PHASE III: INTEGRATED TESTING STRATEGY (ITS) FOR GOLD AND GOLD SUBSTANCES: ADDENDUM 1

FINAL REPORT TO PRECIOUS METALS AND RHENIUM CONSORTIUM FROM WCA

FEBRUARY 2015

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Report Title	Phase III: ITS addendum for gold and gold substances: Addendum 1
Date of production	February 2015
Contract/Project Number	P0187
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Report Quality Check

	Printed name & Signature	**Date**
Document Approved by	Becky Marks 	29/01/2015
Document Quality Checked by	Dean Leverett 	04/02/2015

EXECUTIVE SUMMARY

The Precious Metals and Rhenium Consortium commissioned wca to design an Integrated Testing Strategy (ITS) involving the recommendation of any enabling tests or test waiving based on category read-across and weight of evidence arguments for the gold substances.

The final ITS report was completed in April 2012. However, a tiered testing approach is used for many endpoints under REACH and therefore further iterations of the ITS are required following the initial recommendation for use of existing data, waivers and further testing outlined in the original ITS report. This ITS addendum outlines the additional data that has become available since the 2012 ITS report, provides updated proposals for completing REACH endpoints or for any further testing that is required and gives a record of decisions made regarding the REACH strategy for gold and gold substances since the 2012 ITS report.

The substances covered by this ITS addendum, along with their registration intentions, are provided in the table below. There have been no changes to the substance inventory or declared tonnages since the 2012 ITS report.

Name of substance	CAS number	Volume (tpa)	Substance status	REACH registration deadline	PMC registration date
Gold	7440-57-5	10-100	Mono-constituent substance	2018	2015 / 2016
Tetrachloroauric acid (in aq solution)	16903-35-8	10-100	Mono-constituent substance	2018	2015 / 2016
Aurio(1+)2,6,6-trimethylbicyclo[3.1.1]heptanethiolate	68365-87-7	1-10	Mono-constituent substance	2018	2015 / 2016
Balsams, copaiba, sulfurised, mixed with turpentine, gold salts	68990-27-2	1-10	UVCB, transported intermediate not under strictly controlled conditions	2018	2015 / 2016

Two of the gold substances are being registered at 1–10 tpa, aurio(1+)2,6,6-trimethylbicyclo[3.1.1]heptanethiolate and balsams, copaiba, mixed with turpentine, gold salts. Neither substance meets the REACH Annex III criteria and they are therefore subject to Annex III exemptions. Only Annex VII physico-chemical endpoints are required for these substances and no environmental fate, ecotoxicity or mammalian toxicology testing is proposed.

The following updates have been agreed since the ITS report was finalised in 2012:

- Remaining physico-chemical tests have been conducted at Harlan Laboratories. All physico-chemical endpoints are now complete.
- Balsams, copaiba, mixed with turpentine, gold salts as having Annex III exemptions.
- It has been agreed to conduct a limited OECD 106 adsorption / desorption study with tetrachloroauric acid. Testing is ongoing at Fraunhofer.
- Remaining ecotoxicological testing has been completed at Brixham Environmental Laboratory. All ecotoxicological endpoints are now complete for tetrachloroauric acid. Further testing may be required for PNEC refinement following the first screen of the exposure assessment.
- It was agreed that no further ecotoxicity testing will be conducted in order to fill endpoints for gold metal. Instead, waivers based on lack of solubility will be included, based on the results from the T/D test.
- It was agreed that a bio-elution study in artificial gastric fluid would be conducted with gold metal, and this study has been completed at CIMM. Based on the lack of dissolution shown in this study, it was agreed that it is appropriate to waive further studies for gold metal with administration via the oral route.
- The genetoxicity test programme with tetrachloroauric acid has been conducted, including an *in vivo* micronucleaus assay conducted using neutralised tetrachloroauric acid, required following a positive result in the *in vitro* micronucleus assay. The *in vivo* micronucleus assay was negative., therefore no further testing or classification is required for this substance.
- It was agreed to conduct a combined repeat dose / reproductive screening (OECD 422) study using neutralised tetrachloroauric acid, and this test has been conducted at Covance. In-life procedures have been completed and histopathological assessment of tissues is being conducted in advance of reporting.

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1 INTRODUCTION

The Precious Metals and Rhenium Consortium commissioned wca to design an Integrated Testing Strategy (ITS) involving the recommendation of any enabling tests or test waiving based on category read-across and weight of evidence arguments for the gold substances.

The final ITS report was completed in April 2012. However, a tiered testing approach is used for many endpoints under REACH and therefore further iterations of the ITS are required, following the initial proposals for use of existing data, waivers and further testing outlined in the ITS report. This ITS addendum outlines the additional data that has become available since the 2012 ITS report, provides updated proposals for completing REACH endpoints or for any further testing that is required based on this new data, and provides a record of decisions made regarding the REACH strategy for good and gold substances since the initial ITS report.

1.1 Substances in scope

The gold substances covered in this ITS, and their tonnage requirements, are presented in Table 1.1.

Table 1.1 Substances in Scope

Name of substance	CAS number	Volume (tpa)	Substance status	REACH registration deadline	PMC registration date
Gold	7440-57-5	10-100	Mono-constituent substance	2018	2015 / 2016
Tetrachloroauric acid (in aq solution)	16903-35-8	10-100	Mono-constituent substance	2018	2015 / 2016
Aurio(1+)2,6,6-trimethylbicyclo[3.1.1]heptanethiolate	68365-87-7	1-10	Mono-constituent substance	2018	2015 / 2016
Balsams, copaiba, sulfurised, mixed with turpentine, gold salts	68990-27-2	1-10	UVCB, transported intermediate not under strictly controlled conditions	2018	2015 / 2016

Gold and tetrachloroauric acid are 10 – 100 tpa substances and therefore require a REACH Annex VIII dataset. Aurio(1+)2,6,6-trimethylbicyclo[3.1.1]heptanethiolate and Balsams,

copaiba, sulfurised, mixed with turpentine, gold salts are both being registered at 1–10 tpa. Annex III exemptions have been confirmed for both substances (Section 2.1), therefore Annex VII physicochemical data only is required for these substances.

2 TESTING STRATEGY FOLLOWING 2012 ITS REPORT

This section outlines the decisions that have been made with regard to the testing strategy since the 2012 ITS report was finalised. It details the latest status of the Annex III assessment, test programmes and decisions regarding read across and data waiving. A data matrix accompanies this ITS addendum (Gold_Phase III_Data gap matrix_update_18 12 2014) and outlines how each REACH-required endpoint will be completed.

2.1 Annex III exemptions

For substances that are manufactured or imported at 1–10 tpa only the REACH Annex VII physico-chemical endpoints are required, plus any existing data, if the substance does not meet the criteria of REACH Annex III. For clarity, a summary of the Annex III criteria is as follows (for an exact review of the Annex III regulatory text, see the REACH Regulation):

If substances **meet** the following criteria:

a) Prediction (e.g. by QSARs) that they are likely to meet the criteria for category 1 classification for carcinogenicity, mutagenicity, or reproductive toxicity under the CLP Regulation (category 1 or 2 under the Dangerous Substances Directive (DSD)), or PBT/vPvB.

