PHASE III: PROPOSAL FOR CHOICE OF CONTRACT RESEARCH ORGANISATIONS FOR THE RHENIUM TEST PROGRAMME

DRAFT REPORT TO THE PRECIOUS METALS AND RHENIUM CONSORTIUM FROM: WCA ENVIRONMENT LIMITED

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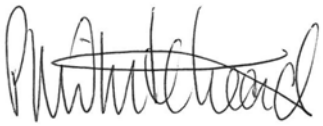
Document Approved by	Paul Whitehead		13 January 2011
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EXECUTIVE SUMMARY

The PMC commissioned wca environment limited to design an Integrated Testing Strategy (ITS) involving the recommendation of any enabling tests, test waiving based on category read-across and weight of evidence arguments, and empirical testing, for the Rhenium group substances. A report was prepared and sent to the PMRC on 25 November 2010.

No further testing is recommended for physicochemical, environmental fate or ecotoxicological endpoints. The following mammalian toxicological tests are recommended for rhenium metal and ammonium perrhenate:

Rhenium metal (CAS 7440-15-5)

- Enabling tests – solubility in low pH (HCl), and/or artificial skin surface liquid (dependent on whether the criteria in REACH Annex III are met). These tests are not considered further in this report and will be discussed separately as required.

Ammonium perrhenate (CAS 13768-11-1)

- *In vitro* test for severe irritation/corrosion (or BCOP - OECD TG437; or ICE - OECD TG438)
- *In vivo* eye irritation (OECD TG405) (if the *in vitro* test indicates that it is not a severe irritant/corrosive)
- Skin sensitization (LLNA - OECD TG429)
- *In vitro* gene mutation in mammalian cells (mouse lymphoma assay - OECD TG476)
- *In vitro* cytogenicity in mammalian cells (micronucleus test - OECD TG487)
- (*In vivo* genotoxicity would need to be considered in the light of a positive result in either of the preceding studies)
- Combined repeat dose oral toxicity study with the reproduction/developmental toxicity screening test (OECD TG422)

No additional testing is recommended for the other substances in the rhenium group substances.

Four GLP laboratories have provided information as to their capabilities, pricing, timing and comments which is presented in this report.

This report provides a recommendation for the choice of laboratories for discussion by the Rhenium Working Group:

Laboratory	Study Allocated	Cost (£)
Harlan Laboratories	a) In vitro eye irritation/corrosion	1,000
	b) In vivo eye irritation (if required on the basis of the in vitro study	950
	c) Sensitisation (LLNA)	2,240
Covance Laboratories	a) In vitro mouse lymphoma assay	14,500
	b) In vitro micronucleus assay	13,900
Charles River Laboratories	a) Dose range finding study	9,000
	b) Combined 28-day repeated dose oral toxicity and reproductive toxicity screen	52,000
	c) Analytical support: • Method validation	8,900-12,500
	• Dose formulation analysis (2 occasions)	3,000
		Total 105,490 – 109,090

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TABLES

1 INTRODUCTION

The Precious Metals and Rhenium Consortium (PMRC) commissioned wca environment to provide consultancy services for the Rhenium REACH Registration project. Phase III of this project consists of the Design of an Integrated Testing Strategy (ITS) involving the recommendation of any enabling tests, test waiving based on category read-across and weight of evidence arguments, and empirical testing.

The approach agreed upon for completion of Phase III for all the categories consisted of the following tasks and considerations:

- a detailed assessment of the potential for test derogation and read-across from one substance to another (main basis for test derogation being the potential for scientifically-based grouping);
- *in vitro* approaches considered as a way of avoiding the need for *in vivo* data generation;
- the design of the test programme to be *de minimis* and draw extensively on arguments for test derogations presented in Phase II and with further refinement and justifications;
- recommendations of the more cost-effective *in vitro* or limit tests where appropriate;
- **at least three GLP-compliant contract research organisations (CRO) to be contacted and compared (on the basis of experience, cost, security, etc);**
- a comprehensive study monitoring programme to be initiated, and
- an Annex III assessment to be conducted in order to minimise testing requirements.

