



Review

Food and feed safety: Cases and approaches to identify the responsible toxins and toxicants

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ABSTRACT

There are food and feed safety monitoring programs to protect consumers. These programs however, are strongly focused on known and regulated substances. New or unexpected substances that might be of risk for consumers will thus escape routine controls. These risks are therefore mainly discovered by human or animal intoxications. All kind of analytical chemical methods, in-vitro bioassays, tracking, and chain analysis are then used to reveal the substance(s) responsible for the intoxication. Only in a few occasions (new) risks were revealed in time by analytical chemical methods or cell based in-vitro bioassays. This paper describes some relevant food and feed safety cases and how the causative substances were identified. This overview strongly indicates that more intense monitoring, including the use of cell based effect bioassays, can reduce the number of intoxications. Moreover, registration and follow-up actions should be arranged in a better way, for example by sharing information within the scientific communities or by establishing a national contact point. In addition, a strategy based on broad screening and bioassay directed identification with liquid chromatography high resolution mass spectrometry is proposed to prevent intoxications and identify toxin and toxicants relevant for food and feed safety.

1. Introduction

Consumers demand that food is safe and free of any risk. Food safety requires that producers guarantee the absence of potentially health threatening compounds in their products. This involves every entrepreneur involved in the food chain, starting with the production of feed for farm animals. It also requires that food of both plant and animal origin is produced in non-polluted areas. The increased demand for keeping animals outside thus pressures farmers to ensure that the soil contains no elevated levels of environmental contaminants that may transfer to food. Besides the feed and soil, farmers should be aware that other materials entering their farm are not contaminated, as shown by a number of cases where the introduction of building debris or presence of contaminated coatings on roofs or walls on farms with laying hens caused non-compliant levels of dioxins and PCBs in eggs (Piskorska-Pliszczynska, Mikolajczyk, Warenik-Bany, Maszewski, & Strucinski, 2014; Schoeters & Hoogenboom, 2006; Winkler, 2015). This also includes litter used in stables for animal welfare, as shown by incidents with dioxins in wood shavings.

But the environment is not the only source of chemical food safety

risks. Also nature itself can be an important source of toxins like marine biotoxins produced by e.g. algae or plant toxins which can become an unwanted constituent of e.g. herbal preparations for use as feed or food supplement. Or even the natural occurrence of the banned antibiotic chloramphenicol in straw and herbs which may lead to non-compliant products of animal origin due to contaminated feed (Berendsen et al., 2010). Finally, we also, frequently indescribably, use veterinary drugs in order to improve the health and production efficiency of food producing animals.

In order to assure that food is safe, consumers demand that authorities check whether producers obey the law and their responsibilities. This is achieved by visiting producers and checking the production process, but also by taking samples at different stages of the production process (chain) and of the end products at farms, companies and shops. These samples are then analysed for the presence of regulated residues and contaminants. In this regard it is important to recognize that illegal practices may result in interesting profits for producers. Raising animals with hormones or other growth promoters, an illegal practice in the EU and many other countries, results in a better feed conversion ratio but is not without risk, e.g. in case of β -agonists like clenbuterol this can lead

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to cardiac hypertrophy in both humans and animals (Brambilla et al., 2000; Brambilla et al., 1997). Adding melamine to infant formula prevented the losses of profits due to poor milk quality, but led to a large number of diseased children and even several deaths (WHO, 2009). In other cases, both producers and authorities may simply be unaware of the presence of compounds that cause a potential risk to animals or consumers e.g. due to the presence of specific plant toxins in grain based products for infants (Mulder, Pereboom-de Fauw, Hoogenboom, de Stoppelaar, & de Nijs, 2015).

It is clear that it is virtually impossible for authorities to check every single feed or food item, also considering the high costs of sampling and analysis. Green waste plans for a future circular economy will make things even more complicated, as more waste streams will enter our food chain. This presents a considerable challenge to determine where and what to look for, a process referred to as risk-based monitoring. It is no surprise that the focus of authorities will be on regulated substances, since it is difficult to follow-up elevated levels of substances with no legal limits. However, the General Food Law (Anon, 2002) in the EU requires or at the least offers the opportunity to follow-up increased levels of any substance that would lead to a risk for the consumer. The latter can only be achieved when toxicological data are available that allow a proper risk assessment. It does not apply to novel and emerging risks.

In practice the question is how such novel and emerging risks can actually be discovered before causing adverse effects in animals and humans. Here new techniques like animal-free in-vitro cell based effect bioassays and untargeted mass spectrometric (MS) analysis may offer some opportunities. In-vitro effect based bioassays are test based on living cells, including bacterial, yeast and mammalian cells. Examples are the bacterial inhibition assays to detect antibiotics and yeast or mammalian cell lines that contain a specific target for a certain compound or compound group (Bovee & Pikkemaat, 2009). An example of the latter is the DR-CALUX bioassay, making use of a rat liver cell line expressing the aryl hydrocarbon receptor (AhR) and upon activation of the AhR by dioxins and planar PCBs also luciferase, which activity can be measured by luminescence (L.A.P. Hoogenboom et al., 2004). The present paper will first describe a number of cases and how they were discovered and handled. These include a number of well-known examples, but also a number of incidents where RIKILT (independent Research Institute on Food Safety with official statutory tasks) was involved in identifying the responsible agents, using an approach of in-vitro bioassays and chemical analytical methods. This will be followed by a discussion on lessons learned and future strategies to discover such emerging risks at an early stage, i.e. before animal or human intoxications.