OR b)

i) Have dispersive or diffuse use(s), particularly where such substances are used in consumer preparations or incorporated into consumer articles; AND

ii) predicted (e.g. by QSARs) that they are likely to meet the classification criteria for human or environmental effects endpoints under the CLP Regulation or DSD.

then all Annex VII test endpoints are required.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1]heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are both 1–10 tpa substances and have no widespread dispersive uses.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1]heptanethiolate

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1]heptanethiolate was confirmed as having Annex III exemptions in the 2012 ITS report. The substance is not considered to be CMR based on a lack of published information to support this conclusion.

Aurio(1+) 2,6,6-trimethylbicyclo[3.1.1]heptanethiolate is not classified as PBT. An estimated log Kow calculated using KOWWIN (USEPA 2008b) of 4.22 would mean that the substance is not classified as B, and therefore not PBT. Harlan Laboratories attempted to determine a measured partition coefficient for this substance (Harlan 2012a), but were unable to determine a result due to the low solubility of the test substance in both octanol and water. As Kow cannot be measured for this substance and the calculated log Kow is <4.5,

aurio(1+) 2,6,6-trimethylbicyclo[3.1.1]heptanethiolate is not considered to meet the screening criteria for bioaccumulation. Estimated biodegradation data calculated using BIOWIN (USEPA 2008a) determined that the organic component of the substance is not readily biodegradable and would be classed as P. This substance would therefore be considered to be P, but not B and therefore not PBT. It should be noted that if ECHA disagree with this assessment further testing may be required. This should be conducted in a sequential manner so that if it is confirmed through testing that the substance is not PBT then the testing can stop before any remaining Annex VII endpoints are fulfilled.

Balsams, copaiba, sulfurised, mixed with turpentine, gold salts

An assessment of balsams, copaiba, sulfurised, mixed with turpentine, gold salts was not possible in the 2012 ITS report as a partition coefficient result could not be obtained for the substance and therefore an assessment of the PBT potential for the substance could not be carried out. However, the assessment has since been conducted and is reported here.

The substance is not considered to be CMR based on a lack of published information to support this conclusion.

Following the completion of the final ITS report in 2012, Harlan issued the full draft report on the physico-chemical properties of balsams, copaiba, sulfurised, mixed with turpentine, gold salts. After reviewing the report from Harlan, it emerged that some solubility data are available that could be used to estimate the partition coefficient of the substance. Further discussion with Harlan determined that it is definitely not possible to conduct further testing as very little compositional information is available. In the water solubility test the organic component was measured as Total Organic Carbon (TOC) in the filtered supernatant, but this could not be used in a partition coefficient study because of interference from the octanol, even in the aqueous phase (as this is saturated with octanol prior to the test). The only way a partition coefficient could potentially be determined would be by monitoring the gold component of the substance. However, it is the organic component and not the metallic component that is considered to be most relevant for assessing the bioaccumulation potential of an organometallic substance. Analysing the gold component would not, therefore, be likely to provide any additional useful information about the bioaccumulation potential of the substance. Estimation of a partition coefficient value based on the solubility data already included in the report could still be an option and it is this option that is considered further in this addendum.

Partition coefficient estimates have been conducted based on the solubility data from the physico-chemical test report for balsams, copaiba, sulfurised, mixed with turpentine, gold salts (Harlan 2012). As this is an organometallic substance water solubility was determined by measuring gold and Total Organic Carbon (TOC). Water solubility results based on gold and based on TOC are presented below.

Measuring the gold concentration: $<1.0 \times 10^{-5}$ g L^{-1} at 20°C, equivalent to a concentration of Balsams, copaiba, sulfurised, mixed with turpentine, gold salts of $<1.9 \times 10^{-5}$ g L^{-1}.

Measuring mean dissolved TOC: 5.28×10^{-3} g L^{-1} at 20°C.

Solubility in octanol was determined based on visual assessment, as follows:

< 9 x 10^{-3} g L^{-1}, shaking at an elevated temperature of 30°C for 21 hours.

Little compositional information is available for this substance therefore some assumptions have had to be made.

The gold content of the substance is known to be 53%. The organic content of the substance is therefore 47%. Assuming that the organic component is an alkane (empirical formula C_NH_{2N+2}) the carbon content of the organic component would be approximately 85%. This would make the carbon content of the substance equal to approximately 40%.

If the carbon content of the substance is assumed to be 40%, the solubility of the substance in water can be determined based on the mean dissolved TOC measurement and the percentage carbon content of the substance, as below:

5.28 mg L^{-1} / 0.4 = 13.2 mg L^{-1}.

The solubility of the substance in octanol (determined by visual assessment) was stated in the Harlan report, as:

<9 mg L^{-1}.

The octanol water partition coefficient can be estimated for the substance by assuming that the identified limit of solubility would be achieved in each phase during a test. The partition coefficient is therefore calculated as the solubility in octanol divided by the solubility in water, i.e.

K_{OW} = 9 / 13.2 = 0.68

Log K_{OW} = $\log_{10}$(0.68) = -0.17.

This estimate of the log K_{OW} value of the substance may be an overestimation as the water solubility is known (although the carbon content of the substance is not precisely known), whereas the octanol solubility is a maximum limit value. A lower solubility in octanol would result in a lower log K_{OW} value for the substance. Overestimation of the carbon (C) content of the substance would lead to an underestimation of the water solubility, which would result in overestimation of the octanol water partition coefficient. Underestimation of the C content of the substance would result in an underestimation of the partition coefficient. Assuming that the C content of the substance is 47% (i.e. the maximum possible proportion based on the gold content of the substance) results in an estimated log K_{OW} value of -0.1.

If the water solubility of the substance, as calculated from the gold concentration, was used as the water solubility estimate for calculation of the octanol water partition coefficient the resulting log K_{OW} value would be 2.68. This is likely to considerably overestimate the true octanol water partition coefficient of the organic component of the substance, because the solubility based on TOC analysis was considerably higher. This high estimate of the log K_{OW} value for the substance is still appreciably below the threshold for identification of substances as potentially bioaccumulative.

The estimated partition coefficient is several orders of magnitude below the threshold for Bioaccumulation (log K_{OW} 4.5). Even the partition coefficient estimated based on the solubility of the gold component, likely to be a considerable overestimate, is well below this threshold. It is therefore extremely unlikely that the substance is bioaccumulative, and should therefore not be classified as PBT.

It is recommended that the estimated K_{OW} value is used for the PBT assessment of this substance, rather than conducting a biodegradation study. Conducting a biodegradation test for this substance would be very challenging due to the low test item solubility and lack of compositional data for the test substance. As the estimated partition coefficient value is so low it would seem appropriate to use this reasoning as justification for identifying the substance as not PBT, rather than to attempt a biodegradation study on a difficult substance such as this. However, if ECHA do not accept this approach then a biodegradation study may need to be conducted at a later stage.

Based on the estimated Kow value, this substance is not considered to be bioaccumulative, and therefore not PBT. Annex III exemptions are therefore considered to apply.