This report presents a more detailed review of the competencies and costs of the selected CRO's, relating to the highlighted action above, together with wca's recommendation for assigning specific studies.

2 METHODS

During Phase I we identified three categories for rhenium (Table 2.1). These were based on oxidation state, potential contribution from the counter ions and potential solubility patterns.

Table 2.1 Rhenium categories

Category	Substance name	CAS number	Type	Tonnage
Re 0	Rhenium	7440-15-5	Substance	1-10
Re 7	Perrhenic acid	13768-11-1	Substance	1-10
	Sodium rhenate	13472-33-8	Intermediate	1-10
	Dirhenium heptasulphide	12038-67-4	Intermediate	1-10
	Potassium perrhenate	10466-65-6	Intermediate	1-10
Re 7a	Ammonium perrhenate	13598-65-7	Substance	10-100

Four UK laboratories that are known to be reputable and competent were contacted and asked to provide a quote for the following tests for ammonium perrhenate.

- *In vitro* test for severe irritation/corrosion (or BCOP - OECD TG437; or ICE - OECD TG438)
- *In vivo* eye irritation (OECD TG405) (if the *in vitro* test indicates that it is not a severe irritant/corrosive)
- Skin sensitization (LLNA - OECD TG429)
- *In vitro* gene mutation in mammalian cells (mouse lymphoma assay - OECD TG476)
- *In vitro* cytogenicity in mammalian cells (micronucleus test - OECD TG487)
- (*In vivo* genotoxicity would need to be considered in the light of a positive result in either of the preceding studies)
- Combined repeat dose oral toxicity study with the reproduction/developmental toxicity screening test (OECD TG422)

A copy of the request that was sent has been presented in Appendix 1.

The four laboratories were:

Harlan Laboratories, Shardlow, Derbyshire
Charles River Laboratories, Tranent, Edinburgh
Covance Laboratories, Harrogate, Yorkshire
Huntingdon Life Sciences (HLS), Huntingdon, Huntingdonshire

3 RESULTS

Tables 3.1 and 3.2 summarise both the preliminary responses from the CRO's, and additional information subsequently requested, and permits comparison between the laboratories. As would be expected, the prices for individual studies vary. Considered as a total package of studies, the range is ca. £103K - £150K. This excludes the cost of analytical support for the repeat dose studies but these additional figures are presented in the tables.

Each laboratory is fully GLP compliant and has been in operation for many decades. Similarly a significant number of studies will have been successfully submitted to international regulatory bodies over this period. Thus from a quality perspective, it would be expected that each one would fulfil the necessary requirements.

Experience with testing inorganic substances including metals varies between them, with both Harlan and HLS claiming extensive background. Charles River and Covance indicated limited experience in this area.

There are a number of criteria regarding the choice of appropriate test laboratory. These include cost per study/package, technical expertise of the staff, reputation, communication processes, and PMRC member preferences. Some facilities will be better than others for certain types of study, although each one would be capable of conducting the full package necessary for ammonium perrhenate.

There is a variation in the quantity of test material suggested by the laboratories. Due to the limited amount of relevant information currently available (vis. acute oral toxicity) it is very difficult to estimate the volume required for the 28-day oral toxicity/reproductive screen. Further discussions with the laboratory of choice will be required. A number of the studies require only fixed amounts of test material and are generally those studies that do not involve repeated dosing procedures.

It is clear that there is still a level of uncertainty over the applicability of the LLNA for skin sensitisation for metal substances, but in a recent review of the LLNA protocol, ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) in the USA indicated that, although experience with metals and their salts is limited, there is no reason at this stage why the method is contraindicated.

The laboratories agreed with the oral route of administration on the basis of the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the ammonium perrhenate. Charles River provided costs for both oral and inhalation (including tracheal instillation) routes, which indicates very clearly the additional costs for using routes other than oral (£58K versus £140K for oral and inhalation, respectively).

