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Response to Guzzi & Pigatto's *Comments on Migration of mercury from dental amalgam through human teeth by H. H. Harris et al. (2008). J. Synchrotron Rad. 15, 123–128*

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The following is a response to the points raised in the comment by Guzzi & Pigatto on our recent paper on Hg in teeth (Harris *et al.*, 2008*b*). The first issue they raised was that our results might have been influenced by our method of preparing the teeth cross sections using a high-speed dental drill to remove the amalgam before the tooth was cross-sectioned. They suggest that their 'lift-on technique' for removal of the amalgam (Guzzi *et al.*, 2003, 2004) would have been a better choice. Specifically, they state their concern that our method of removal would have contaminated the sample and have used as a reference point the observation that dental amalgam removal by this technique causes high levels of Hg vapour in intraoral air (Richards & Warren, 1985), and that it is deposited in tooth space compartments (*e.g.* enamel, dentine, roots). Both of these papers refer to teeth that are drilled *in situ*, not to teeth that had been extracted and the dental amalgam removed (in a non-confined well ventilated space, with nowhere for Hg vapour to accumulate). Moreover, the latter paper is a brief discussion of the amount of Hg vapour produced by different filling removal techniques. It does not discuss, nor provide any experimental evidence, that Hg migrates through teeth from the dental amalgam during the drilling procedure on extracted teeth. They also claim that the mercury atoms can diffuse through the dentinal tubules during drilling because of the size of the Hg atoms. Such arguments lack any considerations of the chemistry of diffusion. The dentinal tubules are filled with an aqueous solution of organic material (Pashley, 1996) and Hg atoms do not diffuse through water very well (unless under pressure). Specifically, the solubility of metallic Hg in water is 0.000056 mg g^{-1} and it takes 24 h to reach this equilibrium concentration (Spencer & Voigt, 1968). Therefore, the idea that Hg can diffuse millimetres into the tooth through the aqueous solution in the dentinal tubules during the short periods of drilling is unrealistic and the solubility is far too low to produce the observed Hg concentrations that we found in teeth. In addition, Hg vapour could not be deposited onto the sections because there was no Hg amalgam or vapour when the teeth were sectioned. Moreover, the fillings were lined so that significant diffusion of Hg into the dentine could only occur after the lining was removed, at which time any Hg vapour would have largely diffused away from the site of the drilling. By contrast, hydraulic conductance in dentinal tubules increases under pressure (Camps *et al.*, 1997), such as that applied during dental amalgam fillings where liquid Hg is produced. Hg in dentinal tubules of amalgam-filled teeth has also been observed recently using transmission electron microscopy (Venclikova *et al.*, 2007).

Their claims seem to be based on their unpublished observations that they have found Cu (600 μg g^{-1}), Ag (215 μg g^{-1}) and Pd (3.9 μg g^{-1}) in the discoloured areas of teeth using their approach for removal of the amalgam. The idea that other metals such as Zn and the above contained in the amalgam could also diffuse during the drilling process is also chemically unrealistic, especially since some of these were substantially incorporated into the hydroxyapatite, as well as the tubules, many millimetres from the drilling site in the teeth that we analyzed. Thus the unpublished results reported in the comment provide support for our analytical procedures in that their results are entirely consistent with our results (Harris *et al.*, 2008*b*) where Fig. 3 of this reference shows that a maximum Cu hotspot concentration of 4 mg g^{-1} was reported in areas where there was no dental amalgam. The average concentrations over the discoloured areas (results to be published) were similar to those reported in the comment by Guzzi & Pigatto. Moreover, as reported in our paper (Harris *et al.*, 2008*b*), the X-ray fluorescence also determines the concentration of other elements, such as Ag (see Fig. 4 of Harris *et al.*, 2008*b*), and these metals in the area of discolouration are consistent with those in bulk dentine found by them.

Other facts in terms of our research (Harris *et al.*, 2008*b*) that negate the speculation by these authors are as follows.

(i) If elemental Hg vapour was only deposited in the tubules during the removal of the amalgam, it is unlikely that it would be rapidly oxidized to Hg^{2+} as we observed (Harris *et al.*, 2008*b*).