2.2 Physico-chemical testing recommendations

Physico-chemical endpoints are completed for gold and gold substances using existing data, data waivers and new test data carried out as part of the CLP and REACH test programmes. All physico-chemical endpoints are now complete for gold substances. The test results obtained as part of the REACH physico-chemical testing programme are provided in Tables 2.1 and 2.2. The data matrix accompanying this ITS addendum outlines how REACH-required each endpoint will be filled.

Table 2.1 Final physico-chemical test results conducted for the CLP test programme

Test material	Test	Results or test status	Reference
Gold	OECD 110 Granulometry (Screening Test)	4.895% < 100µm.	Harlan (2011a)
	N.1 Readily Combustible Solid	Test Item failed to ignite	Harlan (2011a)
	N.4 (modified method) Self-Heating Substances	No signs of self-ignition or self-heating throughout test	Harlan (2011a)
Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate	OECD 102/ A1 Melting/Freezing Temperature	Decomposed from approximately 210°C	Harlan (2011b)
	OECD 110 Granulometry (Screening Test)	13.632% < 100µm.	Harlan (2011b)
	N.1 Readily Combustible Solid	Classified under 4.1, Packing group II	Harlan (2011b)

Test material	Test	Results or test status	Reference
	N.4 (modified method) Self-Heating Substances	No signs of self-Ignition or self-heating throughout test	Harlan (2011b)
Tetrachloroauric Acid (in aq. solution)	UN O.2 Oxidising Properties (Liquid) Expert Statement	Expert statement prepared	Harlan (2011c)

Table 2.2 Physico-chemical test results conducted for the REACH test programme

Test material	Test	Results or test status	Reference
Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate	OECD 109 Relative density	2.01 at 21 +/- 0.5°C	Harlan (2012a)
	OECD 105 Water solubility	< 2.5×10^{-6} g L^{-1}	Harlan (2012a)
	OECD 107 / OECD 177 Partition coefficient	Partition coefficient could not be determined due to the low solubility of the test item in both octanol and water	Harlan (2012a)
	OECD 104 Vapour pressure	4.4×10^{-14} Pa at 25°C	Harlan (2012b)
Balsams, copaiba, sulfurised, mixed with turpentine, gold salts	OECD 102 Melting / freezing temperature	Decomposed at 177°C	Harlan (2012c)
	Boiling temperature	Not determined – decomposed before melting	Harlan (2012c)
	OECD 109 Relative density	2.37 at 21.0 +/- 0.5°C	Harlan (2012c)
	OECD 105 Water solubility	<1.9×10^{-5} g L^{-1} (based on monitoring of gold) 5.2×10^{-3} g L^{-1} (dissolved Total Organic Carbon)	Harlan (2012c)
	OECD 107 / OECD 177 Partition coefficient	Partition coefficient could not be determined due to the low solubility of the test item in both octanol and water	Harlan (2012c)
	OECD 110 Particle size distribution	Proportion <100 μm is 41.3%	Harlan (2012c)
	OECD 104 Vapour pressure	4.1×10^{-8} Pa at 25° C	Harlan (2012d)
	N.1 Readily Combustible Solid	Category 1 flammable solid	Harlan (2012d)
	Explosive properties	Predicted negative based on the components of the test item	Harlan (2012d)

Test material	Test	Results or test status	Reference
	Oxidising properties	Predicted negative based on the components of the test item	Harlan (2012d)

2.3 Environmental fate testing recommendations

Gold and tetrachloroauric acid both require Annex VIII environmental fate endpoints to be completed. Waivers were proposed for hydrolysis and biodegradation endpoints in 2012, and these are still considered to be appropriate. Testing was not proposed for adsorption/ desorption in the 2012 ITS report, but it was noted that it may be required if an exposure assessment is needed for either of these substances. The standard test for adsorption/ desorption under REACH is an OECD 121 screening study (K_{OC} estimation by HPLC). However, this study is not appropriate for inorganic substances; the method has not been validated for any inorganic compounds and none of the recommended reference substances are inorganic compounds. In the wca (2014) Summary of the derivation of predicted no effect concentrations (PNECs) for tetrachloroauric acid (draft) report it was proposed that a limited partitioning study following the OECD 106 batch equilibrium method was conducted in order to provide partitioning information that can be used to derive soil and sediment PNECs based on the equilibrium partitioning approach, and to use in the exposure assessment, which is now confirmed to be required for tetrachloroauric acid. Before proposing this test a search of the published literature was conducted, but no suitable partitioning data was found.

The Gold Working Group agreed to conduct a limited OECD 106 study with tetrachloroauric acid and this study is currently ongoing at Fraunhofer. Only the preliminary stages of the test are being performed, with three soils used to determine the partition coefficient. This approach should be sufficient for determination of a K_D value which will be adequate for the required purposes.

2.4 Ecotoxicological endpoints

Gold and tetrachloroauric acid both require Annex VIII ecotoxicological endpoints to be completed.

In the 2012 ITS report published data for acute toxicity to fish was available for tetrachloroauric acid, and this was reviewed and considered appropriate for completing this endpoint. However, no further existing data were available and therefore testing was proposed, and agreed, for acute toxicity to *Daphnia*, toxicity to algae and toxicity to microorganisms. This testing is now complete, and the final test results from these studies are presented in the table below.

Table 2.3 Summary of ecotoxicity results for tetrachloroauric acid

TEST	RESULT
Short-term *Daphnia magna* [static test, results based on mean	48-h EC50 = 4.8 mg TCA/L (95% confidence interval 2.5 – 9.3 mg/L)

TEST	RESULT
measured concentrations]	
Algal growth inhibition *Pseudokirchneriella subcapitata* [static test, results based on mean measured concentrations]	72-h EC50 Growth Rate >9.0 mg TCA/L (95% confidence interval not determined) 72-h NOEC Growth Rate and Yield = 0.9 mg TCA/L
Short-term fish *Oncorhynchus mykiss* [static test, results based on nominal concentrations]	96-h EC50 = 15.7 mg TCA/L (calculated from EC50 = 9.1 mg Au/L) (95% confidence interval 7.0 -11.8 mg/L for Au)
ASRIT [activated sludge taken from domestic sewage treatment plant, results based on nominal concentrations]	3-h EC50 = 27.9 mg TCA/L (95% confidence interval 15.0-33.3 mg/L) 3-h NOEC = 2 mg TCA/L

Gold is a poorly soluble metal. The standard approach for assessing poorly soluble metals is to compare the environmental reference value (ERV, acute or chronic), based on data for a soluble salt of the metal, with the amount of dissolution shown by the poorly soluble metal in a transformation / dissolution (T/D) test. In the 2012 ITS report it was proposed to follow this approach for gold metal by comparing the test results for tetrachloroauric acid to the amount of gold released during a T/D test with gold metal. However, it was acknowledged that using the results from a gold substance in a 3+ oxidation state, such as tetrachloroauric acid, was proposed as a pragmatic approach and that in reality it is not known which oxidation state (+1 or +3) any dissolved gold would be in. As the T/D test conducted with gold metal showed very low levels of dissolution (< limit of detection, 0.3 µg L^{-1}) it is unlikely to be possible to conduct speciation analysis on any dissolved gold and therefore to determine which oxidation state it is in. There was also uncertainty as to which oxidation state (+1 or +3) is more toxic and would therefore be considered as a worst case approach. Due to this uncertainty, wca developed a new proposal and recommended conducting an acute *Daphnia* study with a suitable gold substance in a +1 oxidation state (if one were available) and compare the results to those for tetrachloroauric acid. The results for the worst case oxidation state would then be compared against the amount of dissolved gold from the T/D test.

