TEST CONFIRMING ABSENCE OF FREE MERCURY IN SILVERFIL ARGENTUM

Type of Test Conducted	:	Excess Mercury Test
Test Conducted by	:	Mr. J. D. R. Walker Bsc., Ph.D., M.I.M
Test Conducted at	:	City University, London
Date of Report	:	September 1990

Report Summary

X-ray Mapping and Analysis by Edax

Using the electron microscope an x-ray map of the spectra produced by electron beam bombardment was created. The electron microscope can also be used to obtain a semi-quantitative analysis of the material for element of Atomic Number greater than about 10. The spectrum obtained and the approximate analysis from an amalgamated sample are shown in the Appendices. It can be seen that the material contained about 33% of silver and about 67% of mercury. No other elements were seen in detectable amounts.

Metallography

No evidence for the presence of free mercury was detected by metallographic examination. If free mercury was present, then this would be expected to show up on metallographic examination as porosity (as it will drop out during preparation). While some porosity was seen the amount found was small and similar to that found in other amalgam alloys amalgamated and condensed according to BS2938:1985.

Discussion of Results

Analysis of the amalgamated material semi-quantitatively showed that the material was approximately two thirds mercury and one third silver (Atomic %). No other elements were detected. This is in confirmation to the information provided by the manufacturer.

Examination of the amalgamated material by X ray mapping showed no evidence of free mercury present as if areas rich in one particular constituent existed they would show up on the "map". It can be seen in Plate II that there has been no particular clustering detected by this mapping technique.

Examination of the amalgamated material metallographically showed no evidence of free mercury present and in fact the porosity seen was less than normally seen in conventionally amalgamated alloys.

Conclusion

Examination of the amalgamated material by X ray mapping showed no evidence of free mercury present.

Examination of the amalgamated material metallographically showed no evidence of free mercury present.

TEST CONFIRMING ABSENCE OF FREE MERCURY IN SILVERFIL ARGENTUM

Type of Test Conducted	:	Excess Mercury Test
Test Conducted by	:	Prof. S. Radhakrishna
Test Conducted at	:	Institute of Advance Studies, University of Malaya, Kuala Lumpur, Malaysia
Date of Report	:	20th October 1995

Report Summary

X ray diffraction tests were conducted on all five samples by using Philips X ray diffraction unit at room temperature.

SEM and Electron dispersive X ray (EDAX) studies were conducted on all the samples by using Philips SEM unit with EDAX attachment.

Optical absorption studies were conducted on all the samples by using Shimadzu 3100 UV-Vis-IR Spectrophotometer. Differential thermal analysis was done using Perkin-Elmer equipment.

All the tests were meant to detect the presence of free mercury likely to be left after the amalgamated alloy has been formed.

Test Results

All the tests failed to detect any free or unreacted mercury when samples were made with the recommended mixing ratio by the manufacturer. The EDAX experiments showed Mercury : Silver in the proportion 68 : 32 while X ray diffraction studies confirm the existence of a silver-mercury alloy (Ag-Hg). No other elements were detected (up to 0.2 ppm). Differential thermal analysis of the samples gives a single peak at 122 C, indicating the presence of a single component. The samples marked A, B and C did not show any optical absorption peaks which can be attributed to the presence of any free or unreacted mercury.

Conclusion

In conclusion it can be stated that there was no free or unreacted mercury in the samples prepared.

TESTS ON BIOCOMPATIBILITY OF SILVERFIL ARGENTUM

Type of Test Conducted	:	Primary Skin Irritation
Test Conducted by	:	Dr. Shahnaz Murad
Test Conducted at	:	Allergy & Immunology Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia
Date of Report	:	15th May 2003

Report Summary

Introduction

Chemicals released from material that contact the body may produce skin irritation. Primary irritation of the skin can be defined as localised inflammatory response to application of test substance. The macroscopic manifestations of irritation are oedema and erythema. The albino rabbit is the most commonly used animal for this test. In experimental animals, a primary skin irritant is defined as a chemical substance that produces an irritant response in a majority of test subjects.

