absorbed by all tissues. The lethal oral dose of soluble chromates in humans is 50-70 mg/kg, target organs being the liver, kidney and haematopoietic system. The mechanism of action of Cr VI is thought to be by oxidation of biological tissues to form a variety of radical species including alkyl and oxygen radicals.

Toxicological studies reported on Cr VI are generally of short duration. Oral administration to rats at 10-14 mg/kg/day for 14-20 days produced several effects including reduced growth rate, increased lipid content of the liver, and alterations in the activity of liver and kidney enzymes. Longer studies, generally with no adverse effects, involve administration via drinking water at concentrations ranging from 25-200 ppm. The NOEL for a one-year rat study in which animals received water containing potassium chromate was 2.4 mg Cr/kg/day (Mackenzie *et al*, 1958).

Cr VI, but not Cr III has been shown to be genotoxic in a number of test systems. Cr III (as Cr_2O_3) was non-tumorigenic when administered to rats in the diet at up to 5% Cr_2O_3 . Cr VI has not been evaluated by the oral route for carcinogenic potential, but Cr VI following inhalation has been shown in animal studies and through epidemiological studies to be carcinogenic in the respiratory tract.

Regulatory Assessments

US EPA oral RfDs for Cr III and Cr VI are 1.5 and 0.005 mg Cr/kg/day respectively.

Conclusion

A conservative approach employing the NOEL for the more toxic Cr VI (2.4 mg Cr/kg/day) with a safety factor of 100 is employed, giving an oral PDE of 25 µg/kg/day. An uncertainty factor of 500 has been employed by the US EPA and the Dutch National Institute of Public Health and Environment

(Baars *et al*, 2001) in terms of chronic oral exposure to CrVI. Employing a lower safety factor of 100 (leading to a PDE of 25 µg Cr/kg/day) is considered appropriate in the context of this NfG for a variety of reasons including: Cr is more likely to be present as CrIII than CrVI; exposure via pharmaceutical products is intermittent rather than chronic, and only 7% of the oral PDE is allocated to the calculation of concentration limits.

Estimated parenteral PDE: 2.5 µg/kg/day (based on a bioavailability of 10%).

MANGANESE (Mn)

Introduction

Mn is an element of the first transition series (Group VIIa). It can exist in eleven oxidation states from -3 to +7, the normally encountered valences being +2 (most common form in nature), +4 (as in MnO₂) and +7 (as in permanganate ion). Mn is considered to be an essential element with a minimum intake of 2.5 mg/day.

Dietary Intake

Mean daily UK intake: 4.5 mg; 97.5 percentile daily intake: 8.2 mg. (Ysart *et al*, 1999).

Toxicological Data

Most studies have been undertaken with Mn²⁺ (MnCl₂ or MnSO₄). Virtually all data relate to the oral route. Oral absorption is low (*ca* 3.5% in the rat). Absorbed Mn is excreted largely via the bile and is eliminated in the faeces. Mn can accumulate in the brain at high intake levels.

Carcinogenicity studies in rats and mice used dietary doses of MnSO₄ up to *ca* 700 and 2000 mg Mn/kg/day respectively. There was no evidence of carcinogenicity in rats but in mice the evidence was considered to be equivocal based mainly on forestomach focal squamous hyperplasia (accompanied by ulceration/erosion and inflammation). NOELs are estimated at *ca* 70 and 200 mg Mn/kg/day in rat and mouse respectively.

Genotoxicity studies on Mn²⁺ are equivocal, some positive results being obtained *in vitro* (e.g. TA1537, mouse lymphoma assay) but not *in vivo*.

A number of epidemiological studies have been reported, the most extensive by Kondakis *et al* (1989) in the Northwest Peloponnesus area of Greece. Intakes via drinking water were up to 4.6 mg/day.

Regulatory Assessments

US EPA : RfD = 0.14 mg/kg/day (20 mg/day considered safe in diet).

RfD = 0.2 mg/l (drinking water criterion based on paper by Kondakis *et al*).

ESADDI – set by US National Resource Food Council and Nutrition Board: 2-5 mg/day for adults.

ATSDR: 0.07 mg/kg/day (based on upper range of ESADDI).

Conclusion

Given the various assessments based on human data, results of chronic rodent studies (NOEL of 70 mg/kg/day in the rat, higher in the mouse) and estimates of dietary intakes, an oral PDE of 100 µg Mn/kg/day is considered to be appropriate.

Estimated parenteral PDE: 5 µg Mn/kg/day (based on a bioavailability of 5%).