2. Cases

2.1. Aflatoxins in feed and food

One of the first incidents described in literature on poisoning of animals via feed was the so-called turkey-X-disease. The “disease” occurred in the early sixties, when a large number of turkeys died after consuming feed prepared from Brazilian peanut meal (Blount, 1961). Only after an intensive search it could be concluded that a mycotoxin produced by the mould *Aspergillus flavus* caused the acute toxic effects in the animals (Sargeant, Sheridan, & Okelly, 1961). Subsequent studies revealed that this compound, called aflatoxin B1, caused liver tumours in rats and was a genotoxic compound (Barnes & Butler, 1964). These observations then triggered further studies on a potential relation between the intake of this toxin and elevated incidences of human hepatocellular carcinomas in certain areas of the world. Indeed, epidemiological studies confirmed such relationship and as a consequence, aflatoxins (aflatoxin B1, B2, G1, G2 and M1) are regarded as human carcinogens (Ming et al., 2002; Ostry, Malir, Toman, & Grosse, 2017; Qian et al., 1994; R. K.; Ross et al., 1992). However, based on the effects

observed in studies with aflatoxins, it also became apparent that the original toxic effects observed in the turkeys could not be fully ascribed to aflatoxins (Cole, 1986). This led to the discovery of high levels of cyclopiazonic acid (CPA) in the original feed material, a mycotoxin produced by many *Aspergillus* strains, including *Aspergillus flavus* (Gallagher, Richard, Stahr, & Cole, 1978). Up to now there are no limits for CPA and most analytical methods for mycotoxins are unable to detect this toxin.

This case on aflatoxins clearly shows that follow-up studies on poisoning of animals are essential to discover and unravel an emerging risk. Subsequent studies on the properties of the compound led to the discovery of its carcinogenic properties in humans, which might otherwise have been unnoticed since such long-term effects are difficult to relate to consumption of certain food products. This case also demonstrated that there is a serious risk of tunnel vision, once a potential candidate is in the spotlight. Further studies to demonstrate the relationship between exposure and effect are thus required to avoid that other toxins are overlooked, such as CPA in this case. It is unclear to what extent this applies to other species of moulds, algae, bacteria or plants, capable of producing a mixture of toxins. It should be emphasised that in some cases it may be difficult to obtain the required amounts of toxins to perform a study with animals and data can only be obtained by using the contaminated food or feed product itself. In-vitro effect based bioassays for the different natural toxins might actually be helpful to confirm causal relationships between intoxications and the presence of toxins in feed or food.

2.2. Melamine in feed and food

One of the most well-known and tragic human intoxications due to food fraud is the melamine scandal in China in 2008 (EFSA CONTAM Panel and EFSA CEF Panel, 2010). Milk powder was adulterated with melamine, a compound high in nitrogen. As quality control of milk powder often includes the determination of total nitrogen content as a measure of the protein content, adulteration with melamine suggests a high protein milk powder. Melamine can cause crystal formation in the urinary tract, leading to kidney damage. In severe cases, consumption of food or feed contaminated with melamine is lethal. In 2007, cats and dogs died in the US after consumption of pet food contaminated with melamine and cyanuric acid. More precise, the pet food contained a rice protein concentrate imported from China which contained melamine and cyanuric acid. In 2008, 300,000 human infants were intoxicated in China by an infant formula adulterated with melamine. Intoxicated infants had melamine related crystalline stones in kidney, ureter and bladder and six deaths were confirmed due to melamine intoxication (WHO, 2009). Currently, melamine is analysed mainly by LC-MS/MS and in 2017 a successful inter laboratory validation study was published by Fry et al. for the determination of both melamine and cyanuric acid in animal feed (Fry et al., 2017). For milk powder several validated methods have been published of which some beside melamine also include the detection and measurement of other nitrogen-rich adulterants (Desmarchelier, Cuadra, Delatour, & Mottier, 2009; Frank, Bessaire, Tarres, Goyon, & Delatour, 2017).

2.3. Acrylamide in food

Another incidental finding from a chemical analysis is the presence of acrylamide in many of our food products. This was discovered in a study on haemoglobin adducts in workers in Sweden, using acrylamide containing paints in a tunnel (Hagmar et al., 2001). To be more precise, these workers showed the expected adducts, but the adducts were also detected in a control group. Follow-up studies showed that the acrylamide exposure of the control group was via consumption of all type of food products, in particular those prepared by frying or toasting, like potato fries and crisps, bread and cookies. Acrylamide analysis is mainly performed by LC-MS/MS. De Paola et al. for example showed

the presence of acrylamide in dried fruits and edible seeds (De Paola et al., 2017). However, also GC-MS analysis is feasible for acrylamide (Mastovska & Lehotay, 2006). Acrylamide is a known carcinogen in rodents, causing a number of different tumours (Dearfield, Abernathy, Ottley, Brantner, & Hayes, 1988). Its discovery triggered a huge number of epidemiological studies to examine a possible relationship between increased intake of acrylamide and certain types of cancer (J. G. Hogervorst, Schouten, Konings, Goldbohm, & van den Brandt, 2007; J. G. F. Hogervorst, Schouten, Konings, Goldbohm, & van den Brandt, 2009; Mucci, 2006). The European Food Safety Authority (EFSA) reviewed these studies in 2015 and could not conclude on such a causal relationship. However, EFSA also noted that the exposure assessment is rather difficult and that these studies may not be suited to exclude that acrylamide is a human carcinogen (Benford et al., 2015). Therefore, the EFSA risk assessment was based on the effects in rodents, showing that the margin of exposure was rather small for a compound with genotoxic and carcinogenic properties.

This case on acrylamide shows that human biomonitoring, including more stable markers like haemoglobin adducts, may be a suitable way to detect exposure to unknown contaminants in the food. Human biomonitoring is an area of research in which human matrices, e.g. urine, blood or hair, are used to determine the exposure of humans to a certain toxin or toxicant. Human biomonitoring is an emerging field and novel analytical techniques may allow a more untargeted approach (Choi, Mørck, Polcher, Knudsen, & Joas, 2015).

2.4. Marine toxins in seafood

Marine biotoxins still cause a lot of intoxications worldwide. Recently Nicolas et al. reviewed the worldwide published incidents with these toxins (Nicolas et al., 2017). The reference method for the determination of marine biotoxins in shellfish was traditionally based on the use of a mouse bioassay (MBA). Nowadays, due to ethical and methodological concerns, this is changed in the EU to analytical chemical methods. Furthermore, the route of administration in the MBA, intraperitoneal injection (*ip*), is completely different than when consuming shellfish (i.e. oral) and several marine biotoxins, such as yessotoxins, are more toxic when *ip* injected than when orally administered (Aune, Sorby, Yasumoto, Ramstad, & Landsverk, 2002). As a result, the MBA will probably overestimate the real risk of some marine biotoxins. Proponents of the MBA are afraid that with the applied analytical strategies new or unknown toxins remain undetected and that, as a consequence, consumers are exposed to potential risks. Cell based in-vitro assays such as the neuroblastoma cell bioassay might be a better and definitely more ethical alternative (Canete & Diogene, 2008; Manger, Woodle, Berger, & Hungerford, 2003).