However, the gold working group did not agree to conduct further testing on a gold (+1) salt and instead preferred to complete the ecotoxicity endpoints for gold metal using waivers based on the lack of solubility for gold metal. It was therefore agreed that no further testing would be conducted and waivers will be drafted for ecotoxicity endpoints for gold metal.

All acute ecotoxicity studies are now complete for gold substances. No further testing is proposed at this point. However, based on the results of the first screen of the exposure assessment for tetrachloroauric acid further studies may be proposed at a later date (likely to be chronic studies) in order to refine the PNECs if this is required order to obtain risk characterisation ratios (RCRs) less than 1.

2.5 Toxicological endpoints

Gold and tetrachloroauric acid both require Annex VIII toxicological endpoints to be completed. In the 2012 ITS report it was proposed that for gold metal most endpoints could

be completed based on existing data or data waivers. A bio-elution study was commissioned in artificial gastric fluid in order to determine whether significant dissolution is likely, and therefore to determine whether studies via the oral route could be waived. It was agreed to conduct the testing at CIMM (Chilean Mining and Metallurgy Research Center), using gold powder as a worst case test substance.

A sample of gold powder was sent to CIMM in early 2012. The metal was subjected to a medium that represented gastric fluid for an exposure time of one hour with agitation. It was then incubated for a further hour without agitation, before aliquots were taken, filtered and bio-accessible metal quantified with ICP-MS. All incubations were at 37°C, at a pH of 1.5. The method followed the Eurometaux Standard Operating Procedure (10 November 2010). A full report of the study has been presented by CIMM titled 'Bio-elution of gold in *in vitro* surrogate of gastric juice', November 2012. BET (Brunauer, Emmett, Teller) surface area measurements (Fraunhofer March 2012) and particle size distribution (T/D report from ECTX, February 2012) are relevant for the evaluation of bio-elution data.

The results obtained at the CIMM laboratory showed no release of gold to the synthetic gastric medium above the method detection limit for the metal (1 µg/L). The results, together with the PSD and BET data, are presented in Table 2.4.

Table 2.4 Bio-elution data for gold powder

Substance	PSD (µm)	BET (m^2/g)	% metal release
Gold powder	d50: 83.88 d90: 308.06	0.042 – 0.051	Below method detection limit

The results of the bio-elution study confirm the inert behaviour of gold under simulated gastric fluid conditions, and together with the published data on lack of solubility in artificial sweat, indicate that no oral or dermal toxicity testing is required for registration of gold metal.

For tetrachloroauric acid, a number of endpoints can be completed using existing (published or proprietary) data, or data waivers. In the 2012 ITS report it was proposed that a literature search be conducted in order to determine if any suitable read across data are available that could be used to complete the repeat dose and reproductive toxicity endpoints. A literature search was conducted in March 2012 but no suitable read across data were identified. It was therefore agreed to conduct a combined repeat dose and reproductive toxicity study (OECD 422) by the oral route.

Genotoxicity testing was also proposed, and commissioned, using a tiered testing approach. The testing approach that was followed is summarised below.

1. Conduct a screening Ames test to assess toxicity to bacterial cells,

- If no unacceptable toxicity is observed, then conduct a full Ames test, including strain TA102.

- If unacceptable toxicity in the screening test is evident, omit the Ames test and proceed to a mammalian cell gene mutation assay (see below).

2. Conduct an *in vitro* micronucleus assay in human lymphocytes, assessing the impact of low pH and pH shift using the pH indicator in the assay,

   - If a pH shift is observed, then perform the assay with and without NaOH neutralization.

3. If the Ames test and micronucleus assay are negative, then perform an *in vitro* mammalian cell gene mutation eg HPRT assay in mouse lymphoma L5178Y cells. Again, the buffering capacity of the media should be evaluated using the pH indicator, and neutralization considered if necessary.

The Ames screen and full Ames test were conducted and were negative (Covance 2012a, Covance 2012b). However, the *in vitro* micronucleus assay was positive (Covance 2013). FISH analysis was conducted in order to determine whether the result was a clastogenic or aneugenic response. The outcome was that the result was a genuine clastogenic response. As such, an *in vivo* micronucleus assay was required for tetrachloroauric acid under REACh regulations (either Annex VIII or Annex IX). For Annex IX studies a testing proposal needs to be submitted to ECHA prior to conducting the test whereas such a testing proposal is not necessary for studies conducted as part of Annex VIII. In order to clarify this position advice was obtained via the UK REACh Helpdesk and it was agreed within the Working Group that the study should be conducted.

Previously, a discussion was held within the Working Group as to whether an *in vivo* micronucleus assay could be combined with the OECD 422 study, in order to save on animal usage. However, there is no standard protocol for the combined study and the Working Group agreed that the studies should be conducted separately (this decision is outlined in the paper 'TCA Genetox/Repeat dose program: Consensus viewpoint Umicore / Johnson Matthey toxicologists (reviewed 1 July 2013)').

Due to the extremely low pH of tetrachloroauric acid (<1) the form of test substance used in the toxicology studies needed to be considered, due to the potential corrosivity of the test item. The Gold Working Group agreed that a neutralised form of tetrachloroauric acid should be used in the studies, and that the solution should be prepared weekly in order to ensure stability of the test item.

The *in vivo* micronucleus assay has now been completed, and the result was negative. The final report for this study will soon be available. No further testing or classification is therefore required for tetrachloroauric acid. The OECD 422 study with tetrachloroauric acid at Covance Laboratories UK has currently completed the in-life phase and is in reporting and awaiting extended histopathological assessment of some tissues.

3 SUMMARY OF UPDATES SINCE 2012 ITS REPORT

The following updates have been agreed since the ITS report was finalised in 2012:

- Remaining physico-chemical tests have been conducted at Harlan Laboratories. All physico-chemical endpoints are now complete.
- Balsams, copaiba, mixed with turpentine, gold salts as having Annex III exemptions.
- It has been agreed to conduct a limited OECD 106 adsorption / desorption study with tetrachloroauric acid. Testing is ongoing at Fraunhofer.
- Remaining ecotoxicological testing has been completed at Brixham Environmental Laboratory. All ecotoxicological endpoints are now complete for tetrachloroauric acid. Further testing may be required for PNEC refinement following the first screen of the exposure assessment.
- It was agreed that no further ecotoxicity testing will be conducted in order to fill endpoints for gold metal. Instead, waivers based on lack of solubility will be included, based on the results from the T/D test.
- It was agreed that a bio-elution study in artificial gastric fluid would be conducted with gold metal, and this study has been competed at CIMM. Based on the lack of dissolution shown in this study, it was agreed that it is appropriate to waive further studies for gold metal with administration via the oral route.
- The genetoxicity test programme with tetrachloroauric acid has been conducted, including an *in vivo* micronucleaus assay conducted using neutralised tetrachloroauric acid, required following a positive result in the *in vitro* micronucleus assay. The *in vivo* micronucleus assay was negative, therefore no further testing or classification is required for this substance.
- It was agreed to conduct a combined repeat dose / reproductive screening (OECD 422) study using neutralised tetrachloroauric acid, and this test has been commissioned and is nearing completion at Covance Laboratories UK.