Test Methods

Tests were conducted between 10-03-2003 to 15-03-2003.

1. The study was carried out in batches with 2 rabbits per batch.

2. To each rabbit, three areas, each roughly twice the size of the patch vehicle, were clipped. The areas clipped were on the shoulders and left flank. The clipped area on the right shoulder was designated for Silverfil, and the left shoulder and left flank were designated for either positive or negative control.

3. Using the amalgamator, a capsule of Silverfil was triturated. The resulting paste was shaped into a thin layer and placed on the patch applicator (gauze). This was then applied to the clipped area on the right shoulder and secured by wrapping hypoallergenic tape around the rabbit's body.

4. For positive control, a gauze was soaked with 1 ml of 5% formalin and then secured either to the left shoulder or left flank using the adhesive tape. A gauze soaked with 1 ml of normal saline was used as negative control.

5. The patches were left in place for 24 hours. Reading were carried out at 1 hour (1st reading) and 24 hours (2nd reading) after patch removal.

Conclusion

Dermal irritation is a result of direct damage to epidermal cells, with no immunologic (allergic) mechanism involved. Positive control sites demonstrated irritation, score ranging from 1 to 3. No irritation was detected at negative control sites and sites tested with Silverfil dental amalgam within the 72 hours of observation.

TESTS ON BIOCOMPATIBILITY OF SILVERFIL ARGENTUM

Type of Test Conducted	:	Skin Sensitization
Test Conducted by	:	Dr. Shahnaz Murad
Test Conducted at	:	Allergy & Immunology Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia
Date of Reports	:	15th May 2003

Report Summary

Introduction

Sensitization tests are undertaken to investigate the potential of a material to cause type IV hypersensitivity following its repetitive or prolonged contact with the skin. Guinea pigs have been shown to be the best animal model for human allergic contact dermatitis. Sensitization tests involve induction and challenge phase with a rest period of 2 weeks in between the phases.

Test Methods

Tests were conducted between 17-03-2003 to 12-07-2003.

The study was carried out in 2 phases: induction phase and challenge phase, with 2 weeks resting period in between phases. The purpose of induction phase was to expose the guinea pigs (epidermally) to the material. If the material was a sensitizer, the physiological processes required to ultimately allow the generation of an immunological response would be initiated during this phase. The challenge phase that followed the resting period was to investigate the elicitation of a mature immunologic response.

The study was carried out in 2 batches. Each batch consisted of 16 guinea pigs to which 10 were assigned to test group (expose to Silverfil both in induction and challenge phases), 5 were assigned to naïve group (only exposed to Silverfil in challenge phase) and 1 was assigned as positive control (exposed to a known sensitizer such as formalin in induction and challenge phases). Skin changes in the naïve group would represent irritation phenomenon should any reaction take place, thus acted as baseline comparison (control) to the real sensitization reaction in test group. The positive control group was for the purpose of validation of the model and techniques.

The test group guinea pigs were assigned T1 to T20, the positive control as C1 and C2 and the naïve group N1 to N10. The first 3 weeks was the induction phase in which animals in the test and positive control groups were exposed to Silverfil dental amalgam and 5% formalin respectively. Naïve group was left untreated. The exposures were for 3 times a week (Monday, Wednesday and Friday), each lasting for six (6) hours and importantly, to the same site throughout. Once induction phase was over, 2 weeks resting period was allocated followed by a challenge phase. In the challenge phase, the guinea pigs from test and naïve group were exposed to Silverfil dental amalgam for six hour duration whereas for positive control animals, were exposed to 5% formalin. It should be noted that in the challenge phase, the exposures were to the skin areas that had not been exposed previously (virgin sites).

For exposure procedure, an area slightly bigger than the patch (gauze) located over the shoulder was clipped. In exposure to Silverfil, a capsule of Silverfil dental amalgam was shaped into a thin layer and placed on the gauze and immediately secured to the clipped area by way of wrapping with adhesive tape. For formalin exposure, 0.3 ml of 5% formalin was placed on the gauze and secured to the clipped area (this amount had been pre-determined as the highest amount possible as not to cause irritation reaction). Each exposure lasted for six hours. Virgin sites were used for challenge phase.