Table 3.1 Comparison of laboratories

Study type	Lab 1			Lab 2			Lab 3			Lab 4		
	Harlan Laboratories			Charles River Laboratories			Covance Laboratories			Huntingdon Life Sciences		
	Costing	Sample required	Notes	Costing	Sample required	Notes	Costing	Sample required	Notes	Costing	Sample required	Notes
in vitro eye corrosion (e.g. OECD TG 437)	£1,000	5 g / 5 ml		£4,500	1 g/1 ml		£1,100 - £2,300	2-3 g	Both studies performed as one	£2,750	2 g	
in vivo eye irritation (OECD TG 405)	£950	5 g / 5 ml		£4,500	2 ml					£2,305	10 g	
skin sensitisation (LLNA OECD TG 429)	£2,240	25 g / 25 ml	Note 1a	£5,500	10 g / 10 ml		£2,630	20 g		£6,500	2 g	
mouse lymphoma assay (OECD TG 476)	£8,910 - £16,060	10 g / 10 ml	Note 1b	£12,000	5 g		£14,500	7 g		£11,000	5 g	
in vitro micronucleus assay (e.g. OCED TG 487)	£8,070 - £15,150			£15,550	70 mg	Note 2a	£13,900	5 g		£11,040	25 g	
Range finding study	£9,470			£9,000	ca. 150 g		£19,990	150 g				
28-day repeat dose toxicity study in rats	£35,770		Analytical support: £3,910. Note 1c	Inhalation: £140,000 Intratracheal Instillation: £123,000 Oral: £38,000		Note 2b	£41,356	260 g		£61,650	500 g	
Reproductive screening study in rats	£49,300	2 kg	Analytical support: £3,910	£35,000		Analytical support as for oral	£67,000	1,155 g		£63,900	500 g	

Study type	Lab 1			Lab 2			Lab 3			Lab 4		
	Harlan Laboratories			Charles River Laboratories			Covance Laboratories			Huntingdon Life Sciences		
	Costing	Sample required	Notes	Costing	Sample required	Notes	Costing	Sample required	Notes	Costing	Sample required	Notes
Combined Twenty-Eight Day Repeated Dose Oral (Gavage) with Reproduction/ Developmental Toxicity Screening Test	£78,910	2,500 g / 2,500 mL	Analytical support: £3,910	£52,000	ca. 700 g	note 2c	£98,000	1.115Kg	Note 3a	£105,250	1,000 g	

Extra Notes

Re: Harlan Laboratories

Note 1a: Pooled Method. If required for notification in USA, individual method should be performed. A concurrent positive control group may also be required (£660). Ear thickness (£400) and/or ear weight (£660) measurements may be added.

Note 1b: Lower price if 1st experiment clearly +ve.

Note 1c: Additional charges for histopathological examination of lesions and tissues from low/intermediate groups.

Depending on the nature of the test material, and in particular, information with regard to its likely corrosivity/irritancy potential, it may be necessary to conduct in vitro/ex vivo screening tests in order to avoid testing of corrosive/severe irritants in animals. Harlan can advise on selection of appropriate screening tests, Prices of which range from £950 - £2,510 for skin irritation and £650 - £1,340 for eye irritation.
Method implementation and development charge £1,020 - £4,090.

Re: Charles River Laboratories

Note 2a: Dose formulation analysis (if required) £4,320.

Note 2b: For inhalation study: Method Validation [HPLC/GC/UV]: £12,500 - £20,000, Method Validation [LC/MS/MS] £15,000 - £24,000.For oral gavage, Method Validation £8,900 - £12,500. Dose formulation analysis-per occasion £1,150 - £1,660.

Note 2c: Method Validation: £8,900 - £12,500; Dose Formulation analysis (2 occasions): £3,000.

All prices are exclusive of VAT. Method validation (Dec/Jan) requires ca. 5g of test item. Start dates of tests Jan/Feb 2011 - Early Q2 2011.

Re: Covance Laboratories

Note 3a: Formulation analysis: (GC)	
Method development:	£1,100
Method validation:	£2,190
Stability (3 time points):	£2,540
Homogeneity (2 levels):	£1,375
Achieved concentration (1 occasion, 4 levels):	£995

Note: The text in red in the table refers to information obtained subsequently to the initial enquiry and is additional to the data in the Phase III ITS report.