ZINC (Zn)

Introduction

Zn is a group IIB element. It is regarded as a non-transition element since it forms no compounds in which the *d*-shell is other than full. Zn II is the predominant oxidation state; the aqua ion is quite a strong acid and is partially hydrolysed in water to hydroxy complexes. Zn forms a range of covalent organo compounds such as Zn (0) alkyls that are useful as reagents in organic synthesis. Zn has a variety of critical functions in humans: it is essential for growth and development (particularly of the brain), maintaining appetite, wound healing, immunocompetence, etc. Zn is a cofactor of the superoxide dismutase enzymes. It is an important component of DNA, acting to stabilise phosphate groups and co-ordinate with organic bases.

Dietary Intake

Mean UK intake: 11 mg/day; 97.5 percentile 20 mg/day (Ysart *et al*, 2000). Zn is an essential element and various bodies have published guidance on recommended intakes. In the US the RDA is 15 mg/day for adult men and 12 mg/day for adult women, with an additional 3 mg/day during pregnancy and 7 mg/day during lactation. UK RNIs (Reference Nutrient Intakes) for Zn are slightly lower (Department of Health, 1991).

Toxicological Data

Uptake of Zn from the gastrointestinal tract occurs by both passive diffusion and by a membrane-associated carrier-mediated process. During digestion Zn is released from its dietary ligands and complexes with low-molecular-weight intestinal ligands that impede or enhance Zn bioavailability depending on their relative affinity for Zn with respect to the membrane carrier. Gastrointestinal absorption of Zn is higher when body stores are lower, and is also higher from refined diets. In the intestinal cell, following internalisation, Zn associates with metallothionein.

Several metal-metal interactions are known to occur with Zn. There is mutual antagonism between Cu and Zn in terms of uptake owing to similarities in electronic configuration of their common ions (d^{10}). Elevated levels of dietary Zn can have a negative effect on Cu balance, which is exploited therapeutically to “de-copper” Wilson disease patients. Long-term oral intakes of 18.5-25 mg/day can interfere with Cu absorption.

A similar interaction occurs between Zn and Fe owing to mutual competition for absorption sites. Studies in rats indicate that a high level of dietary supplementation with Zn can cause anaemia due to increased Fe turnover.

Overall, the bioavailability of oral Zn can vary widely owing to several factors, but it seems reasonable to assume that at least 10% would be absorbed on average.

The toxicological database on Zn is extensive. Rodent LD₅₀s for soluble salts of Zn II (e.g. acetate, sulphate, nitrate) range from *ca* 100-600 mg/kg. Data from sub-chronic and chronic toxicity studies are also available, but are superseded by human data.

Regulatory Assessments

Recommendations on limits for the tolerable intake of Zn can be confusing, sometimes conflicting to some extent with recommended nutrient intakes.

WHO proposed a PMTDI of 0.3-1.0 mg/kg, corresponding to 18-60 mg/day for a 60 kg adult.

In the US the MRL and the RfD have been set at 0.3 mg Zn/kg/day (derived from a LOEL of 50 mg/day which caused slight decreases in red cell parameters in young women).

Conclusion

An oral PDE of 300 µg Zn/kg/day is recommended based on a human LOEL of 1 mg Zn/kg/day (assuming a body weight of 50kg in young women) and an uncertainty factor of 3. Estimated parenteral PDE: 30 µg Zn/kg/day (based on a bioavailability of 10%).

IRON (Fe)

Introduction

Fe is a group VIII element of the first transition series. Its principal oxidation states are +2 (ferrous ion) and +3 (ferric ion). Fe is an essential human nutrient with a variety of physiological roles including those associated with haemoglobin, myoglobin, ferritin and Fe-containing enzymes.

Dietary Intake

Mean UK intake 15 mg/day; 97.5 percentile intake 26 mg/day (Ysart *et al*, 1999). The USA RDA for Fe is derived using an adequate body store of 300 mg, estimated losses of 1 mg/day in men and 1.5 mg/day in women, and an oral absorption fraction of 10-15%, leading to a recommendation of 10 mg/day for adult males and 15 mg/day for adult females, with an additional 15 mg/day recommended during pregnancy.

Toxicological Data

Fe has been studied extensively in a myriad of animal and human studies. Human toxicity is well documented, particularly in respect of fatalities in children associated with ingestion of adult Fe supplements. A single dose of 20 mg Fe/kg is sufficient to produce gastrointestinal symptoms.

Regulatory Assessments

US EPA has not derived any toxicity values for Fe.

Conclusion

In the absence of any regulatory assessment, it is proposed that the oral PDE for Fe should be based on the US RDA of 15 mg/day (250 µg/kg/day). However, the whole of this PDE is accounted for by the average dietary intake, and so the recommended PDE should be considered as a safe additional amount. This is supported by the fact that the 97.5 percentile dietary intake is 26 mg/day. In addition, a significant proportion of dietary Fe will be in the form of haem Fe that is well absorbed compared with non-haem Fe (the form likely to be encountered as a catalyst residue in pharmaceuticals).

Estimated parenteral PDE: 25 µg/kg/day (based on a bioavailability of 10%).

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