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Reply

Letter to the editor by Dekant et al. concerning the manuscript "Analysis of some breakfast cereals on the French market for their content of ochratoxin A, citrinin and fumonisin B_1: Development of a method for simultaneous extraction of ochratoxin A and citrinin" by Molinie et al., this volume

Dear Dr. Birch,

The letter from Professor Dekant requires the following clarifications and comments. This work was carried out for the "Institut National de la consommation" ("INC") in our laboratory by Anne Molinié during her Ph.D. thesis and samples were analysed by a method which we developed for simultaneous extraction of OTA and citrinin. Samples were collected blindly in 2002 (march) by "INC" in various shops and supermarkets in France. It is the usual procedure of "INC" to submit their results to the producers before publication and this resulted in a strong reaction from some of them, who after publication in '60 millions de consommateur' in January 2003, requested an answer 'droit de réponse' in the journal "60 millions de consommateurs". In view of the strong polemic about/some of the positive results, the "INC" asked Dr. Castegnaro to make an expertise judgement of the data and to re-analyse some samples. This was performed using the method of Entwisle et al. (Entwisle, A. C., Williams, A. C., Man, P. J., Slack, P. T., Gilbert, J. (2000). Liquid chromatographic method with immunoaffinity column clean up for determination of ochratoxin A in barley: collaborative study, *Journal of AOAC International, 83*, 1377–1383.), originally developed for barley and validated for recoveries, repeatability and reproducibility for some of the breakfast cereals. Dr Castegnaro's report was published on the web site from the "INC" www.60millions-mag.com and in the journal '60 millions de consommateur' in march 2003. The results confirmed those produced in our laboratory, especially for cereal 28 (7.3 μg/kg against 8.8 μg/kg) and cereal 16 (1.6 μg/kg against 3.4 μg/kg, the lower value detected being explained by the fact that the cereals contained bicarbonate; see explanation on the web site of INC)". In the meantime, "INC" proposed, to the expert chosen by the industry, a double expertise (Dr. Castegnaro and their expert analysing, in parallel at the same time and place, the samples questioned). The INC is still awaiting an answer!

This clarification being done, we will more specificailly answer to Dekant's letter to the editor. It is true that Virginie Faucet went to Dr Dekant's laboratory to work with H. Zepnick on analysis of OTA and metabolites produced in vitro on December 2002. H. Zepnick was developing his method and V. Faucet asked him if he would agree to include some samples (those extracted from breakfast cereals) with a view to confirm the presence of OTA. He kindly analysed them himself and provided her with a fully detailed report, including the method description, the chromatograms and quantifications. This was more than was requested by us. We then applied the dilution factors and compared these results with those obtained by our HPLC/spectrofluorimetry results.

When we prepared the first drafts of the manuscript in February, 2003, we included the HPLC method description provided by H. Zepnick and a table which presented the raw data and the calculations. The table disappeared during the various exchanges with the editor and, in view of the new polemic sustained by Pr. Dekant, we requested, in 8 October, 2004, that the following table should be included as addendum to the manuscript. The various remaining volumes of the extracts (see Table 1), were dried, and redissolved in 500 μl water before LC–MS/MS therefore the calculation cannot be directly extrapolated from raw data. This clarifies the calculations from the raw data.

With regard to W. Dekant's comments, first it would have been difficult to cite the manuscript by Zepnick et al. (Zepnik, H., Volkel, W., Dekant, W. (2003). Toxicokinetics of the mycotoxin ochratoxin A in F 344 rats after oral administration. *Toxicol Appl. Pharmacol., 192*, 36–44.) which appeared in the September issue since we were not appraised of this at the time. We could have done it later and acknowledged it in this answer. We are very surprised by the selection of some chromatograms by Dr. Dekant. You will find below copies of two chromatograms provided to us (see Figs. 1 and 2).

These two chromatograms attest to the quality of the work performed by H. Zepnick during the set up of the method in Dr. Dekant's laboratory and its application to real samples. This, in fact, prompted us to use the

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Table 1
Analysis by LC–MS/MS

Cereals[a]	Volume before drying (μl)	Concentration/ml of solution injected in the LC–MS/MS	OTA μg/kg of cereal
3	80	0.67	0.1
10	20	6.43	4.2
12	2.5	0.392	2.5
14	20	7.35	4.8
15	20	5.8	3.8
16	8	1.90	3.1
17	8	1.44	2.3
28	40	25.75	8.4
31	40	36.44	11
36	40	9.2	3

[a] Samples detected above 2 μg/kg by HPLC were analysed by LC–MS/MS. Sample 3 was used as negative control.