Conclusion

Both the guinea pigs (C1 and C2) from the positive control group exhibited grade 1 reaction following the challenge phase. These results validate the model. The skin changes remained the same even at t = 48 hours. This suggests formalin is rather a weak sensitizer. All the guinea pigs in naïve and test groups showed a result of zero (o). This suggests that Silverfil (at the does applied) did not cause any irritation or sensitization to the guinea pigs. As mentioned earlier, sensitization, should it take place, was the result of immunologic process, as opposed to direct damage mechanism in irritation reaction.

TESTS ON BIOCOMPATIBILITY OF SILVERFIL ARGENTUM

Type of Test Conducted	:	Cytotoxicity
Test Conducted by	:	Dr. Noor Rain Abdullah
Test Conducted at	:	Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia
Date of Reports	:	28th October 2002

Report Summary

Test Methods

Initial Dilution was 20 mg/ml Nitric Acid (Nitric Acid is the diluent) and it was serial diluted to a series to dilution. The concentrations in Nitric Acid was further diluted in water (5 fold dilution) and then further diluted to culture medium (20 fold and 2 fold). The concentration of Silverfil for the test was from 25 ug/ml to 0.3 ug/ml.

Cytotoxicity test was carried out using the Cytotoxicity Detection Kit (Roche). The test was set up using 96-wells plate. Test substance Silverfil and Nitric Acid were tested individually and carried out in triplicate. The control was culture medium with cells alone (negative control) and cells in the present of 1% Triton X 100 in culture medium (positive control). The test was carried out according to the manufacturer's manual. The test was read at 490 nm (with reference wavelength at 630nm).

Results / Analysis

The % Cytotoxicity at each concentration of tested material Silverfil land Nitric Acid were calculated.

$$\text{The \% Cytotoxicity} = \frac{\text{Experimental value – Low Control (negative control)}}{\text{High control (positive control) – Low control}}$$

Conclusion

Silverfil, at 25 ug/ml showed a % toxicity to the MDBK as 15%, the other lower concentration demonstrate a lower % of toxicity. Also tested was Nitric Acid and it demonstrated a negligible effect to the cells within the concentrations tested (25 ug/ml to 0.03 ug/ml).

Cell growth inhibition will take place with concentration of extract at toxicity of 50%. The % toxicity with Silverfil extract was 15% and below, thus Silverfil can be considered to have no significant toxic effect on cell growth at all.

TEST ON IN VITRO GENOTOXICITY OF SILVERFIL ARGENTUM

Type of Test Conducted	:	Test on In Vitro Genotoxicity
Test Conducted by	:	M. D. Muhammad Hidayat, H. Akram, A. Zaihan, A. Aziz, M. A. Khadijah
Test Conducted at	:	School of Dental Sciences, University of Science Malaysia, Kelantan, Malaysia
Date of Reports	:	Presented at the 8th Dental Student's Scientific Conference held at the University of Malaya on 15-16 December 2006.

Report Summary

Objective

The objective of this study was to identify the genotoxic characteristsics of Silverfil Argentum by using Bacterial Reverse Mutation Assay (Ames test)

Test Methods

The test material was evaluated in two mutated strains of Salmonella typhimurium (TA98, TA100) with and without an external metabolic activation system (S9 mix). The bacteria were incubated for 48 hours at 37 $^{+/-}$ 0.5 ^{0}C before the colony growth or revertant colonies were counted.

Results

The investigations of the genotoxic reaction on the test material revealed that the number of revertant colonies in both strains with and without S9 mix was less than twice of the negative control even in the presence of high Silverfil Argentum concentrations (5.0 ug/ml).

Conclusions

The results of the tests of two different Salmonella strains featuring either base pair substitution (TA98) or frameshift mutation (TA100) were considered to have no genotoxicity activity of Silverfil Argentum.