REFERENCES

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APPENDIX 1

This appendix is a review of the data currently available for these substances. It is accompanied by the data gap matrix for gold and gold substances (Gold_Phase III_Data gap matrix_update_18 12 2014).

Current registration intentions are as follows: gold and tetrachloroauric acid as 10-100 tpa substances, Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate as a 1-10 tpa substance and balsams, copaiba, sulfurised, mixed with turpentine, gold salts as a 1–10 tpa transported intermediate, not fulfilling the criteria for Strictly Controlled Conditions. Annex III exemptions are currently considered to apply to aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts.

A1.1 Physico-chemical properties

A1.1.1 Appearance/ physical state/ colour

Data are available from peer-reviewed handbooks indicating that gold is a soft, yellow metal (Lide 2008, O'Neil 2006). No additional testing is proposed.

Data are available from peer-reviewed handbooks indicating that tetrachloroauric acid exists when solid as yellow to reddish hygroscopic monoclinic crystals (Lide 2008, O'Neil 2006). No additional testing is proposed.

Proprietary data indicate that aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is a pale yellow powder (Harlan 2011b). No additional testing is proposed.

Proprietary data indicate that balsams, copaiba, sulfurised, mixed with turpentine, gold salts is a brown powder (Harlan 2012a).

A1.1.2 Melting point/ freezing point

Data are available from peer-reviewed handbooks indicating that the melting point of gold is 1064°C (Lide 2008, O'Neil 2006). No additional testing is proposed.

Proprietary data indicate that aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate decomposes from approximately 210°C (Harlan 2011b). No additional testing is proposed.

A melting point / freezing point test does not need to be conducted for tetrachloroauric acid as the substance is in aqueous solution. The freezing point of this substance will therefore be slightly less than that of the pure solvent.

Proprietary data indicate that balsams, copaiba, mixed with turpentine, gold salts, decomposes from 177°C, without melting (Harlan 2012c).

A1.1.3 Boiling point

Data are available from peer-reviewed handbooks indicating that the boiling point of gold is 2856°C (Lide 2008) or 2700°C (O'Neil 2006). No additional testing is proposed.

A boiling point test does not need to be conducted for tetrachloroauric acid as the substance is in aqueous solution. The boiling point of the solution will be slightly elevated above that of the pure solvent.

A boiling point test is not required for aurio (1+) 2,6,6-trinmethylbicyclo[3.1.1] heptanethiolate as, in accordance with column 2 of REACH Annex VII, the boiling point study does not need to be conducted as the substance decomposes before boiling.

A boiling point test is not required for balsams, copaiba, sulfurized, mixed with turpentine, gold salts as the substance decomposes before melting.

A1.1.4 Density

Data are available from peer-reviewed handbooks indicating that the density of gold is 19.3 g/cm^3 (Lide 2008) or the relative density is 19.3 (O'Neil 2006). No additional testing is proposed.

Tetrachloroauric acid is being registered as a solution and therefore a density test does not need to be conducted as the relative density will be higher than that of the solvent. As the substance is being registered as an aqueous solution this means that the relative density will be >1.

Proprietary data are available indicating that the relative density of aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is 2.01 at 21.0 +/- 0.5°C (Harlan 2012a).

Proprietary data are available indicating that the relative density of balsams, copaiba, sulfurised, mixed with turpentine, gold salts is. 2.37 at 21.0 +/- 0.5°C (Harlan 2012).

A1.1.5 Particle size distribution (granulometry)

Proprietary data indicates that the proportion of gold < 100 µm is 4.9% (Harlan 2011a). No additional testing is proposed.

Proprietary data indicate that the proportion of aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate <100 µm was 13.6% (Harlan 2011b). No additional testing is proposed.

The particle size distribution of tetrachloroauric acid does not need to be determined, in accordance with Column 2 of Annex VII, as the substances are marketed and used in a non-solid or granular form. No additional testing is proposed.

Proprietary data indicate that the proportion balsams, copaiba, sulfurised, mixed with turpentine, gold salts <100 µm was 41.3% (Harlan 2012c).

A1.1.6 Vapour pressure

A vapour pressure test is not required for gold in accordance with Column 2 of Annex VII, as the melting point is above 300°C. No additional testing is proposed.

A vapour pressure test does not need to be conducted for tetrachloroauric acid as it is in aqueous solution. As the substance is only available as a solution, the vapour pressure is essentially that of the solvent.

Proprietary data are available to indicate that the vapour pressure of aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is 4.4 x 10^{-14} Pa at 25°C (Harlan 2012b).

Proprietary data are available to indicate that the vapour pressure of balsams, copaiba, sulfurised, mixed with turpentine, gold salts is 4.1 x 10^{-8} Pa at 25°C (Harlan 2012d).

A1.1.7 Partition coefficient

The partition coefficient for gold and tetrachloroauric acid does not need to be determined in accordance with Column 2 of Annex VII, as the substances are inorganic. No additional testing is proposed.

In accordance with Column 2 of Annex VII, the partition coefficient does not need to be determined for inorganic substances. Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is an organometallic substance. The log Kow for the organic component of aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is estimated as 4.22 using KOWWIN (USEPA 2008). A partition coefficient study was commissioned at Harlan Laboratories, but it was not possible to determine a partition coefficient value due to the low solubility of the test item in both octanol and water (Harlan 2012a).

In accordance with Column 2 of Annex VII, the partition coefficient does not need to be determined for inorganic substances. Balsams, copaiba, sulfurised, mixed with turpentine, gold salts is an organometallic substance. The structure of the substance is not sufficiently well defined to allow the partition coefficient of the organic component to be calculated. A partition coefficient study was commissioned at Harlan Laboratories, but it was not possible to determine a partition coefficient value due to the low solubility of the test item in both octanol and water (Harlan 2012c).

A1.1.8 Water solubility

A transformation/dissolution test has been conducted on gold metal (ECTX 2011). The results indicate that dissolved gold was not detected at levels greater than the limit of detection of 0.3 μg l^{-1}. No additional testing is proposed.

Data are available from peer-reviewed handbooks to indicate that tetrachloroauric acid is very soluble in water (> 10,000 mg L^{-1}) (Lide 2008, O'Neil 2006). No additional testing is proposed.

Proprietary data indicate that the water solubility of aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is < 2.5 x 10^{-6} g L^{-1} (Harlan 2012a).

Proprietary data indicate that the water solubility of balsams, copaiba, sulfurised, mixed with turpentine, gold salts is <1.9 x 10^{-5} g L^{-1} based on monitoring gold. The mean dissolved

Total Organic Carbon concentration of the sample solutions was 5.20 x 10^{-3} g L^{-1} (Harlan 2012c).

