# DIOXIN ANALYSIS: NEED FOR A NORMALISATION OF THE EXTRACTION TECHNIQUES OR TEQ UNITS AND USEFULLNESS OF MULTIVARIATE STATISTICS FOR PROFILES STUDIES

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## Introduction

Regarding dioxin analysis in feedstuff from animal origin, the TEQ has to be expressed relatively to the total sample weight, but no normative extraction method is recommended. As different extraction processes do not present the same efficiency, particularly in term of fat recovery, the amount of extracted analytes is highly dependent on the method used. The first objective of this work was to show a consequence of this problem in term of legal decision for a sample, considering the quantitative result obtained with two different extraction techniques compared to the maximum authorised level. The second purpose, based on congener profiles in various matrices ($n$=181), was to demonstrate the usefulness of multivariate statistics as a tool to build a descriptive and decisional model.

## Material and Method

*Samples.* The comparison between different extraction procedures was realised on three samples, including one raw fish flour and two derivative commercial products from a fish industry. For the contamination profile studies using multivariate statistical analysis, 181 samples (30 cheeses, 91 milks, 3 eggs and 57 trouts) coming from different French veterinary services were analysed. *Statistics*. Statistical analysis were conducted using Statistica Software v. 5.5 (Statsoft). *Apparatus.* A Dionex ASE 300 was used for the extraction, and the GC-HR-MS detection was performed on a HP 6890 gas chromatograph, equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 mm film thickness), and coupled to a Jeol JMS-700D high resolution mass spectrometer. *Reagent and Chemical*. All the organic solvents (Promochem) were Picograde® quality. Silica (Fluka), sodium sulfate and dipotassium oxalate (Merck), acetic acid and sulfuric acid (SDS) were of superior analytical quality. Native and 13C-labeled PCDD and PCDF standards were purchased from Promochem. All standard solutions were prepared in toluene and stored in darkness at < 6 °C. *Method.* 10-20 g of fresh sample (equivalent to 0.5 g of fat) were lyophilised, pulverised, and transferred in ASE cells. Pressure and temperature were set to 100 bar and 120 °C respectively. Basically, the extraction solvent was a mixture toluene/acetone 70:30 (v/v), and four successive extraction cycles (5 min each) were performed. The extract was evaporated to dryness, permitting the estimation of the fat weight. A three steps purification process was then performed, using successively silica, florisil and celite/carbon columns. TEQ values were calculated considering the total weight or the fat weight.,Contamination profiles, i.e. the relative intensities of each congeners, were also studied. In addition, the fish flour and the two derivative products were analysed again replacing the extraction solvent toluene/acetone by toluene/acetic acid 94:6 (v/v).

**Results and Discussion**

The fat content and the TEQ values obtained after analysis of the fish flour and the two transformed commercial products, performed either with extraction solvents acetone or acetic acid, are presented in Table 1. Concerning these results, the following conclusions can be made. **(1) :** a significative difference between the two solvents in term of fat extraction efficiency was noticed. Indeed, it appeared that acetic acid increase the extracted fat amount from a factor 1.3 to 2.9. This observation was in accordance with the European decision 71/393/EEC, that recommends an acid hydrolysis for the total fat analysis in foodstuffs of animal origin. **(2) :** a significative difference between the two extraction procedures in term of TEQ reported to the total weight. Indeed, this value (between 1.2 and 2.6) appeared more important with the acetic acid extraction. This observation could be linked to the previous result, considering that acetic acid permit to extract a high amount of fat, so consequently a high amount of dioxin contained in this fat. The TEQ value reported to the fat weight was effectively similar between the two methods. **(3) :** the contamination profiles, i.e. the relative abundances of the different congeners reported to the total signal, were found very similar with the two extraction procedures (Figure 1). This observation indicated that the selectivity of the two solvents were equivalent and that the difference noticed in term of TEQ reported to the fresh weight was not due to one particular congener with high influence on the TEQ calculation. **(4) :** for product 2, the TEQ value reported to the total weight appeared below the authorised limit in the case of acetone extraction ($0.53<1.25$, $p<0.001$), but above this limit in the case of acetic acid extraction ($1.37>1.25$, $p<0.005$). Finally, this results demonstrated the need to determine a normative way for the extraction or to determine precisely the unit associated to the quantitative result. In the last case, it seems more judicious to report the dioxin amount to the fat weight. **(5) :** the contamination profiles appeared quite different between the three samples (Figure 1). In fact, this phenomenon was already noticed for a long time in this laboratory during routine analysis, including samples of different nature and different geographical areas. Then, it was decided to use multivariate statistics in order to obtain a synthetic graphic representation of a large number of samples, based on their global contamination profile, and to look for a possible discrimination of these samples according to the type of matrix or the origin.