The limitation of analytical chemical methods is reflected in the discovery of tetrodotoxins (TTX) in Greek shellfish by the MBA (Vlamiš et al., 2015). When introducing the chemical method, HPLC-FLD, for the detection of paralytic shellfish poisons (PSPs), i.e. saxitoxins, samples causing death of mice were investigated with this chemical alternative. Not all deaths could be explained by the presence of PSPs and application of other analytical chemical methods revealed the presence of TTXs. TTX is now seen at a European level as a new emerging toxin in shellfish (Turner, Powell, Schofield, Lees, & Baker-Austin, 2015). It should be emphasised, that application of the neuro-2a bioassay in routine control would certainly have led to positive test results and the possible detection of the TTXs when following-up these results.

2.5. Dioxins

In 1957 large numbers of chickens died or became diseased in the US. The hens showed low fat levels (wasting syndrome) and increased oedema, a disease called chicken oedema disease (Sanger, Scott, Hamdy, Gale, & Pountden, 1958; Schmittle, Edwards, & Morris, 1958). The effects were related to the use of a toxic fat in the feed, a fat that

was scraped from the inside of cow hides. It took about ten years to elucidate that dioxins were the real cause, which required new analytical techniques (Firestone, 1973; Higginbotham et al., 1968). Dioxins were introduced into the fat by the treatment of the cow hides with chlorophenols, containing low but nevertheless significant amounts of dioxins. This was the first reported incident with dioxins.

In January 1999, farmers in the South-West of Belgium noticed problems in chickens like a decreased hatching of eggs, but also chicken oedema disease (Bernard et al., 1999; van Larebeke et al., 2001). A veterinarian at one of the feed companies started investigations but was initially unable to reveal the cause of the problems. After several months he was informed that oedema in chickens was a known effect of dioxins. Even though dioxins in the food chain were no longer assumed to be a real issue, he decided to send samples of chicken fat, eggs and feed to the RIKILT laboratory for dioxin analysis. Here the presence of very high dioxin levels in the samples were confirmed, and subsequently also PCBs (Bernard et al., 1999; L.A.P.; Hoogenboom, Traag, & Mengelers, 1999). It was estimated that up to 200 L of a PCB oil had ended up in 60 tons of vegetable fat that was used for the production of feed for chickens and pigs (L. A. P. Hoogenboom et al., 2004). The discovery caused a huge crisis in Belgium and a complete change in the EU policy on contaminants in the food chain.

In humans, a relatively high exposure to dioxins results in another typical effect, being the skin disease chloracne. This became e.g. apparent in the Seveso incident in the North of Italy in 1976 where 193 children developed chloracne after being exposed to TCDD (Mocarelli et al., 1986). Similar effects on the skin were observed in 1967 and 1979 in two incidents with rice oil in, respectively, Japan and Taiwan, termed YuSho and YuCheng (Hsu et al., 1985; Kuratsune, Yoshimura, Matsuzaka, & Yamaguchi, 1972). In both incidents, about 2000 people were affected. The incidents were shown to be caused by the leakage of a PCB-oil used in the heat-exchange equipment to purify the rice oil. In addition, already at the start of the incident in 1967 large numbers of chicken were affected, due to the preparation of animal feed with a fatty acid distillate obtained during the purification (Kuratsune et al., 1972). In 2004, Victor Yushchenko, the presidential candidate in the Ukraine, showed the very typical skin disease, leading to the suggestion by the British toxicologist John Henry that he was “exposed” to dioxins (Castellani, 2004). This was initially not further investigated until a laboratory in Amsterdam offered to examine his blood with the DR-CALUX bioassay, a test developed for detecting Ah-receptor agonists (Aarts et al., 1993; Denison et al., 2004). The very high test response indicated the presence of dioxins, which was then confirmed by two laboratories applying gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS) analysis (Brouwer et al., 2005; Sorg et al., 2009). A few mg of TCDD were enough to cause the effect.

These dioxin examples show that incidents are often disclosed by effects in animals or humans. They also show that a quick follow-up is essential to elicit the cause and end the exposure of more animals and/or humans. Due to the establishment of maximum levels in the EU and increased monitoring, various other dioxin incidents have been discovered, in most cases by applying GC-HRMS. This includes the Irish dioxin incident in 2008, with levels very similar to those in the Belgian incident, but unnoticed for about 3 months since the pigs and cows fed with the contaminated feed did not show any obvious adverse effects. However, a small increase in levels in a mixed fat derived from gelatine production, although below the maximum level, could have pointed out that something was going on (Heres, Hoogenboom, Herbes, Traag, & Urlings, 2010). The reason to monitor that by-product was actually a previous incident, that was discovered by increased monitoring, applying a high-throughput bioassay for dioxins, i.e. the DR-CALUX (L. A. P. Hoogenboom et al., 2007). Follow-up of a slight increase in levels, in particular pooled milk, actually led to the discovery of the incident with Brazilian citrus pulp caused by contaminated lime in 1999 (Malisch, 2000) and the one in 2004 with potato peels due to the use of contaminated kaolin clay in the sorting process of potatoes (L.A.P.

Hoogenboom et al., 2010).

2.6. Flame retardants in feed

In 1973, farmers in Michigan state observed health problems in their cows, i.e. decreased milk yield, still-born calves and hoof problems (Carter, 1976; Fries, 1985; Reich, 1983). It was hypothesized that the cause was some kind of contamination of the feed. Later it became clear that about 30,000 cattle, 1.5 million chickens, 5000 pigs and 1500 sheep were fed with the same feed and also showed adverse effects. However, it took some time before investigations were started, initially without any success. Samples were e.g. examined for the presence of PCBs, which were still widely used at that time. Only by incident it was discovered that the feed actually contained polybrominated biphenyls (PBBs), compounds with an extremely long retention time on the GC-MS used for the PCB analysis (de Kok, de Kok, Brinkman, & Kok, 1977). They were nevertheless discovered since the technician forgot to turn off the recorder when going to lunch. Knowing about the long retention time, another researcher made the link with the PBBs. Further investigations showed that the company producing the flame retardant mixture, called FireMaster BP-6, had mixed up the bags with that of a feed ingredient called NutriMaster. This caused the very tragic event with the cows and other farm animals.