Note:

A joint panel of outside experts ,brought together by the US FDA on 6 Sept. 2006), voted on Thursday (7th Sept, 2006) to reject the draft report that concluded that dental amalgam fillings used by millions of people are safe. Dr. Karl Kieburtz, Chairman of one of the panels, and other panelists said that:

'Research is needed on the effect of dental mercury on children, the foetuses of pregnant women with fillings and others whose bodies may absorb, distribute, process and eliminate mercury differently'.

TEST ON TIME TAKEN FOR COMPLETION OF CHEMICAL REACTION (AMALGAMATION)

Type of Test Conducted	:	Test on Completion of Amalgamation Process
Test Conducted by	:	Dr. Wan Jeffrey Basirun
Test Conducted at	:	Department of Chemistry University of Malaya, Kuala Lumpur, Malaysia
Date of Reports	:	15th August 2003

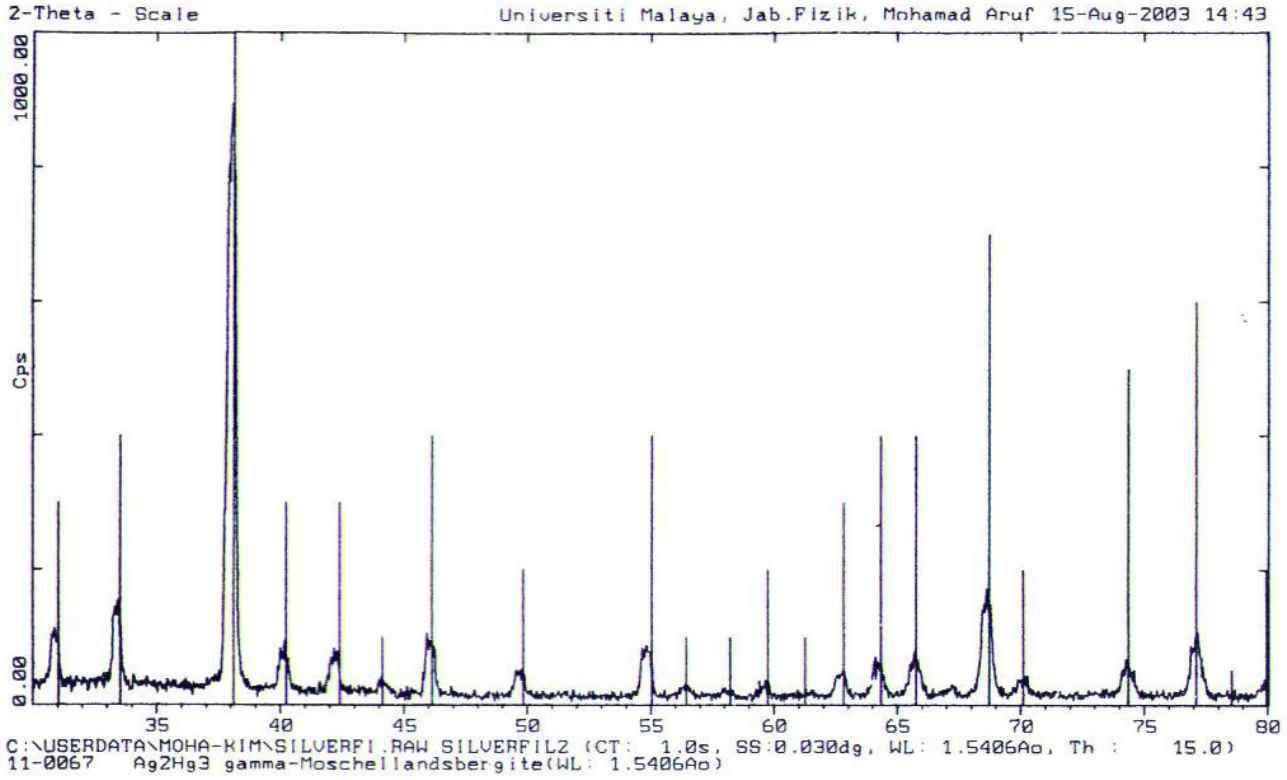
Report Summary

Test Methods

Analysis by X-Ray Diffraction was done on Silverfil after mixing the Silverfil powder with mercury. It was found that after 30 minutes, the XRD diffractograms matched exactly to that of Ag_2Hg_3 which is also known as gamme-Moschellandsbergite and there was no free mercury in the amalgam as the XRD diffractograms did not resemble readings to those of free mercury.