**Table 1.** Fat content and TEQ values obtained for three samples and two extraction methods.

| **Sample** | | **Fat content (%)** | | **TEQ (pg/g total)** | | **TEQ (pg/g fat)** | |
|---|---|---|---|---|---|---|---|
| Extraction solvent | (*nb of analysis*) | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| **Fish Flour** | | | | | | | |
| Toluene/Acetone | (*n=3*) | 8,83 | 0,43 | 1,04 | 0,03 | 13,00 | 0,46 |
| Toluene/Acetic acid | (*n=2*) | 12,45 | 0,13 | 1,27 | 0,01 | 11,20 | 0,00 |
| **Product 1** | | | | | | | |
| Toluene/Acetone | (*n=6*) | 16,63 | 0,67 | 2,92 | 0,17 | 19,07 | 0,51 |
| Toluene/Acetic acid | (*n=5*) | 21,62 | 1,15 | 3,69 | 0,17 | 18,54 | 0,73 |
| **Product 2** | | | | | | | |
| Toluene/Acetone | (*n=5*) | 3,66 | 0,71 | 0,53 | 0,07 | 15,88 | 2,02 |
| Toluene/Acetic acid | (*n=4*) | 10,51 | 0,98 | 1,37 | 0,09 | 14,23 | 0,94 |

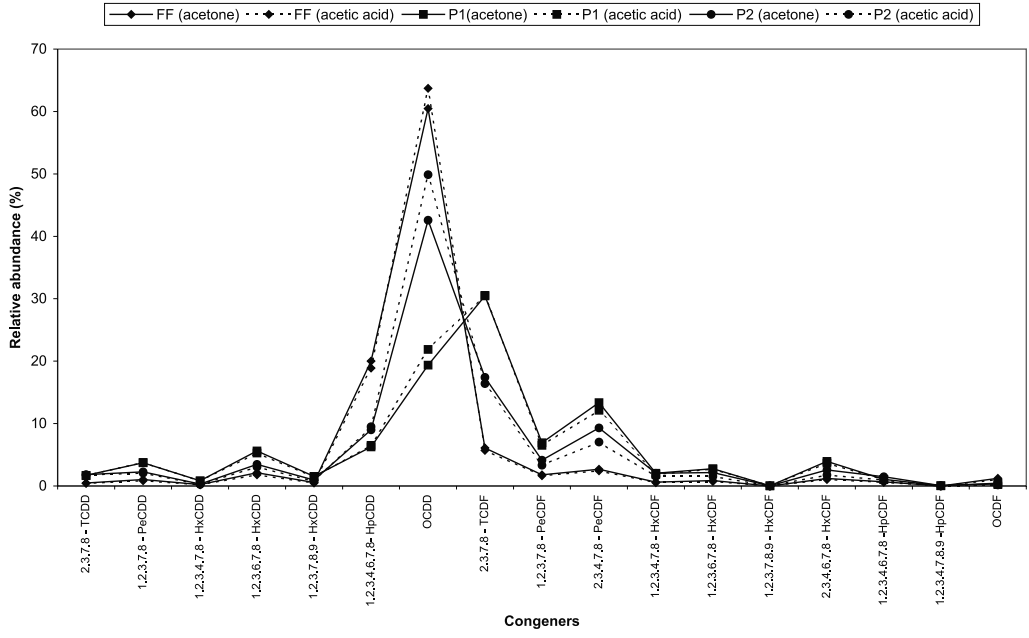


**Figure 1.** Contamination profiles obtained with acetone or acetic acid extraction for the 3 tested samples (FF: fish flour, P1/P2: derived products 1 and 2).

Each sample was considered as one observation defined by the relative abundances of the 17 measured congeners. Each congener was considered as a statistical variable, with a value varying among the different samples, so potentially discriminant between different existing sample groups. Discriminant factorial analysis (FDA) were then realised using the incremental step-by-step method. In a first step, the type of matrix (milk, trout, cheese or egg) was defined as the classification variable. The resulting two first factorial axis were built by linear combination of the 13 most discriminant congeners and released 98.8 % of the total variability. The associated graphic representation (Figure 2) clearly revealed the existence of a “matrix effect” on the observed contamination profiles, at least between trout, milk and egg samples. In a second step, only the milk samples were considered and the geographic origin (French department 29, 35, 59, 73 or 72) was defined as the classification variable. The resulting two first factorial axis were built using 14 congeners and released 81.2 % of the total variability. The corresponding graph (Figure 3) indicated a non homogenous distribution of the different samples, and the possible existence of a “geographic origin effect” on the milk sample contamination profiles. This statistical approach, already used to study the correlation between the physico-chemical properties and the toxicity of organohalogenous pollutants, appeared promising to demonstrate the existence of significant different contamination profile between a large number of samples. But the real interests of this technique are double. On one side, the model can be only used as a diagnostic tool, permitting to confirm a result, for example in case of a doubt on a possible contamination between fish and milk samples analysed on a same batch. On the other side, it can be considered as an exploratory tool, permitting to revealed the existence of different discrimination origins which has of course to be investigated, for example biological, environmental, biochemical or metabolic factors.