In 2007, the use of the so called DR-CALUX bioassay for screening of food and feed for the presence of dioxins and dioxin-like PCBs resulted in a “false-positive” test result for the feed additive choline chloride, widely used in feed for chickens. Despite a high bioassay test result, no chlorinated dioxins or dioxin-like PCBs could be discovered in the sample. Initially there was no follow-up, but a similar test result by another laboratory was mentioned at a workshop, merely as proof that the test might not be so useful in practice for some matrices. As there was no sample remaining to examine the cause of what was thought to be a false-positive test result, RIKILT advised the Netherlands Food and Consumer Product Safety Authority (NVWA) to collect additional samples of choline chloride. The false-positive test result was reproduced in a substantial part of the samples (Malisch & Kotz, 2014; Traag, Kotz, van der Weg, Malisch, & Hoogenboom, 2009). The product was then further investigated with GC coupled to time-of-flight mass spectrometry (TOF MS), an untargeted technique. This revealed the presence of a number of brominated flame retardants, like PBDEs and 2,4,6-tribromophenol, and also an unknown compound with a high mass and eight bromine atoms. This compound could not be identified until “octabromo” was googled, revealing only one hit, being a novel flame retardant (OBIND), that was not yet on the market. The compound was ordered and its presence in the feed additive was confirmed. However, this compound was negative in the DR-CALUX assay, similar as the other detected flame retardants. The presence of 2,4,6-tribromophenol led to the hypothesis that the samples might in addition also contain brominated dioxins. Also this was confirmed by GC-MS analysis. It is hypothesized that the company producing the choline chloride also produces the brominated flame retardants, resulting in cross contamination. The levels detected were unlikely to cause a major incident, also regarding the levels of choline chloride in feed (Traag et al., 2009). However, the fact that production lines of industrial chemicals and feed ingredients are not always well separated, indicates a potential risk.

2.7. Medroxyprogesterone acetate and estradiol in sugar syrup

In 2002, pig farms reported problems with pigs becoming infertile and severe problems with pregnant sows regarding the delivery of their piglets (van Leengoed et al., 2002). The effects suggested the presence of an estrogen active compound in the feed. The veterinary faculty of the University of Utrecht (The Netherlands) therefore sent samples to RIKILT, that had just reported the development of a yeast estrogen bioassay for detection of estrogenic compounds. The samples showed a

clear positive response and fractionation of a sample extract resulted in a positive fraction exactly at the retention time where estradiol eluted from the column (Bovee, Bor, Heskamp, Hoogenboom, & Nielen, 2006). It was shown that besides medroxyprogesterone acetate, the feed was also heavily contaminated with estradiol at levels high enough to explain the adverse effects in the sows. The feed contained a sugar syrup that was a rest product derived from a pharmaceutical company producing tablets for the treatment of postmenopausal complaints. This syrup caused a major feed incident in the Netherlands and shows that waste streams can lead to adverse effects in animals and pose a real risk for the animal production. The problem would easily have been picked up by applying the above mentioned validated estrogen bioassay for the testing of feed samples. Moreover, this case is perhaps a presage for what will happen more often when circular economies are extending and emphasizes the need of using strategies and methods that can detect new risks.

2.8. Pyrrolizidine alkaloids

Over 6000 plant species are known to contain toxic pyrrolizidine alkaloids (PAs). For the PAs it is well known that farm animals such as pigs and horses are more susceptible than smaller animals such as rabbits, sheep and goat. Crews & Anderson, 2009 reported on a case where horses were intoxicated by hay containing Common ragwort (*Senecio jacobaea*) (Crews & Anderson, 2009). This intoxication was not fatal, but severe diarrhoea was observed. Analytical chemical analysis revealed the presence of a total PA concentration of 10 mg/kg hay. PAs are hepatotoxic, but in animal studies also shown to be genotoxic and carcinogenic. It is known that the 1,2-unsaturated PAs are metabolically activated to reactive pyrrolic species which are responsible for the hepatotoxicity and genotoxicity (Li, Xia, Ruan, Fu, & Lin, 2011). Human intoxication with high levels of PAs can lead to the so called acute hepatic veno-occlusive disease (HVOD) (Kakar et al., 2010). Most human intoxications are related to the consumption of food supplements, herbal medicines and tea when the harvested crops like grain and tea leaves are contaminated with toxic plants, e.g. from *Crotalaria*, *Senecio* or *Heliotropium* families. For example, grain contaminated with *Heliotropium* seeds have led to poisoning incidents including deaths in India and Afghanistan (Mattocks, 1986; WHO-IPCS, 1988). In the 1970s in North-Western Afghanistan over 1500 people suffered from HVOD after consuming bread prepared from PA contaminated wheat flour (Kakar et al., 2010). Incidents like these indicate the importance of control and monitoring of PAs in various commodities. Previously, mainly commodities were tested in which transfer of PAs occurred into products such as milk and eggs, nowadays also pollen products such as honey, food supplements and tea are of interest. E.g. the latter were investigated by Bodi et al., analysing 274 different teas for total PAs (sum of 17 individual PAs) (Bodi et al., 2014). The total PA concentrations in these teas ranged between below the limit of detection (LOD) (highest LOD was 2 µg/kg for lycopsamine) and 5647 µg/kg.

Methods for detecting PAs strongly improved in the last decade due to new mass spectrometric technologies and the availability of reference standards, which often hampers the development of methods to detect biotoxins. The first methods described in literature to detect PAs were based on thin layer chromatography (TLC) and on spectrophotometric detection (Mattocks, 1967; Sharma, Khajuria, & Atal, 1965). These methods had a poor LOD, in the order of mg/kg. Currently applied methods based on liquid chromatography tandem mass spectrometry (LC-MS/MS) have LODs in the order of µg/kg (Mulder et al., 2018). Due to the improved sensitivity and selectivity of these LC-MS/MS based methods it is feasible to get more insight in the occurrence of PAs in various products, like for example herbal tea infusions. Mulder et al. analysed 168 samples of (herbal) teas and 191 samples of food supplements (Mulder et al., 2018), showing that 54% of the teas and 31% of the food supplements contained PAs above the LOD.

