



Protecting our food: Can standard food safety analysis detect adulteration of food products with selected chemical agents?



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ABSTRACT

The food we eat and water we drink is routinely tested for a range of biological and chemical contaminants, which can be hazardous to human health, as part of food safety legislative requirements. The vulnerability of the food industry to deliberate contamination events, rather than naturally occurring events, was explored as one aspect of the EU FP7 project EDEN (End-User Driven Demo for CBRNe). We wanted to investigate if routine food safety testing could detect deliberate contamination with three chemical contaminants and three matrices (cooked ham, sugar and water). The contaminants selected had to be hazardous to human health at levels in the final food product that could occur with a deliberate contamination event.

Standardised reference panels were developed and homogeneity and stability were tested prior to distribution for food safety chemical testing, as required by EU legislation, in the meat food chain (cooked ham and water) and the sugar food chain (sugar and water). Each reference panel contained 11 samples analysed in triplicate (33 analyses per matrix). The meat food chain panels contained bromadiolone (a rodenticide) in the meat and sodium trifluoroacetate (a simulant for a toxic pesticide) in the water at levels from 0 to 4000 parts per million (ppm). The sugar food chain panels contained mercury chloride in both the sugar and water, at levels from 0 to 12 500 ppm. The food safety standard chemical analysis methods were compared to the following external laboratory methods for the meat food chain panels: liquid chromatography coupled to mass spectrometry for meat and nuclear magnetic resonance spectroscopy for water. Inductively coupled plasma with mass spectrometry was used to analyse the sugar food chain panels containing both sugar and water samples. Neither the meat nor the sugar food safety methods detected contamination in any of the samples whilst the external laboratory correctly identified and quantified the contaminants in all the samples.

The results for these three contaminants (bromadiolone, sodium trifluoroacetate and mercury chloride) are not surprising given that they are not the target of today's food safety testing procedures. These limited results are of note and highlight food chain vulnerability to deliberate contamination events with novel contaminants. The EDEN project is exploring a 2-level approach: screening food with non-specific detection tools which are supplemented by targeted detection tools when an alert is triggered. This approach could lead to increased consumer protection whilst simultaneously reducing the economic burden of testing and product recall for the industry.

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Abbreviations: CBRNe, Chemical, Biological, Radiological, Nuclear and explosive; EDEN, End-User Driven Demo for CBRNe; EU, European Union; EU FP7, European Union Seventh Framework Programme; FAO, Food and Agriculture Organization; HACCP, Hazard Analysis and Critical Control Points; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; ICUMSA, International Commission for Uniform Methods of Sugar Analysis; GC-MS, Gas Chromatography Mass Spectrometry; GFAAS, Graphite Furnace Atomic Absorption Spectrometry; LC-MS, Liquid Chromatography Mass Spectrometry; MRLs, Maximum Residue Levels; NMR, Nuclear Magnetic Resonance; PCB, Polychlorinated Biphenyl; WHO, World Health Organisation.

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1. Introduction

Routine food safety testing is carried out, according to legislative requirements [1–8], in food products to detect biological and chemical contaminants that can occur naturally or accidentally during the food production process. Deliberate contamination of our food chain is thankfully a very rare event. However, the potential consequences of a deliberate attack can be disproportionately large [9]. The European Union (EU) Bio-preparedness Green paper [10] concluded that the existing food safety framework needed to be complemented by a new framework that included security aspects, such as food defence practices.

The asymmetrical threats that food defence practices hope to prevent, or respond to, stand in contrast to naturally or accidentally occurring contamination events (Fig. 1). Food safety testing is based on scientific knowledge of the critical points during the food production process combined with an understanding of the likelihood of natural and accidental contaminating agents in that food chain, the HACCP (Hazard Analysis and Critical Control Points) principles [11]. Using the same approach in food defence could be problematic where the motivation for an attack can be political, criminal or economic and the agents used may be novel to the food chain in question [12,13]. Historically we have global evidence of malicious contamination events from both a criminal and terror perspective ranging from the addition of foreign matter to food and drink products (physical, like metal objects, as well as chemical contaminants), contamination of an allergen free production facility with allergenic material, to the infection of salad bars with *Salmonella* bacteria by a cult [12,14].