A1.1.9 Surface tension

The surface tension study does not need to be conducted for gold, tetrachloroauric acid, aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate or balsams, copaiba, sulfurised, mixed with turpentine, gold salts in accordance with Column 2 of Annex VII, as surface activity is not expected or predicted and surface activity is not a desired property of the materials. No additional testing is proposed.

A1.1.10 Flash point

The flash point study does not need to be conducted for gold or tetrachloroauric acid in accordance with Column 2 of Annex VII, as the substances are inorganic. No additional testing is proposed.

The flash point study does not need to be conducted for aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate or balsams, copaiba, sulfurised, mixed with turpentine, gold salts, in accordance with ECHA (2008) Guidance on information requirements and chemical safety assessment, chapter R7a: endpoint specific guidance, as these substances are solid at room temperature. No additional testing is proposed.

A1.1.11 Autoflammability

Proprietary data for gold showed no signs of self-ignition or self-heating throughout the test (Harlan 2011a). No additional testing is proposed.

A self-ignition study does not need to be conducted for tetrachloroauric acid based on expert judgement, as the substance is in an aqueous solution. No additional testing is proposed.

Proprietary data indicate that aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate showed no signs of self-ignition or self-heating throughout the test (Harlan 2011b). No additional testing is proposed.

A self-ignition study does not need to be conducted for balsams, copaiba, sulfurised, mixed with turpentine, gold salts as the substance is never transported in large enough volumes for this endpoint to be appropriate.

A1.1.12 Flammability

Proprietary data indicates that gold is not classified as a readily combustible solid under Division 4.1 (Harlan 2011a). No additional testing is proposed.

Flammability does not need to be assessed for tetrachloroauric acid based on expert judgement, as the substance is in an aqueous solution. No additional testing is proposed.

Proprietary data indicate that aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate is a readily combustible solid under Division 4.1 (Harlan 2011b). No additional testing is proposed.

Proprietary data indicate that balsams, copaiba, sulfurised, mixed with turpentine, gold salts is a Category 1 flammable solid (Harlan 2012d).

A1.1.13 Explosive properties

The explosive properties study does not need to be conducted for gold, tetrachloroauric acid, aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate or balsams, copaiba, sulfurised, mixed with turpentine, gold salts, in accordance with Column 2 of Annex VII, as there are no chemical groups associated with explosive properties present in the molecules. No additional testing is proposed.

A1.1.14 Oxidising properties

The oxidising properties study does not need to be conducted for gold, aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate or balsams, copaiba, sulfurised, mixed with turpentine, gold salts, in accordance with Column 2 of Annex VII, as the substances are incapable of reacting exothermically with combustible materials on the basis of their chemical structures.

Proprietary data indicate that tetrachloroauric acid does not possess oxidising properties based on the results of a preliminary test clearly showing that the test substance does not have oxidising properties as no "vigorous" burning was observed (Johnson Matthey 2010). No additional testing is proposed.

A1.2 Environmental Fate

A1.2.1 Hydrolysis

The hydrolysis study does not need to be conducted for gold or tetrachloroauric acid, in accordance with section 1 of REACH Annex XI, as these substances are not expected to undergo hydrolysis in the environment due to a lack of hydrolysable functional groups. Testing does not therefore appear necessary. No additional testing is proposed.

This study is not required for the other gold substances as they require an Annex VII dataset only.

A1.2.2 Biodegradation

The ready biodegradability study does not need to be conducted for gold or tetrachloroauric acid, in accordance with Column 2 of Annex VII, as these substances are inorganic. No additional testing is proposed.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and Balsams. copaiba, sulfurised, mixed with turpentine, gold salts are confirmed as having Annex III exemptions. Only available environmental fate data is therefore required for these substances.

A1.2.3 Adsorption / desorption

No data are available for gold or tetrachloroauric acid for this endpoint. A modified OECD 106 adsorption / desorption study is ongoing at Fraunhofer and the results will be used to complete this endpoint. The results will also be read across to provide information on the adsorption potential of gold metal.

This study is not required for the other gold substances as they require an Annex VII dataset only.

A1.3 Ecotoxicology

A1.3.1 Short-term toxicity to fish

One published study is available for tetrachloroauric acid (Buhl and Hamilton 1991) to fulfil this endpoint. Data available from this study have been assigned a Klimisch score of 2 and report the short-term toxicity of tetrachloroauric acid to arctic grayling, coho salmon and rainbow trout. The 96-hour LC50s from this study range from 9.1 to 33.5 mg Au L^{-1} (nominal concentrations).

No data are currently available for gold to fulfil this endpoint. The standard approach for assessing the toxicity of a poorly soluble metal would be to compare the amount of dissolution determined in a TD test with the ecotoxicity reference values (either EC50 values from acute tests or NOEC (or EC10) values from chronic tests) for a soluble salt of the metal in order to indicate whether or not toxicity is possible as a result of the dissolved levels of gold. However, the quantities of gold which are released into solution from gold metal during a TD test are diminishingly small (<0.3 $\mu g\ l^{-1}$) and it is unlikely to be possible to determine the speciation of any dissolved metal. It was therefore proposed and agreed by the Gold Working group that ecotoxicity endpoints for gold metal will be waived, based on the lack of solubility.

This study is not required for the other gold substances as they require an Annex VII dataset only.

A1.3.2 Short-term toxicity to aquatic invertebrates

No existing data were available for tetrachloroauric acid to fulfil this endpoint. An OECD 202 *Daphnia* sp. acute immobilisation test was commissioned at Brixham Environmental Laboratory and determined an EC50 of 4.8 mg L^{-1} (Brixham Environmental Laboratory 2012a).

No data are currently available for gold to fulfil this endpoint. The standard approach for assessing the toxicity of a poorly soluble metal would be to compare the amount of dissolution determined in a TD test with the ecotoxicity reference values (either EC50 values

from acute tests or NOEC (or EC10) values from chronic tests) for a soluble salt of the metal in order to indicate whether or not toxicity is possible as a result of the dissolved levels of gold. However, the quantities of gold which are released into solution from gold metal during a TD test are diminishingly small (<0.3 μg l^{-1}) and it is unlikely to be possible to determine the speciation of any dissolved metal. It was therefore proposed and agreed by the Gold Working group that ecotoxicity endpoints for gold metal will be waived, based on the lack of solubility.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and Balsams. copaiba, sulfurised, mixed with turpentine, gold salts are confirmed as having Annex III exemptions. Only available environmental fate data is therefore required for these substances.

A1.3.3 Toxicity to aquatic algae and cyanobacteria

No existing data were available for tetrachloroauric acid to fulfil this endpoint. An OECD 201 Algae and cyanobacteria growth inhibition test was commissioned at Brixham Environmental Laboratory and determined an EC50 of >9.0 mg L^{-1} and a NOEC of 0.9 mg L^{-1}, based on growth rate (Brixham Environmental Laboratory 2012b).