Although the carcinogenic properties of some of the PAs are

undisputed, it should be mentioned that it was shown that the mutagenicity of extracts of *Senecio jacobaea* in the so-called *Salmonella*/microsome test (Ames-test), were due to the presence of quercetin rather than PAs (Bovee et al., 2015). This demonstrates that combinations of bio/effect screening and MS identification are crucial in order to identify the compounds being responsible for, or contributing most to, the observed effects of complex mixtures.

2.9. Tropane alkaloids in cereal food intended for children

Current developments in mass spectrometric equipment allow the use of multi-class, multi-compound analysis. With these methods it is possible to include more compounds of interest in one single run. Mulder et al. developed an LC-MS/MS method for the analysis of ergot alkaloids in cereals but also included a number of tropane alkaloids (TAs) (i.e. atropine and scopolamine) (Mulder et al., 2015). This newly developed method was subsequently used to analyse a sample set of cereals for children. The majority of the samples investigated contained ergot alkaloids at various concentrations. However, a striking observation was the presence of high levels of TAs in some of the samples. These data were used by EFSA in a new risk assessment on TAs and as such coupled to a new study on adverse effects in human volunteers showing a decreased heart rate at relatively low exposure (EFSA CONTAM Panel, 2013). It was concluded that levels observed in some of the cereals formed a serious health concern as they would lead to an exposure in the range where the effects in healthy young men were observed. The problem is most likely caused by the presence of weeds like Jimson weed (*Datura stramonium*) in the fields where the cereals are grown. The discovery led to an increased monitoring of these cereal products and strict regulation in the EU to eliminate this contamination (Anon, 2016). Similar multi-methods are now also being used to test food supplements for various plant toxins but also include a number of pharmacologically active substances that may be illegally added to support claims of such supplements (e.g. increased energy, increased strength, and loss of weight).

2.10. Illegal use of beta-agonists

Beta-agonists are mimics of adrenalin, widely used to treat asthmatic disease. Well-known examples are clenbuterol and salbutamol, but actually there is a whole range of related compounds. Very low doses are already sufficient to obtain the desired effects. Application in calves led to the discovery that these compounds have an interesting side-effect, being the reduction of the fat content and increase in muscle tissue (Muir, 1988). This resulted in the routine treatment of cows, but also a number of incidents in consumers eating animal derived products with elevated levels of these compounds (Brambilla et al., 2000; Brambilla et al., 1997; Martinez-Navarro, 1990). The use of these compounds as well as all other growth promoting substances became illegal and they were included in routine national monitoring programmes in 1988 by some EU countries (Anon, 1985). In 1996 this was followed by a inclusion in EU legislation and the compounds were banned for use as growth promoters in the animal production in all EU member states (Anon, 1996). The first sensitive detection methods for beta-agonists were based on thin layer chromatography (TLC), for example as described by van Ginkel et al., whom developed a method for trenbolone acetate in cattle urine (van Ginkel, van Blitterswijk, Zoontjes, van den Bosch, & Stephany, 1988). After TLC, new method developments were mainly based on LC-MS analysis and antibody based screening methods and both found their way in routine monitoring (Haasnoot et al., 1996). In 2003, a sample of calf urine tested positive in an assay with antibodies against clenbuterol (Nielen et al., 2003). Follow-up analysis with LC-MS/MS failed to show the presence of a known beta-agonist, thus implying a false-positive result in the enzyme linked immunosorbent assay (ELISA). It was decided to test the sample in a novel assay based on the binding of beta-agonists to the

beta2-adrenergic receptor harvested from a newly engineered cell-line. The sample showed a positive test result in this competitive receptor binding assay too, clearly indicating the presence of an unknown beta-agonist. An intensified search with LC-TOF MS technology eventually led to the identification of a completely new compound (real unknown), having a clenbuterol-like structure but coupled with an unknown side-chain.

It should be emphasised that the application of such unknown analogues may imply an even greater risk than the abuse of known drugs which have been tested for adverse effects. This new beta-agonist for instance contained a phenylhydrazine side-chain that was likely to be released at a low pH as present in the stomach. Phenylhydrazine is genotoxic and causes tumours in animals (Muller, Engelhart, Herbold, Jackh, & Jung, 1993).

As in animals, beta-agonists are also attractive for athletes, body builders and other people trying to reduce their body fat. In 2013, several cases were reported in the Netherlands of people with heart problems and even one cardiac arrest (Bovee et al., 2016). People had been using a food supplement and investigations were started to reveal the cause of the adverse effects. Initially this was focussed on plant toxins but without any indication of a toxin or other compound (e.g. drugs) that could be related to the observed effects. Based on the reported adverse cardiac effects and claimed beneficial effects on the supplements' label, it was decided to test the supplement for the presence of beta-agonists, using the above mentioned competitive beta2-adrenergic receptor assay. This test showed a clear positive result and a new search was started for the most likely candidate. This led to the suspicion of the β 3-agonist N-isopropyltopamine, which was confirmed by LC-MS/MS analysis (Bovee et al., 2016).

2.11. Diethylstilboestrol in a food supplement

A similar case, i.e. the presence of a pharmacologically active substance in a food supplement, occurred in 2009 in The Netherlands (Toorians, Bovee, De Rooy, Stolker, & Hoogenboom, 2010). A patient with a family history of prostate cancer visited the hospital because he started to develop breasts (gynecomastia). He was operated for this but only after additional visits to an endocrinologist admitted that he was using a natural food supplement, called Prostatosol, to reduce his PSA levels. There was a clear suspect for the presence of an estrogenic substance and investigations were started to confirm this. Again, as with MPA and estradiol in the syrup, the yeast estrogen bioassay, expressing the human oestrogen receptor α , was applied and showed a very high response. This triggered further internet searches revealing some cases with a similar supplement that contained diethylstilbestrol (DES), whose presence was confirmed by NMR and LC-TOF MS analysis. The levels were actually very high and could easily explain the adverse effects observed in the patient.