The EDEN project, End-User Driven Demo for CBRNe, is a large EU FP7 project in the field of societal security with one aspect ad-

ressing potential CBRNe incidents in the food chain. One of the aims of EDEN is to shorten response time after an event as well as increasing food chain resilience with the development of affordable and rapid detection tools. End-users were asked to identify gaps and needs in prevention, preparedness, response and recovery to CBRNe incidents in the food chain [15,16]. Scenarios were developed based on exploring these further [17] and novel tools are currently under development to meet some of the gaps. The first step towards measuring an effect of EDEN was the establishment of the baseline response and resilience within the food chains being studied. The EDEN project wanted to explore how vulnerable different food products were to deliberate contamination and whether current food safety methods would be able to detect contamination in the final food products. The food chain products chosen for testing were processed ham, granulated white sugar and water. Water was chosen as it is used in the production process as well as being a simpler analysis matrix than meat and sugar. The efficacy of standard food safety testing methods at detecting the chemical agents, chosen during scenario development, was compared to testing at an external chemical identification laboratory, not affiliated with the food industry.

2. Standard food safety methods

Food safety programs prevent unintentional contamination of food products and refer to conditions and practices able to preserve the quality of the food. They aim to prevent contamination and foodborne disease. The EU food safety programs are based upon the HACCP principles. HACCP is a systematic risk analysis approach used for the identification, evaluation, and control of food safety hazards [18].

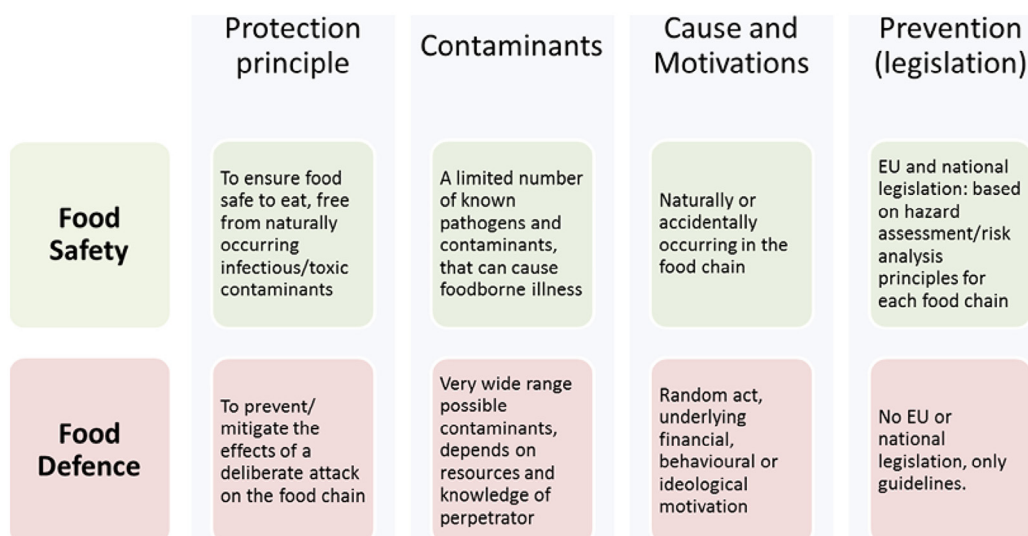


Fig. 1. The differences in food safety and food defence regarding protection principle, contamination, cause and motivations and prevention.

In the meat sector, in particular, the main focus is on the control of microbiological hazards such as bacteria and parasites [19]. Meat industries perform, during the whole production process, a wide range of microbiological analyses aimed at detecting the most common pathogenic microorganisms associated with meat and meat products [20]. Analysis of the raw ingredients, as well as additional analyses during production and in the final product, ensures food quality, food safety and that general hygiene measures are maintained [21]. Food safety testing is carried out from farm to fork including official controls performed by the national food safety authorities at the abattoir [22]. The control of the main chemical hazards are also guaranteed by checks and analyses carried out by the national food safety authorities. The list of chemicals to be tested for includes antibacterial substances and other veterinary drugs, environmental contaminants, such as PCBs and organophosphorus compounds, as well as mycotoxins and dyes [23]. For this reason, as well as cost, the standard chemical analyses performed by meat industries are only aimed at establishing the nutritional content and evaluating the quality profile of the raw material including auditing suppliers. The main chemical analyses performed by the meat industries are: pH (method PD23); determination of water activity (method UNI UNI 11302:2009); moisture content (method UNI ISO 1442:2010); total lipids (method UNI ISO 1444:2010); total proteins (method UNI ISO 937:1991); ash content (method UNI 10590:1997); sodium chloride content (method PD25); and collagen content (method ISO 3496:1994).

The sugar industry adheres to Codex Stan 212–1999 [24] for food safety testing purposes for sugar that is to be commercially available, either directly as sugar or if used in other food products. It lays down requirements to ensure that the concentrations of heavy metals and pesticides are not hazardous to human health. Consequently, sugar factories have implemented sampling and analysis programs in order to ensure that their final products comply with current regulations before being dispatched.

