To: Mr Frans Verstraete European Commission
From: EURL mycotoxins & plant toxins; Hans Mol, Patrick Mulder, Monique de Nijs
Date: July 2, 2021

Brief comments on the question posed by the European Commission to the EURL mycotoxins & plant toxins on the method for analysis of HCN in almonds.
On June 15, BDSI, Caobisco, ESA and Frucom sent a joint letter to the commission in which they ask if the Commission could provide advice concerning the preferred detection method to be used for quantification of HCN in almonds.

For the quantification of total hydrocyanic acid (HCN) (free and originating from cyanogenic glycosides) in food and feed samples various methods exist. Many of them involve enzymatic hydrolysis of cyanogenic glycosides (HCN-precursors) from the sample to HCN, followed by determination of HCN. Alternatively, intact cyanogenic glycosides can be determined as such (and then reported as HCN equivalents). In the literature, also methods have been reported that determine only free HCN. These may result in an under estimation of total HCN.

The methods based on enzymatic hydrolysis and determination of HCN have several critical steps which need to be optimized and controlled. These include potential autolysis during homogenisation in case of intact commodities, quantitative release of HCN from its precursors (parameters: type/amount of enzyme used, incubation conditions), and potential loss of HCN (volatile) from the extract after hydrolysis. Regarding the determination of cyanide as a reaction product, the reaction needs to be quantitative, and the derivative needs to be selectively determined.

At the moment, the EURL considers CEN16160 as the preferred method of analysis for determination of total HCN in almonds. Other methods might be suited but equivalence would need to be demonstrated. In addition, it is recommended that samples are analysed by laboratories that have an ISO17025 accreditation specifically for the quantification of total HCN in almonds.

The CEN16160 method was originally developed for the analysis of total HCN in feed samples, and has been interlaboratory validated for various matrices including almonds. The method involves extraction by acid solution, enzymatic hydrolysis of HCN-precursors to HCN at controlled pH, steam distillation of HCN into alkaline solution, derivatization of cyanide with a reagent (NDA) to form a fluorescent complex. This derivative is then measured by HPLC with fluorescent detection.