2.12. Anabolic steroids in food supplements

In a study to investigate the practical use of a yeast based androgen bioassay to detect anabolic steroids in food or feed, 18 supplements, already analysed by LC-MS/MS, were tested. The conventional LC-MS/MS multimethod showed the presence of known anabolic steroids in 11 out of these 18 supplements. These eleven also tested positive in the yeast androgen bioassay. However, 2 out of the 7 supplements that were negative with LC-MS/MS, showed clear androgenic activities in the bioassay. It was hypothesized that it was most likely that there were androgenic compounds present in the supplements which were not included in the LC-MS/MS method. Using a bioassay guided fractionation approach and investigating the bioactive fractions, eventually led to the identification of 1-testosterone in one supplement, and of androstenediol and androstenediol in the other one. This clearly shows the added value of a bioassay based screening strategy, as LC-MS/MS alone resulted in over 10% of false negative outcomes (2 out of the 18

supplements) (Rijk et al., 2009). The LC-MS/MS method was then expanded towards these newly identified compounds. Also this case shows that a combination of bioassays and MS methods offers the possibility to detect and identify both known and yet unknown bioactive compounds, which can form real threats to consumers.

2.13. Sildenafil and its analogues in food supplements

There are many (natural) supplements for man's sexual performance that were shown to contain either undeclared sildenafil (Viagra), or worse, sildenafil-analogues (Reeuwijk et al., 2013; Venhuis, Blok-Tip, & de Kaste, 2008). The latter can be problematic, as it is often unclear why these analogues did not reach approval as a drug. Such analogues might even cause severe side-effects, like previously demonstrated by the unknown clenbuterol-analogue (Brambilla et al., 2000). The mechanism of action of sildenafil and its analogues is based on the inhibition of phosphodiesterase type 5 (PDE5) (Boolell et al., 1996). Based on this principle a commercial enzyme inhibition assay is available. In our institute food supplements were screened by this PDE5 inhibition assay and findings were confirmed with LC-hrMS analysis. Analysis revealed that several supplements were adulterated with sildenafil and/or analogues but in several occasions also indicated the presence of natural compounds. For example, the prenylated flavonol glycoside icariin is a weak PDE5 inhibitor and is sometimes used at high concentrations in these kind of supplements. Also some food supplements were active in the assay without detection of a known substance in the LC-hrMS analysis. These supplements are currently under investigation in order to identify the unknown bioactive substance.

2.14. Antimicrobial compounds in feed

Microbial screening methods are often used for the broad detection of antimicrobial compounds in feed and food products. These microbial inhibition assays are based on the inoculation of a medium with an antimicrobial sensitive bacterium (Pikkemaat, 2009). The format can be either a tube or a plate. In the tube format, growth of the bacteria will lead to acidification of the medium and in the presence of an indicator a colour change will occur. When samples (fluidics like milk) contain antibiotics, the growth of the bacteria will be inhibited resulting in no acidification and no colour change of the medium. The main other test is based on plates with medium inoculated again with bacteria sensitive towards antimicrobial compounds. On these plates, solid samples (for example meat) can be placed. If antimicrobial compounds, to which the bacterial strain used in the plate is sensitive, are present, a so called inhibition zone occurs. Both approaches, tubes and plates, are a cost effective manner to screen for the presence of antimicrobial substances present in food and feed. After the screening a confirmation step is required for the suspect screened samples, which is often based on targeted LC-MS/MS analysis of the regulated antimicrobial compounds. This approach has been shown to be very successful, but in some cases the inhibition of bacterial growth cannot be explained by the targeted analysis, meaning that another substance responsible for the observed inhibition is present. At RIKILT a non-targeted approach was used to identify an antimicrobial compound present in animal feed (Wegh et al., 2017). The compound showed antimicrobial activity in a *Micrococcus luteus* inoculated plate which is sensitive to a wide variety of drugs such as tetracyclines and β -lactams. After HPLC fractionation, the activity of the fractions was determined with the antimicrobial plate test. Two different HPLC mobile phase systems were used for the fractionation, respectively under acidic and slightly alkaline conditions, in order to increase the selectivity and reduce the number of mass candidates. The active fractions together with blank fractions were analysed with LC-hrMS. After sophisticated data analysis various quaternary ammonium compounds were tentatively identified. These ammonium compounds can be used for various applications such as pesticide, herbicide and disinfectant. From the

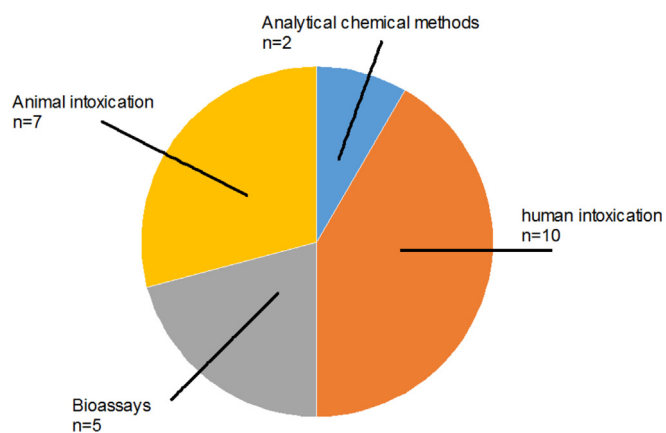


Fig. 1. Overview of cases and how they were discovered.

tentative identified compounds, one compound (didecyltrimethylammonia (DDAC)) was purchased and subsequently confirmed to be present in the animal feed. This compound was used as an example to confirm the activity and identification strategy (Wegh et al., 2017).

3. Lessons learned

Of the 24 example cases described above, 10 were discovered by human intoxications, 7 by animal intoxications, 5 by bioassays and 2 by analytical chemical analysis. This clearly demonstrates that novel risks are rarely discovered by chemical analysis, and in most cases, unfortunately, after causing effects in humans or animals (Fig. 1). Of the 17 cases discovered by effects in humans or animals, 15 could have been detected by monitoring using at that time available bioassays or chemical methods.