The EU has harmonised the maximum residue levels (MRLs) of pesticides [7] in sugar products (e.g. sugar beet, sugar cane, maple, palm etc., collectively termed sugar plants). The sugar plant category includes over 380 pesticides and their corresponding MRLs. EU legislation describes the quality control and validation procedures for the analysis of pesticide residues in food and feed products [25]. Gas and liquid chromatography mass spectrometry (GC-MS and LC-MS) systems are normally used. Heavy metal analysis is described by the International Commission for Uniform Methods of Sugar Analysis [26]. The sugar industry routinely tests for the following heavy metal contaminants: arsenic, copper and lead using atomic absorption spectroscopy (AAS), colorimetric methods and

graphite furnace atomic absorption spectroscopy (GFAAS) respectively.

3. Chemical contaminants

The choice of chemical contaminants, which could be used in a malicious attack on the food industry, consisted of several evaluation steps in order to identify the most suitable agents. The initial list consisted of over 50 chemical compounds pre-selected if they fulfilled one or more of the following criteria:

- Used in previous food poisoning incidents [14]
- Listed as a toxic industrial chemical
- Listed as pesticides, including herbicides and rodenticides
- Listed as pharmacological substances, including veterinary medicines

This list was refined based on a number of other criteria including physical and sensory properties, availability as well as chemical stability and toxicity in the selected food chain and production processes. The agents chosen needed to be hazardous to human health, at concentration levels that could easily be achieved in a deliberate contamination event, whilst not affecting the smell, texture, colour or taste of the food product. On the basis of these criteria, we chose the following two chemicals for contamination of the meat food chain: bromadiolone (a rodenticide) in cooked ham and sodium trifluoroacetate (a simulant for a more toxic pesticide sodium fluoroacetate) in water. We chose mercury chloride (a heavy metal salt) for contamination of the sugar food chain, both in granulated white sugar and water.

4. Food safety analysis and chemical contaminants

First of all homogeneity and stability testing was carried out for contaminated meat and sugar samples under different storage conditions and for up to four weeks of storage. Then reference panel sets were produced for the meat food chain (cooked ham and water) and the sugar food chain (sugar and water). Each set contained 11 samples. The meat food chain panels consisted of minced cooked ham and water contaminated with 0 to 4000 parts per million (ppm) of bromadiolone for the cooked ham and sodium trifluoroacetate for the water. The sugar food chain panels were made up of sugar and water contaminated with 0 to 12 500 ppm of mercury chloride. Each sample of cooked ham and sugar weighed 20 grams while 20 ml water samples were prepared and subsequently analysed in triplicate (Table 1).

Table 1

The different reference panel sets each containing 11 samples per food matrix and contaminant. Each sample weighing 20 g (meat, sugar) or 20 ml (water) and analysed in triplicate

Food Chain	Contaminant	Contamination level	Concentration (ppm)	No. samples prepared	No. analyses
Meat	Bromadiolone (cooked ham) Sodium trifluoroacetate (water)	None	0	3	9
		Low	4	3	9
		Medium	40	2	6
		High	400	2	6
		Very High	4000	1	3
		Total no. meat samples			
Total no. water samples					33
Sugar	Mercury chloride (sugar and water)	None	0	3	9
		Low	13	3	9
		Medium	125	2	6
		High	1250	2	6
		Very High	12 500	1	3
		Total no. sugar samples			
Total no. water samples					33

The meat samples were analysed according to Adamowicz et al. [27]. A sub sample of 1 gram was taken from the meat samples and extracted three times with 3 ml of acetone followed by centrifugation for 5 minutes at 1562 relative centrifugal force (rcf). The extracts were combined and after evaporation until dryness, on a heat block at 60°C under a gentle flow of nitrogen gas, 1 ml of acetonitrile was added. The samples were shaken on a whirl mixer for 30 seconds and filtered through a 0.22 µm filter, before analysis on a Dionex Ultimate 3000 RS liquid chromatograph coupled to a Bruker Daltonics MicroTOF-Q III mass spectrometer (LC-MS) system in negative electrospray ionisation (ESI) mode.

The water samples of the meat food chain were analysed using quantitative ¹⁹F nuclear magnetic resonance (NMR) spectroscopy on a Bruker Avance III 600 MHz NMR spectrometer. Sub samples of 0.4 ml were analysed with deuterium oxide contained in coaxial insert tubes for lock. Each experiment consisted of between 64 and 512 scans depending of the level of contamination. To ensure quantitative spectra the relaxation delay was set to 13 seconds.

