# Letter to the Editor

*Annie Pfohl-Leszkowicz*

## Formation of 2′-deoxyguanosine-carbon 8-bound ochratoxin A adduct in rat kidney DNA

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We have carefully read the article by Delatour *et al.* entitled "Absence of 2′-deoxyguanosine-carbon 8-bound ochratoxin A adduct in rat kidney DNA monitored by isotope dilution LC-MS/MS" [1], in which the authors present negative evidence for formation of the ochratoxin A (OTA) adduct in kidney DNA isolated from rats using LC-MS/MS. The DNA adduct in question, 2′-deoxyguanosine-carbon 8-bound ochratoxin A (dG-OTA), was originally characterized by us [2] and subsequently shown to comigrate with the major adduct detected in rat kidney, as evidenced by $^{32}$P-postlabelling [3]. Thus, we are keenly interested in the results presented by Delatour *et al*. and have noted a number of flaws in the article that we feel are important to point out to the readership of *Molecular Nutrition & Food Research*.

(i) In the article the LOD is claimed to be 10 fmol that is sufficient for detecting 3.5 adducts/$10^9$ nucleotides. This LOD value was determined using an artificial matrix and included sample spiking with 1.25 pmol (1250 fmol) isotopically labeled dG-OTA that was generated using isotopically labeled dG of 96-98% isotopic purity. As discussed previously [4], this high amount of spiking creates a problem because the isotopically labeled sample contains the non-isotopically labeled dG-OTA adduct as an impurity (shown with an arrow in the blank sample in Figure 5 of the paper of Delatour *et al.*, [1]). Based on the isotopic purity of the starting dG (96–98%) the samples easily contain 1% impurity, which means that all the samples analyzed contain ~12.5 fmol dG-OTA. When the sample is spiked with 10 fmol of dG-OTA (Figure 5) the peak noted is not significantly different than the peak present in the blank. However, they claim that 10 fmol is the LOD, when in fact the amount of dG-OTA in the sample is not 10 fmol, but is probably (10 + 12.5 fmol) ~22.5 fmol due to the impurity present in the isotopically labelled sample. This LOD value of 10 fmol is also used as the LOD for the actual rat samples that have also been spiked with 1250 fmol isotopically labeled dG-OTA and thus contain the non-isotopically labeled impurity (~12.5 fmol). However, unlike the data presented for the artificial matrix (Figure 5) in which the non-isotopically labelled impurity is detected in the blank sample, this impurity is not detected in the rat kidney samples (Figure 6) and so clearly the LOD value for the rat samples is higher than the value determined using the artificial matrix, which is also inaccurate. This is important because the LOD of 10 fmol is used in Table 1 to rule out dG-OTA formation. Interestingly, despite much higher LOD than reported in the article, the data presented in Figure 6 for the rat kidney DNA could be viewed as a POSITIVE result for detection of dG-OTA. Here they actually observe a peak for the dG-OTA adduct following 90 days incubation using the 633.4–429.1 fragmentation transition in MS/MS detection. This fragmentation is very adduct (dG-OTA) specific and a peak above background is clearly observed that comigrates with the adduct standard. That the adduct peak does not appear in the 633.4–517.3 chromatogram does not present a problem because this fragmentation pathway is less adduct (dG-OTA) specific and matrix effects create more background noise. In our view these drawbacks prohibit Delatour *et al*. to categorically rule out formation of dG-OTA in rat kidney.

(ii) Throughout the article the authors focus on negative results concerning OTA metabolism and OTA-mediated mutagenicity/genotoxicity and fail to draw attention to the large body of evidence in support of DNA damage mediated by OTA metabolism (reviewed in Pfohl-Leszkowicz *et al.* [5]). This bias is particularly evident in their discussion of OTA-mediated mutagenicity/genotoxicity in which they fail to reference two recent (2007) papers that show convincing evidence for DNA damage [6] and mutagenicity [7] by OTA. Odell *et al*. [6] have examined the DNA ploidy distribution in renal tumours and have concluded that the elevated DNA ploidy distribution in renal carcinomas must be associated with genetic change and thus OTA is genotoxic. They also state that the genetic damage initiated by OTA is necessary for subsequent renal tumourigenesis. Palma *et al*. [7] show that OTA induces an increase of mutation frequency at two gene loci, hypoxanthine-guanine phosphoribosyl-transferase and thymidine kinase. Although the data

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support a model where OTA is mutagenic *via* oxidative DNA damage, these two recently published papers confirm the genotoxicity of OTA and suggest that OTA-mediated DNA damage is responsible for tumour formation in the rat.

In conclusion, this paper fails to demonstrate the lack of covalent binding by OTA on DNA. On the contrary, the data presented positively confirm formation of dG-OTA adduct in rat kidney.

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## References

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