It is clear that eventually all feed and food ends up in a bioassay, being unfortunately humans and animals. However, only in some cases contaminated food and feed result in clear and directly recognised effects, in particular with acute toxic compounds. The general lack of clear direct toxic effects may give the false impression that these contaminants present in our food are safe. This is probably true in most cases but not necessarily so for every contaminant. Chronic effects may only be discovered after dedicated studies, as in the case of liver cancer caused by the very potent aflatoxins. For many other compounds, such studies may not be able to demonstrate the adverse effects, due to the high variability between humans and the relatively small differences in exposure between low and high exposed people. This may be the case for acrylamide, being a clear carcinogen in animal studies, but epidemiologically not proven to have led to increased numbers of cancer cases.

Analytical chemical techniques are very suitable for detecting the presence of known contaminants. The use of multi-methods in combination with libraries actually allows the simultaneous detection of many different toxins and toxicants. This is demonstrated by the detection of tropane alkaloids in cereals consumed by children, following their inclusion in a method for ergot alkaloids (Mulder et al., 2015). However, in case of marine biotoxins, the fact that only part of the marine toxins have been identified and that there are standards for only a few, are the major reasons that many countries still rely on the mouse bioassay for monitoring shellfish. Nowadays, there are alternatives for the mouse bioassay, e.g. the neuro-2a bioassay to detect the diarrhetic shellfish poisons (DSPs) (Bodero, Bovee, et al., 2018; Canete & Diogene, 2008). In some cases such cell based bioassays may actually be more sensitive than chemical analysis, as is e.g. the case with ciguatoxins where the neuro-2a assay can detect levels which are thought to be relevant for humans, whereas LC-MS methods lack the sensitivity (Caillaud et al., 2010). In the case of substances used illegally in food producing animals but also in food supplements, it is evident that

everything is done to avoid or delay their discovery. Such novel compounds are unlikely to be included in existing libraries and thus missed by MS based methods per definition. Only one thing is certain, they are used because of their biological effects. As such, only assays aiming at these effects, will be able to detect their presence. Application of such effect based bioassays may thus protect us to a larger extent against the use of unknown analogues, which in addition to their intended effects, may also show additional adverse effects for which they were never tested (see the described case of the unknown clenbuterol in section 2.10.). This was also a major conclusion of EFSA in a series of opinions on meat inspection (EFSA BIOHAZ Panel, 2013; EFSA BIOHAZ Panel and CONTAM Panel and AHAW Panel, 2011, 2012). The additional value of bioassays for such a risk-based monitoring was already proved, as e.g. demonstrated above for the newly discovered clenbuterol analogue in feed by an ELISA and beta-adrenergic receptor assay. Similar applies in the field of food supplements. At present there are many “natural” food supplements on the market with added pharmacologically active substances, just to ensure that they show the intended effect (Reeuwijk et al., 2013). Also here effect based bioassays have proven their added value, as e.g. demonstrated by the detection of DES in a “natural” supplement for healthy prostate function by a yeast estrogen bioassay, the detection of the β 3-agonist N-isopropyltopamine in a supplement for increased energy and strength by the adrenergic receptor assay, and the detection of androgens (1-testosterone, androstenediol and androstenediol) by the yeast androgen bioassay in food supplements for athletes intercepted by the Belgian inspection service.

At present there are quite a number of dedicated bioassays available that can be used for testing feed and food. The main reason for their use is that these bioassays, just like tests with animals, seem the only way to really ensure the absence of toxins. Cell based tests are being developed and validated in all kind of (research) areas in order to reduce and replace the use of laboratory animals. There is a set of in vitro bioassays capable of detecting various hormonal activities. A number of these hormone bioassays have been validated for screening of feed and food, but also urine, and the competitive beta-adrenergic receptor assay has been validated for feed, while bacterial inhibition assays to detect all kind of antimicrobials have been validated for all kind of matrices (Bovee & Pikkemaat, 2009). The DR-CALUX assay has also been validated for all kind of matrices and has been successfully used for screening of food and feed for dioxins and dioxin-like PCBs. As demonstrated above, its use actually would also pick up brominated and mixed bromo-chloro dioxins, meaning that negative test results also confirm the absence of high levels of these compounds in our food chain. In addition to these dedicated bioassays, the use of gene expression based techniques allow the screening of samples for a very broad array of effects (Bodero, Hoogenboom, et al., 2018; Schmeits, Katika, Peijnenburg, van Loveren, & Hendriksen, 2014; Schmeits et al., 2013; Shao et al., 2013).

In other cases, more generic bioassays may be applied initially, e.g. in the case of marine biotoxins, where a generic assay based on the reduction of MTT by neuroblastoma cells seems suitable for testing shellfish for both DSPs and PSPs. This was shown for the neuro-2a bioassay to detect the DSPs (Bodero, Bovee, et al., 2018). This assay is also capable of detecting the PSPs, ciguatoxins, and the TTXs (Okumura, Tsuzuki, & Tomita, 2005). If positive, samples may be examined with chemical analytical methods and more dedicated specific bioassays to reveal the identity and nature of the toxins causing the effect, e.g. a dedicated array with specific markers for several marine biotoxins (Bovee et al., 2011). The assays may also be used to identify the most relevant compounds in mixtures of compounds with related effects but different potencies and occurrence.

In vitro bioassays can also be used to avoid tunnel vision with respect to the agent causing the intoxications or observed effects. This was e.g. shown for the mutagenic effects caused by extracts of ragwort, at first ascribed to the presence of PAs. Follow-up studies showed that quercetin is actually causing the positive test result (Bovee et al., 2015).

Similarly, by using the DR-CALUX assay, it was shown that the positive test result obtained in the case of the acid polymerization products of indole-3-carbinol cannot be explained by the relative low levels of ICZ in this mixture, even though this compound is probably the most potent reaction product in this mixture (unpublished).

The good news is that many of the bioassays are relatively cheap and sensitive and can easily be applied on large numbers of samples. However, it should be avoided that such tests cause too many false-positive results. There are e.g. many natural compounds that, like dioxins, are able to bind to the Ah-receptor. The DR-CALUX assay was e.g. used to identify bergapten as the most active Ah-receptor agonist in oranges (van Ede et al., 2008). A major difference is however, that in vivo these natural compounds are easily degraded by first-pass metabolism, whereas the relevant dioxins are poorly metabolized and easily reach their target in the body, or accumulate to body levels that may become adverse. Only by using a specific but simple clean-up, the DR-CALUX-assay can actually be applied successfully for detecting chlorinated dioxins, dioxin-like PCBs and also their brominated analogues in food and feed. On the other hand, there also is the risk of false-negative results in case biological samples like tissues or excreta are analysed. Levels of contaminants or residues in such matrices can be extremely low, at the border of current high-end instrumental MS-techniques. Such levels frequently cannot be confirmed with a bioassay.