No data are currently available for gold to fulfil this endpoint. The standard approach for assessing the toxicity of a poorly soluble metal would be to compare the amount of dissolution determined in a TD test with the ecotoxicity reference values (either EC50 values from acute tests or NOEC (or EC10) values from chronic tests) for a soluble salt of the metal in order to indicate whether or not toxicity is possible as a result of the dissolved levels of gold. However, the quantities of gold which are released into solution from gold metal during a TD test are diminishingly small (<0.3 μg l^{-1}) and it is unlikely to be possible to determine the speciation of any dissolved metal. It was therefore proposed and agreed by the Gold Working group that ecotoxicity endpoints for gold metal will be waived, based on the lack of solubility.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and Balsams. copaiba, sulfurised, mixed with turpentine, gold salts are confirmed as having Annex III exemptions. Only available environmental fate data is therefore required for these substances.

A1.3.4 Toxicity to microorganisms

No existing data were available for tetrachloroauric acid to fulfil this endpoint. An OECD 209 Activated sludge respiration inhibition test was commissioned at Brixham Environmental Laboratory and determined an EC50 of 27.9 mg L^{-1} and a NOEC of 2 mg L^{-1} (Brixham Environmental Laboratory 2012c).

No data are currently available for gold to fulfil this endpoint. The standard approach for assessing the toxicity of a poorly soluble metal would be to compare the amount of dissolution determined in a TD test with the ecotoxicity reference values (either EC50 values from acute tests or NOEC (or EC10) values from chronic tests) for a soluble salt of the metal in order to indicate whether or not toxicity is possible as a result of the dissolved levels of gold. However, the quantities of gold which are released into solution from gold metal

during a TD test are diminishingly small (<0.3 μg l^{-1}) and it is unlikely to be possible to determine the speciation of any dissolved metal. It was therefore proposed and agreed by the Gold Working group that ecotoxicity endpoints for gold metal will be waived, based on the lack of solubility.

This study is not required for the other gold substances as they require an Annex VII dataset only.

A1.4 Toxicology

An Annex VII/VIII dataset is required for gold and tetrachloroauric acid. The other gold substances are currently believed to have Annex III exemptions and therefore only require available data. Background information for the approach taken for the mammalian toxicology endpoints for gold is presented below. Sections 1.4.1 to 1.4.10 detail the available information for each endpoint for the gold substances and the specific approach proposed for filling any data gaps.

The default approach for assessing the hazards of metals in the zero valency state is to evaluate the hazards of salt(s) of the metal in question and then read across the information to the metal itself. The specific classification of the metal will then depend on its bioaccessibility to the appropriate environmental and body compartments when compared to the bioavailability of the tested salt.

Gold substances are used therapeutically in the treatment of rheumatoid arthritis and other rheumatic diseases. There is also interest in using gold(I) and gold(III) complexes in cancer chemotherapy. Administration is either by the oral or parenteral routes, and is designed to permit appropriate blood levels of ionic soluble gold to be achieved by the use of bioavailable forms, i.e. deliberate introduction of gold into the bloodstream and therapeutic target organs. Under these conditions, with deliberate introduction into the body, ionic gold in the relevant valency state is known to have specific toxicity potential particularly on the immune system. Other soluble gold salts such as gold chloride for example, are known to be hepato- and nephro- toxic.

However, zero valent gold is considered inert and hence there will be a lack of transformation and dissolution into soluble forms within the mammalian body. Thus the toxicities observed with ionic gold would not be apparent with gold metal since it would not be expected to enter any body compartment and would be excreted unchanged following oral ingestion.

Gold has been extensively used in human contact situations for millennia in the form of jewellery articles, and in dental restoration (as an alloy). It is also used in food as a decoration, in alcoholic drinks, and is an approved food additive in the EU (E175). This acceptance of metallic gold as a food additive is due to its chemical inertness and lack of transformation into soluble salts within the mammalian body.

Two forms of metallic gold are produced by PMC members, massive and powder. Particle size screening of the powder by Harlan as part of the CLP notification (Harlan 2011a)

indicated that only 4.9% was below 100 um. Hence inhalation is not considered a relevant route of exposure, and the two physical forms of the metal will be considered together.

A1.4.1 Skin irritation

No proprietary studies were identified on gold metal itself. The guinea pig Buehler study (Arcelin 1995) for sensitisation referred to below showed no evidence of dermal irritation when the Galvanogold was placed on the skin for 24h, three times per week during the induction period (or during the challenge procedure).

There is clear evidence from the history of use in jewellery, etc. as indicated previously that gold metal does not cause skin irritation that would require classification under EU legislation. No further testing is recommended for this endpoint and a weight of evidence argument will be used.

Proprietary data are available to indicate that tetrachloroauric acid is corrosive (Berthold 1993). No additional testing is proposed.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.2 Eye irritation

For gold metal, the HSDB database cites Grant, Toxicology of the Eye. 2nd ed. Springfield, Illinois: Charles C. Thomas, 1974, p. 530. Metallic gold dust has been tested in rabbit eyes. The metal persisted for many months and caused invasion by leucocytes, vascularisation, and accumulation of giant cells. Particles too big to be ingested by leucocytes become encapsulated, whereas fine dust is removed.

No additional testing is recommended for this endpoint. Gold (massive or dust) would not be considered a specific eye irritant, although traumatic damage by fine dust particles could be expected.

The eye irritation study does not need to be conducted for tetrachloroauric acid, in accordance with Column 2 of Annex VII, as the substance meets the classification criteria for corrosive to the skin.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.3 Skin sensitization

Two relevant proprietary reports were received by wca on this endpoint for gold. The first (Arcelin 1995) was a modified Buehler method using AGC Galvanogold, electroformed material for dental restorations. The reliability was assessed as Klimisch 1. The test material was identified as 99.9% pure. No sensitisation response was seen.

The second study (Albrecht 1999. Klimisch 1) was a GPMT using a gold dental alloy with a purity of 98.2%. An extract from the material was made using the ISO 10993-12 procedure, with isotonic saline. No indication of gold concentration in the extract is identified, but it is presented as an extract volume per unit area of metal. No evidence of sensitisation was seen under the conditions of the test.

Although there are published reports of possible sensitisation to 'gold' in humans, this is generally considered to be related to other metals with known sensitising potential, such as nickel, that may be alloyed with the gold to produce types of jewellery. No transformation/dissolution, would be expected by dermal contact with gold. Previous T/D testing of gold (ECTX 2011) has shown that any dissolution is below the limit of detection, although this was not conducted in a biologically relevant medium e.g. artificial skin sweat (see bio-elution testing below). No additional testing is recommended for this endpoint.

The skin sensitisation study does not need to be conducted for tetrachloroauric acid, in accordance with Column 2 of Annex VII, as the substance meets the classification criteria for corrosive to the skin.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.4 Genotoxicity

A1.4.4.1 *In vitro* mutagenicity study in bacteria

A proprietary report (Klimisch 1) was received by wca on this endpoint for gold (Donath and Kraft 2008). This was an Ames test performed in accordance with OECD TG471. The test substance was similar to that used in the sensitisation test above except that the gold content was 84.8%. It was tested as an extract in isotonic saline according to ISO 10993-3 and 10993-12, with the 'concentration' presented as an extract volume per unit area of metal. No increases in bacterial revertants were seen either in the presence or absence of S9.