Table 3.2 Laboratories' responses to queries

Query	Lab 1	Lab 2	Lab 3	Lab 4
	Harlan Laboratories	**Charles River Laboratories**	**Covance Laboratories**	**Huntingdon Life Sciences**
Prior experience with inorganic metal salts	Much experience (inc. catalyst industry)	*"Our chemistry department work with 1-2 per year"*	*"We have limited experience with this kind of compound but we have lots of experience with all the study types you require for your project and are confident we will be able to perform the studies."*	*"We have a lot of experience with inorganic materials and have conducted studies to meet the regulatory requirements of authorities throughout the world and have completed over 1000 chemicals notifications."*
Total costings (and discounts)	£124,550 - £141,850 (total in report)	No total given in quotation, hand-calculated total of approximately £128,000 - £141,000 (depending on options) if 28-day tox. and repro combined.	No total given in quotation, hand-calculated total of approximately £160,000 - £162,000.	No total given in quotation, hand-calculated total of £138,845 if Repro and repeated dose combined
Relevance of LLNA for metal salts	*"The predictive capacity of the LLNA for metal salts has to be evaluated case by case, but this is the same with other (e.g. guinea pig) models. The Manager of Acute and Alternative Toxicology would need to study published literature in a bit more depth to provide a more thorough response."*	*"we do not have any experience of performing a LLNA with metal salts and we feel we are not able to provide a sufficient answer to this query"*	*"After speaking with our short-term toxicity experts I can confirm this study is valid for the metal salt."*	*"The OECD guideline indicates that the LLNA is known to give false negative results with some metals, it does not however mention metal salts. The current REACH guidance expects that the LLNA will be used for sensitization testing and we are confident that it will be acceptable in this situation."*
Volume of substance required	2.55 kg if 28d tox. and repro combined: 3.15 kg if separate	ca. 865 g if 28d tox. and repro combined	1.6 kg	1,044 g
Availability of Lab space	Max turnaround time (receipt of test item to draft report) for OECD 476, 487, 437, 405 & 429 is 12 weeks. May rise by 2 to 3 weeks after end Jan 2011	Start dates of up to early Q2 quoted. No turnaround time specified	Tests can be accommodated in Q1 2011	*"Laboratory space in 2011 is currently not a problem for any studies starting from mid February 2011 onwards although we would recommend booking as early as possible if you have submission deadlines in mind."*

Generic test plans for each test have also been sent by the laboratories.

Table 3.2 Laboratories' responses to queries, contd.

Query	Lab 1	Lab 2	Lab 3	Lab 4
	Harlan Laboratories	**Charles River Laboratories**	**Covance Laboratories**	**Huntingdon Life Sciences**
The appropriate route(s) of administration for the 28-day and repro screen	*"The mean particle size above 100 micron plus the fact that percentage distribution does suggest that there will be only a small proportion that would be respirable suggests that it would not be expected that the inhalation route would be requested for these types of studies* *We expect that oral gavage would be acceptable."*	*We can confirm that the choice of the oral dose route is more suitable for the 28-day study given the MMAD and GSD of the Rhenium metal salt."*	*"I have also discussed the repeat dose studies with our inhalation expert and the inhalation route would not be suitable, the route of administration would be the oral route."*	*"Based on the information that you have provided we believe that an inhalation study is not required and that oral gavage administration would be the most appropriate route of administration for the toxicity studies."*

Note: The text in red in the table refers to information obtained subsequently to the initial enquiry and is additional to the data in the Phase III ITS report.