Conclusion

It can be concluded that there was no free mercury in the amalgam after 30 minutes and all the mercury existed in the amalgam for of gamma-Moschellandsbergite which has the molecular formula Ag_2Hg_3



TEST MEASURING COEFFICIENT OF DIFFUSION OF MERCURY

Type of Test Conducted	:	Test on Completion of Amalgamation Process
Test Conducted by	:	Dr. Wan Jeffrey Basirun
Test Conducted at	:	Department of Electro-Chemistry University of Malaya, Kuala Lumpur, Malaysia

Report Summary

The mass transport from a bulk solution to an electrode can occur in three ways:

Diffusion
This arises from concentration gradient that occurs on the surface of the electrode. The bulk solution has a larger concentration of ht electro-active species than the electrode surface and hence there is a concentration gradient that results in the transport of that species from the bulk solution to the electrode surface.

Convection
These are movement due to mechanical force acts on a solution. There are two types. The first is natural convection, which can be present in any solution and arises from thermal gradient and/or density differences within the solution. The second type of movement is forced convection, which is achieved by external forces such as gas bubbling through pumping or stirring.

Migration
The external electric field which exists at the electrode/solution interface as a result of the drop in electrical potential between the two phases is capable of exerting an electrostatic force on charged species present in the interfacial region and thereby of inducing the movement of ions to or from the electrode.

Test Methods
The three headed electrochemical cell was used throughout the experiments. The reference electrode was Ag/AgCl fitted into a Luggin capillary. Hence all potentials are relative to the Ag/AgCl reference electrode. The dental amalgam as the working electrode was fabricated by pressing the amalgam powder into glass tubes and a copper wire was attached in one end to establish electrical contact. The circular working electrode had a diameter of 4mm.

A platinum wire was used as the counter electrode. The BAS BioAnalytical instrument was used as to run the cyclic voltammetry experiments. $NaNo_3$ (Analar grade) with a concentration of 0.5 M was used as the inert electrolyte. $Hg(No_3)_2H_20$ (Analar grade) with a concentration of 5.965×10^{-3} M was used as a source of Hg^{2+} ions. Because the ionic migration of Hg2+ is reduced with the introduction of a large amount of inert electrolyte, the mass transport of Hg2+ ions can be attributed to diffusion only conditions.

Conclusion
The diffusion constant estimated by using the Randles-Secvik equation gave:
7.09×10^{-9} m^2 s^{-1} inferring that Silverfil has a high coefficient of diffusion of mercury.

TEST REVEALING THAT SILVERFIL IS SIMILAR TO A MINERAL IN NATURE

Type of Test Conducted	:	Geological Study based on XRD analysis
Test Conducted by	:	Associate Prof. Dr. Azman A Ghani
Test Conducted at	:	Department of Geology University of Malaya, Kuala Lumpur, Malaysia

Report Summary

Introduction

Silverfil is a new dental amalgam material composed of a mixture of reactive silver particles (ag) and a partially amalgamated silver-mercury powder (Ag_3Hg_2). The Silverfil sample was analysed using XRD machine at the Department of Geology, University of Malaya.

Test Methods

When a material with a regular spaced array of atoms is irradiated by a monochromatic beam of X-ray, there occurs scattering (or diffraction) of the beam. This scattering can be regarded as one of reflection though there is the important difference that the x-rays also penetrate below the surface of the material. The rays reflected from the successive atomic layers can be or not be in phase: the condition for a maximum reflected intensity being that the contribution from successive planes be in phase. As different minerals/material have their own characteristics d-spacing, it is thus possible to identify them by noting the angles at which peak reflections are present.