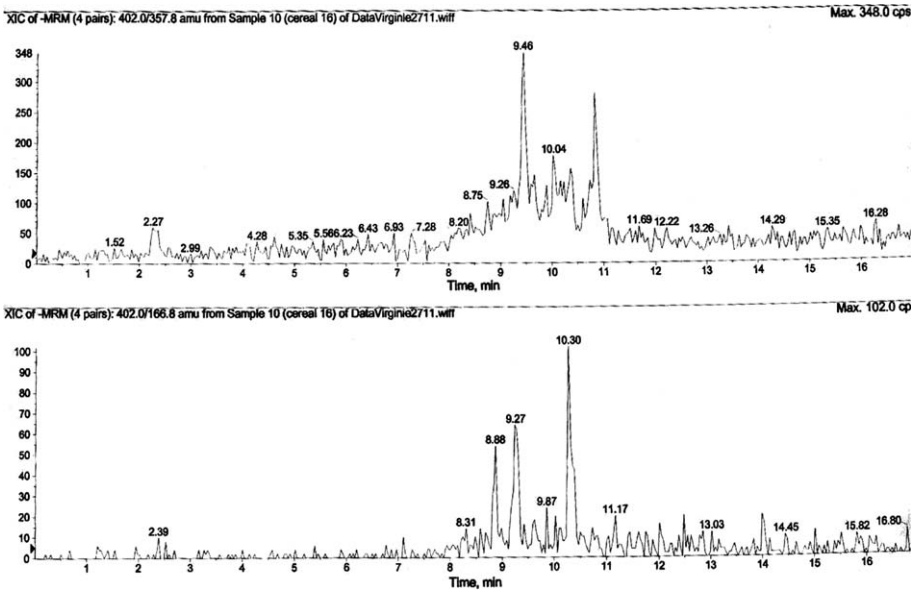

Fig. 1. Example of chromatogram of cereal 16.

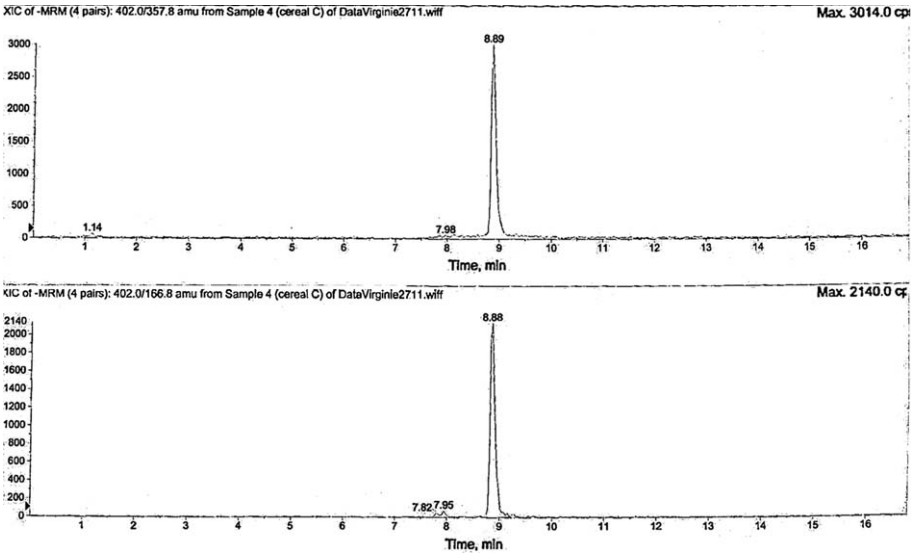

Fig. 2. Example of chromatogram of cereal C (a blanked cereal enriched with pure OTA).

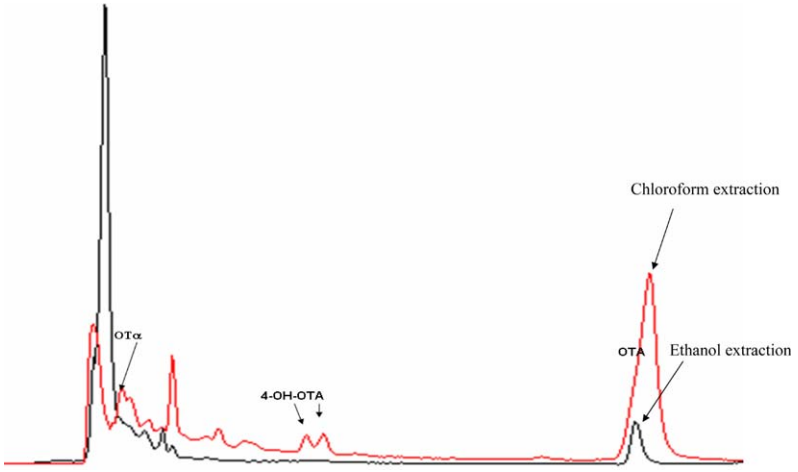

Fig. 3. Comparison of ethanol extraction (lower line) and chloroform extraction (upper line).

data to further support our results, already confirmed by Dr. Castegnaro.