4 RECOMMENDATIONS

As mentioned in section 2 above, the choice of CRO is dependent on a number of factors including cost per study/package, technical expertise of the staff, reputation, communication processes, and PMRC member preferences. Assimilating all these criteria, it is proposed that the following CRO's are considered for conducting the studies:

Table 4.1 Proposal for conduct of studies

Laboratory	Study Allocated	Cost (£)
Harlan Laboratories	a) In vitro eye irritation/corrosion	1,000
	b) In vivo eye irritation (if required on the basis of the in vitro study	950
	c) Sensitisation (LLNA)	2,240
Covance Laboratories	a) In vitro mouse lymphoma assay	14,500
	b) In vitro micronucleus assay	13,900
Charles River Laboratories	a) Dose range finding study	9,000
	b) Combined 28-day repeated dose oral toxicity and reproductive toxicity screen	52,000
	c) Analytical support: • Method validation	8,900-12,500
	• Dose formulation analysis (2 occasions)	3,000
		Total 105,490 – 109,090

The laboratories recommended are considered to be proficient with regard to the studies selected and are known to wca. Cost has not been the primary indicator for selection, but it should be noted that Charles River has quoted the lowest cost for the most expensive study, ie

the combined toxicity and reproductive toxicity screen. For a full cost comparison please see Table 3.1 above. Neither of the CRO's that would be considered for the repeat dose studies (Covance and Charles River Laboratories) claim to have significant experience in the testing of metals/metal salts.

It is recommended that a personal visit by wca is made to Charles River Laboratories to ensure that the needs of the PMRC are understood and that all criteria for successful conduct of the critical study are in place. Harlan and Covance are also known to one or more members of the PMRC as capable of conducting the allocated studies.

While wca is recommending the particular laboratories, further revisions of the allocation is possible following discussions within the Rhenium Working Group.

APPENDIX 1 RHENIUM METAL SALT: REQUEST FOR QUOTATION

The following letter was sent to the identified Contract Research Organisations:

wca environment limited requires to contract out a series of toxicology studies to support registration under REACH. The test substance is an inorganic salt of a precious metal (rhenium) and requires Annex VIII endpoints to be fulfilled. An Integrated Testing Strategy has been defined for the substance taking into account category formation, read-across and weight of evidence strategies. As a result, the following studies are required in the first instance:

- in vitro eye corrosion (eg OECD TG 437)
- in vivo eye irritation (OECD TG 405) if in vitro corrosion study is negative
- skin sensitization (nominally LLNA OECD TG 429)[1]
- mouse lymphoma assay (OECD TG 476)
- in vitro micronucleus assay in an appropriate cell line (eg OECD TG 487)
- 28-day repeat dose study in rats[2] (with appropriate drf study)
- Reproductive screening study in rats[2] (with appropriate drf study)

[1] the suitability of the LLNA for an inorganic metal salt should be discussed. An in vivo rabbit skin irritation study indicates the substance is not a dermal irritant.

[2] Currently an acute oral toxicity study is available. The rat oral LD_{50} is estimated to be > 2 g/kg. No acute toxicity data is available by any other route, although read-across and WoE indicates that a second route can be waived. A particle size distribution analysis has been conducted to determine the aerodynamic diameter of the substance, calculated from laser diffraction data using the tapped macroscopic bulk density of the substance. The results from this indicate that the MMAD is 138.1 um and the geometric standard deviation is 2.21. The additional data points are: d_{10}: 62 um, d_{90}: 241.9 um. On this basis we are assuming that the route of administration for the 28-day rat study will be oral, and therefore a combined repeat dose/reproductive screening study (OECD TG 422) is appropriate. However, if it is felt that the inhalation route should be considered (at least for the repeat dose part), please indicate and cost accordingly.

Please can you provide:

- an indication of your prior experience with inorganic metal salts
- the costs of the studies (separately and together), and indicate if a discount is applicable if all the studies are contracted to you
- the relevance of the LLNA for inorganic metal salts

- the appropriate route(s) of administration for the 28-day and repro screen
- an indication of substance volume required. It is appreciated that only minimal information on toxicity is available (eg acute oral toxicity > 2 g kg^{-1}). However, since the rhenium salt is expensive to produce it is necessary to have some indication of test substance requirement for the programme.
- Laboratory space availability during 2011 (anticipated start Q1, although the date may be modified)
- Electronic copies of relevant generic Test Plans