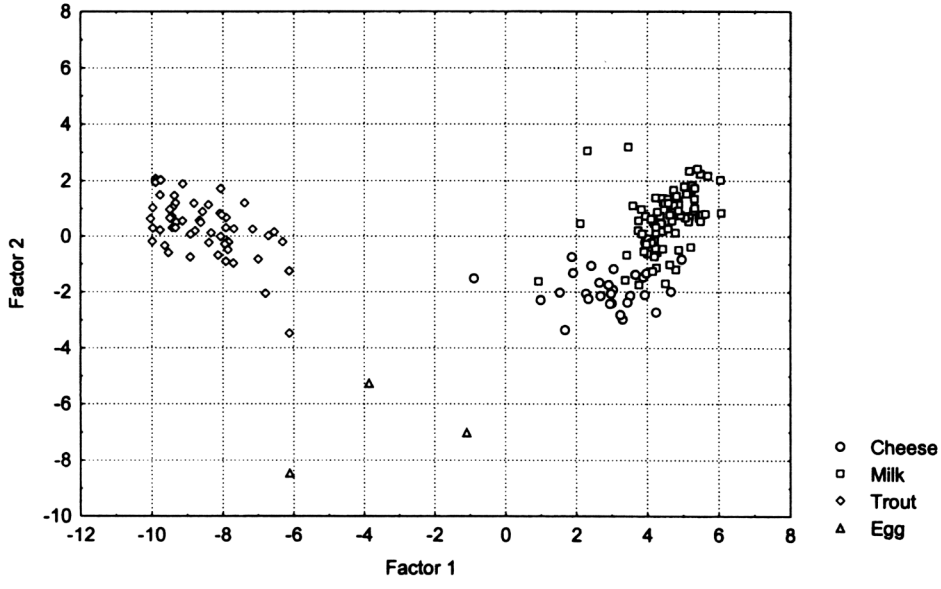


**Figure 2.** Results of the FDA showing the existence of a matrix effect on the observed contamination profiles.

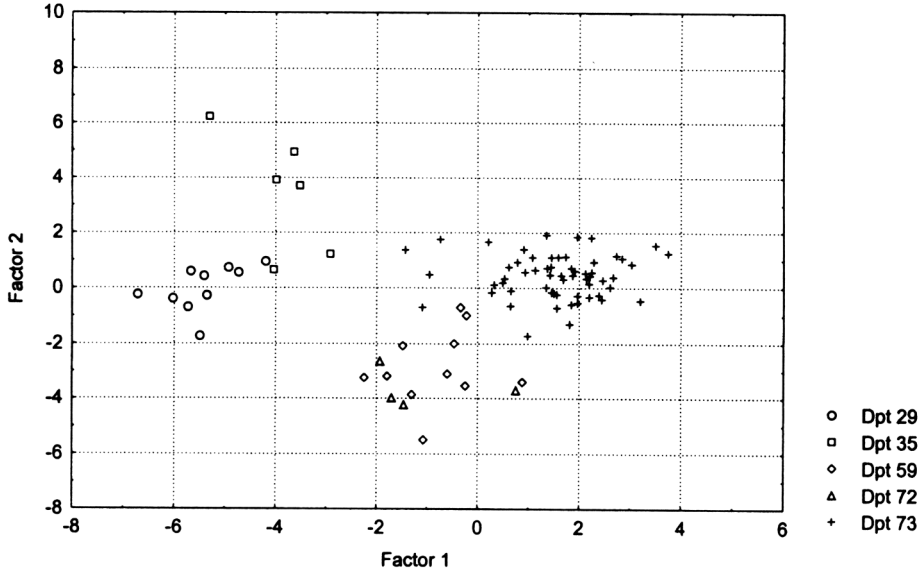


**Figure 3.** Results of the FDA indicating the possible existence of a geographic effect on the observed milk contamination profiles.