(ii) The Hg contamination stops rapidly in the areas of secondary dentine growth where there used to be pulp (shown by the translucent areas in the optical image in Fig. 1 of our reference); this indicates that the major migration occurred before the new dentine was deposited (Harris *et al.*, 2008*b*). There would be no reason for it to stop at this point, if the Hg was deposited from the drilling process, as implied by them.

(iii) The difference in the oxidation state and chemical composition of the Hg-containing amalgam deposit and the dentinal tubules (Fig. 4 of Harris *et al.*, 2008*b*) show that these Hg deposits could not be entering the tooth *via* the same mechanism that would apply if it was due to Hg vapour during the drilling procedure.

(iv) The fact that the composition of the metals in the pulp horn was consistent with those in the dentinal amalgam (Harris *et al.*, 2008*b*) shows that Hg must have had direct contact with the pulp during the filling procedure.

(v) Metals other than Hg are able to penetrate deep into the dentine of the teeth even when dentinal amalgam is not present (Harris *et al.*, 2008*a*), such as when dental cavities are present.

Guzzi & Pigatto also question the results we obtained about the Hg content of the calculus (Harris *et al.*, 2008*b*). Results reported by Pigatto *et al.* (2005) support our hypothesis that microbial activity results in Hg diffusing from the fillings into the calculus, but they report unpublished results from the teeth of one patient where the Hg

levels were lower than we determined. Of more relevance are data from two reports on Hg in plaques and calculus on a large number of patients. In one study (Lytle & Bowden, 1993), a range of 0.01–0.54 μg g^{-1} of Hg was deposited in fresh plaque over 24 h, and was attributed to the action of *Streptococcus mutans* in plaques on teeth filled with amalgams. In another study of 60 patients, concentrations of Hg in microgram quantities of calculus removed from teeth were as high as 0.3 mg g^{-1}, as determined by bulk X-ray fluorescence measurements (von Bohlen *et al.*, 1994). Note that the Hg concentrations that we determined in the calculus from the microprobe scans of teeth cross sections (Harris *et al.*, 2008*b*) were consistent with those measured independently with bulk techniques (von Bohlen *et al.*, 1994) for a large number of patients, but were considerably higher than those in fresh plaque (Lytle & Bowden, 1993). Other research has shown that *Bacterionema matruchotti* accumulate Hg in dental calculus and do so by incorporating Hg compounds into their cell walls when cultured with dental calculus (Fujito *et al.*, 1988; Fujito, 1989). While these were *in vitro* experiments, it demonstrated that bacterial action could concentrate Hg in calculus, which is consistent with both the mechanism and experimental results that we discussed in the paper. This establishes the validity of our mapping analysis on cross sections of teeth. Moreover, if the Hg was deposited into the calculus by deposition of Hg vapour during the removal of the amalgam from the tooth, then it would also have been deposited on the exterior of teeth where there was no calculus and this was not observed (Harris *et al.*, 2008*b*).

Thus the criticisms of Guzzi & Pigatto are based on their speculation on the mechanism of rapid diffusion of Hg vapour through dentinal tubules during drilling. As discussed above, we contend that such diffusion is not feasible when the physical chemistry of Hg diffusion through filled tubules is considered, and, moreover, cannot explain the distribution of other metals over a similar area that we have measured directly by X-ray fluorescence mapping (Harris *et al.*, 2008*b*). Other research groups using other experimental techniques (Fujito *et al.*, 1988; Fujito, 1989; Lytle & Bowden, 1993; von Bohlen *et al.*, 1994) support our conclusions, and furthermore show that our quantification in the elemental mapping of teeth cross sections is consistent with expectations from measurements of plaque that have been removed from teeth (von Bohlen *et al.*, 1994).

In summary, Guzzi & Pigatto assert that all significant Hg ingestion occurs from ingestion of Hg vapour either directly or through the dissolution of the vapour in saliva that is swallowed and that this vapour arises from physical erosion of dental amalgam fillings. They also ignore the possibility that the toxicity of Hg will depend on the form that is ingested and the route of entry; *i.e.* most orally ingested Hg is not absorbed, but if it goes directly into the blood stream then higher toxicity can arise from smaller amounts. There is no direct evidence to exclude the other mechanisms that we have discussed (Harris *et al.*, 2008*b*), which provide other plausible pathways that have not been appropriately considered in evaluating exposure and, hence, we reiterate that they may prove to be confounding factors in epidemiological studies.

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