An Ames screen and full Ames test were conducted with tetrcchloroauric acid at Covance (Covance 2012). Both were negative. Published data are available for tetrachloroauric acid for a "rec assay" with the bacterium *Bacillus subtilis* (Kanematsu et al. 1980). Tetrachloroauric acid produced a negative result. This can only be used as a supporting study.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.4.2 *In vitro* cytogenicity in mammalian cells

No data are available for gold to fulfil this endpoint, and hence this is a data gap. In order to evaluate the potential clastogenicity of gold, a sample could be subjected to the same or

similar extraction procedure as used in the Ames test and GPMT, as indicated above. However, this study is considerably more expensive than the Ames test and, therefore, its toxicological relevance was first established by assessing the dissolution of gold in the bio-elution studies. Since there was no release of gold metal at the limit of detection (1 µg l^{-1}) in bio-elution tests with artificial gastric fluid (CIMM 2012) this endpoint will be waived for gold metal.

An *in vitro* micronucleus study using cultured human peripheral blood lymphocytes was conducted with tetrachloroauric acid and a positive response was observed (Covance 2013). As a result of this response, fluorescence in situ hybridisation (FISH) analysis was conducted in order to determine whether the result was a clastogenic or aneugenic response. The outcome was that the result demonstrated that micronuclei were generated via a predominantly clastogenic (chromosome breakage) mechanism. As such, an *in vivo* micronucleus assay was conducted with tetrachloroauric acid (Section A1.4.4.4)

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.4.3 *In vitro* gene mutation study in mammalian cells

This study is required under Annex VIII if negative results are obtained in the Ames test and the *in vitro* cytogenicity assay.

There are no data available for this endpoint for gold. In order to evaluate the gene mutation potential of gold, a sample could be subjected to the same or similar extraction procedure as used in the Ames test and GPMT, as indicated above. However, this study is considerably more expensive than the Ames test and its toxicological relevance should be established by assessing the dissolution of gold in the bio-elution studies prior to considering the necessity of further mutagenicity testing. No release of gold metal at the limit of detection (1 µg L^{-1}) in bio-elution tests with artificial gastric fluid (CIMM 2012) and therefore this endpoint will be waived for gold metal.

As tetrachloroauric acid showed a positive response in the *in vitro* cytogenicity assay, this study is not conducted for this substance. Instead, an *in vivo* micronucleus assay is conducted.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.4.4 *In vivo* micronucleus assay

An *in vivo* micronucleus assay was conducted for tetrachloroauric acid and the final report for this study will be available soon. A negative result was obtained. No further testing or classification is therefore required for this substance.

This study is not required for any of the other gold substances.

A1.4.5 Acute toxicity, oral route

No proprietary data are available for gold for this endpoint. The HSDB database cites the lack of acute oral toxicity to gold metal dust (Luckey, T.D. and B. Venugopal. Metal Toxicity in Mammals, 1. New York: Plenum Press, 1977, p. 167). This is a peer reviewed database, and hence has been given a Klimisch 2 score.

Metallic gold is considered inert and not bioavailable, which is supported by results from a bio-elution test with gold powder in artificial gastric fluid which showed no metal release above the limit of detection (1 µg l^{-1}) (CIMM 2012). Hence it would not be considered appropriate to repeat this investigation to current OECD and GLP standards.

The acute toxicity (oral) study does not need to be conducted for tetrachloroauric acid, in accordance with Column 2 of Annex VII, as the substance meets the classification criteria for corrosive to the skin. One proprietary study is available for this endpoint for tetrachloroauric acid (Berthold 1993), which was given a Klimisch score of 2. In this study there was not ≥50% mortality in either of two groups of rats administered by gavage with either 215 or 464 mg kg^{-1} bw over 14 days. Therefore the LD50 from this study was determined to be >464 mg kg^{-1} bw.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.6 Acute toxicity, inhalation

The gold powder was subject to particle size screening by Harlan (Harlan 2011a). Less than 10% of the substance passed through a 100 um sieve, and hence a full particle size distribution assessment is not required since the powder is unlikely to be significantly inhalable. This route of administration can therefore be waived since the material is 'granular'.

The acute toxicity (inhalation) study does not need to be conducted for tetrachloroauric acid, in accordance with Column 2 of Annex VII, as the substance meets the classification criteria for corrosive to the skin. Additionally there is no evidence that exposure to an aerosol of tetrachloroauric acid solution occurs.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.7 Acute toxicity, dermal

Since there is a long history of the use of gold metal in jewellery, etc., with extensive skin contact, it is not therefore considered relevant to subject metallic gold to acute dermal toxicity testing. This is supported by the lack of dissolution observed in artificial sweat (see Section A1.4.10).

The acute toxicity (dermal) study does not need to be conducted for tetrachloroauric acid, in accordance with Column 2 of Annex VII, as the substance meets the classification criteria for corrosive to the skin.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.8 Short term repeated dose toxicity study (28 days)

The HSDB reference (Luckey, T.D. and B. Venugopal. Metal Toxicity in Mammals, 1. New York: Plenum Press, 1977., p. 167) mentions that chronic exposure to powdered gold in rats is not oncogenic. However, subcutaneous implantations of gold sheet or injections of gold can induce sarcomas. The latter effect is possibly related to a local irritation response rather than a direct carcinogenic effect of gold metal. On the basis that gold is considered an inert material by oral administration, and therefore not bioavailable (see section 1.4.12), it is recommended that the 28-day repeated dose study is waived as being scientifically unjustified.

No existing data were available for tetrachloroauric acid and therefore testing has been agreed to complete this endpoint and is underway at Covance. A combined repeat dose and reproductive screening study (OECD 422) is being conducted.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.9 Screening for reproductive/ developmental toxicity

There are no studies published or available on this endpoint for gold. However, in view of the lack of bioavailability of gold by the oral route (see Bio-elution testing, A1.4.10), it is proposed that this study should be waived as being scientifically unjustified.

No existing data were available for tetrachloroauric acid and therefore testing has been agreed to complete this endpoint and is underway at Covance. A combined repeat dose and reproductive screening study (OECD 422) is being conducted.

Aurio (1+) 2,6,6-trimethylbicyclo[3.1.1] heptanethiolate and balsams, copaiba, sulfurised, mixed with turpentine, gold salts are subject to Annex III exemptions and therefore require available toxicological data only.

A1.4.10 Bio-elution testing

The T/D testing of gold (ECTX 2011) showed that the level of dissolution was below the level of detection.

Liden et al (1998) investigated the release of gold metal from 13 different gold-containing jewellery materials stored for several weeks in artificial sweat. No release of gold was

detected using ICP and atomic absorption. This published data supports the lack of gold dermal bioaccessibility and no further tests are proposed.

The chemically inert properties of gold metal and consequent lack of bioavailability via oral administration are frequently referred to in the published literature. In order to provide supporting data for waiving som,e endpoints for gold metal, bio-elution testing was conducted at CIMM for gold powder, in artificial gastric fluid (CIMM 2012). This study no metal release above the limit of detection (1 µg L^{-1}) and supports the lack of bioavailability of gold metal.

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