Results

The results show that the amalgam is similar to the naturally occurring metal element Moschellandsbergite [Ag_2Hg_3 Silver Mercury (Silver Amalgam)]. All 20 peak of the Silverfil profile is similar to Moschellandsbergite except at the 65.7” and 74.2”. However, the intensity of nearly all the Silverfil peak is slightly lower compared to the Moschellandsbergite. Moschellandsbergite is a rare mineral named for its type locality at Landsberg (formerly known as Moschellandsberg), Obermoschel, Rhineland-Pfalz, Germany. This locality has produced many specimens of various familiar mercury minerals such as cinnabar, mercury and calomel. It has also produced some rather obscure mercury minerals especially mercury alloys such as Belendorffite, Cu_7Hg_6; Paraschachnerite, Ag_3Hg_2; including mercury, calomel, cinnabar Schachnerite, $Ag_{1.1}Hg_{0.9}$ and of course Moschellandsbergite.

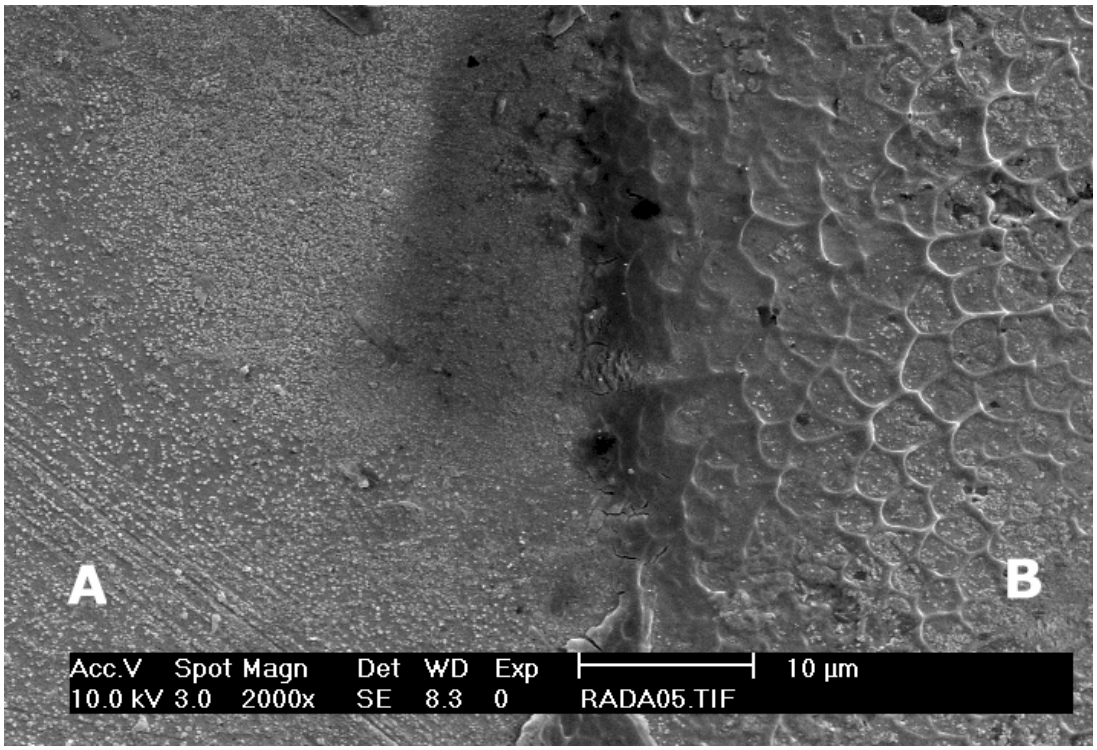
Any excess or waste amalgam made from Silverfil material is safe enough to be disposed onto the earth.

TEST ON SILVERFIL AMALGAM'S WORK HARDENING PROPERTIES

Summary

Laboratory tests using the Rolling Ball technique were conducted on Silverfil amalgam. The results showed that Silverfil can be work hardened, with the Vicker's hardness of the material increasing twofold over 24 hours.

Work hardening with Silverfil amalgam is possible as it is a single-phased matrix, while traditional silver-tin-copper amalgams are multi-phased and cannot be work hardened.



A Surface of the amalgam before work hardening
Average of 53 Vickers

B Surface of the amalgam after work hardening
Average of 106 Vickers