Dr Dekant says that "he reserves the right to check all data generated by his laboratory for accuracy and correctness". We fully agree with such policy which applies also to the work generated in our laboratory. We are, however, surprised that, in the manuscript, which we should have cited, an enormous error occurred. To justify their lack of detection of 4-hydroxy-ochratoxin A, the authors state, "This seems to be in contrast to other studies where higher excretion rates and higher recovery were reported (Castegnaro et al. 1989; Storen et al. 1982, Suzuki et al. 1977), but these studies applied much higher doses which may interfere with renal junction or results in higher yield of Otalpha formation due to slower or incomplete intestinal absorption". In the manuscript by Zepnick et al. (2003), Dekant's group used a treatment with 0.5 mg OTA/kg body weight while Castegnaro et al. (1989) used 0.5, 2.5 and 5.0 mg/kg b.w.; in other words the same dose and two higher doses. These authors detected 4-OH-OTA in a dose dependent manner, confirming the results from Storen et al. (1982) and Suzuki et al. (1977). The statement from Dr. Dekant's group prompted us to compare data by strictly applying the method published by Zepnick et al. and the method in use in our laboratory.

Fig. 3 presents the chromatograms of the HPLC/spectrofluorimetry for the same sample analysed by both methods. Clearly no 4-OH-OTA was detected in the samples analysed by Dr. Dekant's group.

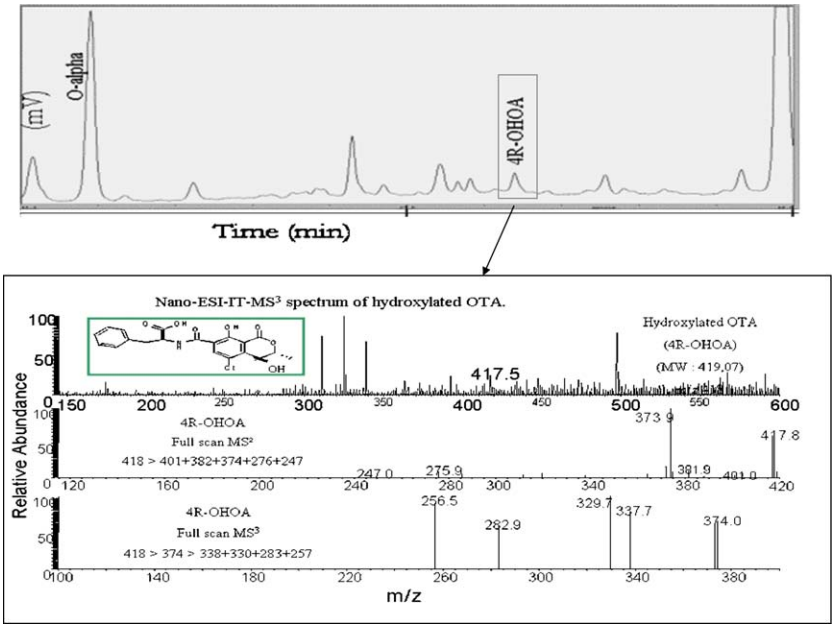

Fig. 4. Separation of OTA metabolites excreted in urine and mass spectrum of 4-OH-OTA isolated from urine.

What could be the explanation? It is well known that OTA binds to serum albumin and most probably 4-OH-OTA and Dekant's group precipitated the proteins before analysing OTA and metabolites! We, therefore, went further in our investigation and analysed the presence of 4-OH-OTA in urine of rats treated by OTA using mass spectrometry. The mass spectrum presented in Fig. 4 was obtained by Virginie Faucet in Toulouse. It clearly demonstrates that 4-OH-OTA is excreted in the urine.

All these data were presented to the E.U. working group. One may wonder how such an article was accepted without modification within 29 days and published within 4 months, while other are held for months.

We hope that this answer clarifies the scientific aspects of the comments from Dr. Dekant. However, we are bound to add that we found the admonition by Dr. Dekant concerning ethics to be rather hollow in view of our experiences of his behaviour to us and other colleagues in the EU Partnership, caused by his own far more serious and damaging ethical breaches.

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