Sub samples of 0.1 gram sugar or 0.1 ml water taken from the sugar food chain samples were added 9.9 ml of water containing 0.5% nitric acid. Samples with low levels of contamination were diluted 1:100, samples with medium levels of contamination were diluted 1:1000 whilst the high and very high level contaminated samples were diluted 1:10 000 and 1:100 000 respectively. All the samples were then analysed using a Thermo Scientific Xseries2 inductively coupled plasma with a mass spectrometer (ICP-MS) system for total mercury content.

All the applied analytical techniques (LC-MS, NMR and ICP-MS) identified the contaminants added to the meat and sugar food chain samples and gave quantitative estimations of the contents based on calibration curves for the actual contaminants on each instrument. Homogeneity and stability testing showed that the meat and sugar sample results were consistent throughout the four week testing period, under optimal storage, when analysed as described above.

A set of meat food chain reference panel was sent to the meat industry partners and a set of sugar food chain reference panel was sent to the sugar industry partners for standard food safety chemical analysis, as described in section 2 above. The food safety tests were not able to detect contamination in any of the samples.

5. Discussions

The probability and severity of natural and accidental contamination will depend on the different food matrices, many of which provide ideal environments for fungi, virus and bacteria [28]. The severity of natural and accidental contamination can range from product losses to serious human health risks, depending on the agents involved and the food chain [29]. Since food analysis comes with intrinsic costs, in terms of investment, personnel and analysis time, the food industry has directed the testing schemes to the most probable hazards and critical control points (HACCP). As a result, food safety testing is highly developed in the food industry as proven by the extensive national and international food safety regulations. In addition to this, a number of stricter private safety schemes are implemented in some food chains to guarantee an even higher level of safety for consumers [30]. The food safety chemical testing carried out here was focused on food quality indicators such as pH, humidity, water activity, collagen and protein content (meat) as well as pesticides and heavy metal analysis (sugar). Deliberate contamination can therefore be difficult to detect with routine food safety testing for chemicals since the contaminants can be foreign to the food chain in question. Both the contaminant and the point of contamination are chosen by an imaginative human mind [13,31].

This study shows that, with these three chemical contaminants and three matrices, the food safety analysis methods were not sufficient, in stark contrast to the external chemical identification laboratory results. Although the sample size was limited, just 33 analyses in each matrix, these results are still of note, since standard food safety testing, for chemicals, would have cleared these samples for human consumption. The negative findings are perhaps not that surprising given that the contaminants used (bromadiolone, sodium trifluoroacetate and mercury chloride) are not the target of today's food safety testing procedures. Unlike the food safety testing, the specialised laboratory did not restrict analysis to only a few potential contaminants. The broad range of potential contaminating substances and different food matrices, in a food defence incident, makes this a challenging subject. Without further testing we cannot be certain regarding other contaminants and other food matrices.

These results highlight the potential vulnerability of the food chain to deliberate contamination events. The food industry therefore needs to implement cost effective measures (food defence principles) that can complement current food safety testing. Current food safety testing needs to be combined with non-specific detection methods, preferably with in-line or at-line detection capabilities. This is especially true for contaminants that do not alter smell, texture, colour or taste of the food product. The EDEN project has explored a number of promising technical solutions for food testing (EDEN unpublished data). This study does not take into account other control measures the food industry may have in place, unrelated to food safety testing, like security cameras and controlled access.

This work has led us to propose a two level approach, combining food safety and food defence, for routine food analysis. The first level of protection uses non-specific (untargeted) detection tools, to detect changes in conformity in the food product and provide alerts when these are out of the accepted range. The second level is triggered by the alert and activates deployment of targeted detection tools that should be able to quickly identify the specific contaminant and allow for a quick response. A contaminated food product using only food safety testing would enter retail whilst, using this two level approach, the contaminated samples would be detained during production. Thus, this two level scheme integrating food safety and food defence could substantially increase consumer protection and simultaneously reduce the overall costs of testing and product recall for the industry. These technological solutions are currently being tested during the EDEN demonstrations to help define the technical, operational and forensic potential of this new proposed approach. The final outcome of the economic feasibility will depend on the non-targeted tools having multi-use potential like determination of food quality, food safety, and food defence aspects.

6. Conclusion

The EDEN work package dealing with food defence identified that routine chemical food safety analysis methods were unable to detect deliberate contamination with three chemical contaminants. This highlights the need to augment routine food safety testing with food defence principles. The EDEN project is exploring a two-level approach combining non-specific detection tools during the production phase together with specific identification tools if an alert is triggered.

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