With respect to the detection of emerging risks, an important issue is how to identify the compounds responsible for “false-positive” effects in bioassays. Samples testing positive should be followed up by chemical analysis to either confirm the presence of compounds known to be positive in the assay, or, if not caused by the known regulated compounds, try to identify the responsible compounds by using an approach called bioassay directed analysis. This means that the positive extract is further fractionated and the bioassay is used again to identify the fraction(s) containing the active compound(s). Analytical chemical methods are then used to identify the responsible compound(s), e.g. by looking for masses present in active fractions and not present in negative fractions. This is especially relevant if the bioassay shows a clearly elevated response.

4. Future strategies

The examples above clearly demonstrate that novel risks are rarely discovered by chemical analysis, but in most cases after causing effects in humans or animals, unfortunately the “bioassays” where eventually all feed and food ends up. To prevent more casualties, it is important to report and follow-up human and animal intoxication cases, where food or feed appears to be involved.

However, the real challenge is how to prevent human and animal intoxications. Increased monitoring efforts are key, but when performed solely with analytical chemical analysis, targeting the known regulated compounds, this will not solve all these problems. Although multi MS based methods are being developed that can determine multi compounds of multi compound classes in a quantitative manner, the examples above show that an approach is needed where animal free in vitro bioassays are combined with analytical chemical methods. In general, such bioassays can best be used for a first broad screening and the analytical chemical methods to identify the responsible compounds in samples screened positive. If positive test results are not caused by known regulated compounds, efforts should be taken to identify the responsible compounds by using an approach called bioassay directed analysis, where extracts are fractionated and positive fractions are further examined by MS based methods (Fig. 2). Key in these approaches is that both the extraction and fractionation method should be as broad as possible, without causing matrix effects in either the bioassays or MS based methods or both. If extraction or fractionation are too specific, it increases the risk that the compound of interest is not extracted or fractionated. The untargeted collected MS data can be processed using chemometric models (unsupervised or supervised) in

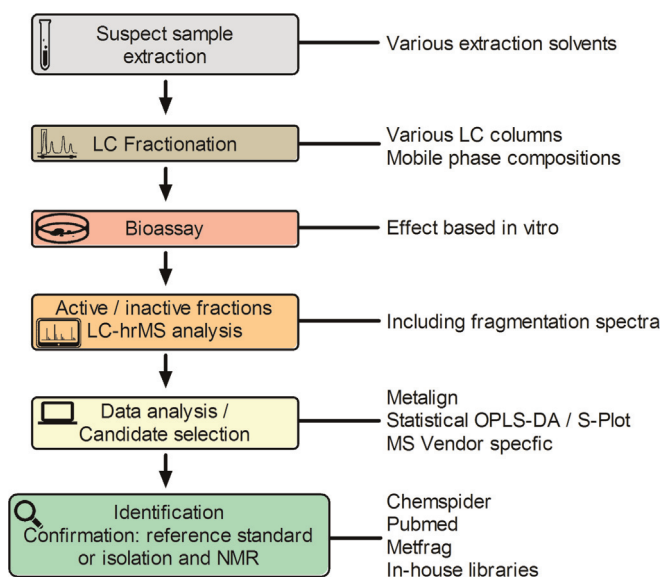


Fig. 2. Identification strategy for bioactive compounds.

order to select the relevant m/z values. These m/z values are used to determine the elemental composition based on the accurate mass data and isotopic distribution present in the spectra. This data can be subjected to large in-house libraries with food safety related compounds, typically containing 100–2000 compounds, or in larger databases with more than 500,000 compounds such as Chemspider or Pubchem. Additionally, MS fragmentation data can be used to minimize the number of hits in these large databases. In silico fragmentation tools such as MetFrag are being used with the MS fragmentation data to further (tentative) identify the bioactive compound. Once a candidate is tentatively identified, the easiest way is to order the pure compound (if available) and use it as a reference standard in order to confirm the results. In other cases a full purification and identification by preparative LC and NMR may be needed.

However, in situations, where it is preferred to perform monitoring with MS based methods, it might be useful to investigate on a regular basis (e.g. every 3 months) a number of compliant samples with the bioassays in order to ensure nothing was missed. Every situation is specific, but can be addressed by a combination of a bioassay with an analytical chemical method. Many research facilities mainly or only cover one of the two areas, which means that intoxications will not be prevented to the extent as needed and possible.

One alternative approach that currently is being used is that of profiling a large range of natural compounds in order to detect any deviations from the expected (reference) profile. An example of this approach is the screening of samples of urine by steroid profiling for effects possibly caused by exogenous hormones. Such hormones will influence the natural profile of steroids and their metabolites. Detecting such a deviating profile can be considered as an effect based screening result. Compared to traditional bioassays for hormones, this is a more expensive and complicated approach. On the positive side, it is more sensitive for a wide range of hormones, representing different classes like androgenic or estrogenic hormones, making the analyses of biological samples feasible.

Another effect-based approach is the use of bioaffinity techniques during sample preparation, using either immobilized antibodies or receptors prior to MS analyses (Aqai, Cevik, Gerssen, Haasnoot, & Nielen, 2013). Bioaffinity of course does not represent the full activity of a molecule, but can be very selective in isolating the active fraction of, e.g. biological, samples.

A new trend is to perform sample analysis on-site measurements, e.g. at the production site (factory or farm) or in the supermarket. On-

site use of cell based bioassays and chemical analytical methods is still too difficult, but many biosensors based on antibodies or receptors can be used on-site easily (e.g. for the detection of antimicrobials, cocci-diostats, mycotoxins, and beta-agonists), just as loop mediated isothermal amplification (LAMP) methods for the detection of specific DNAs (e.g. for species like horse, pig and cow, but also for allergens like peanut protein) and handheld NIR and Raman devices for e.g. pesticides and PAs (Aartse et al., 2017; Sheu, Tsou, Lien, & Lee, 2018; Vermeulen, Ebene, Orlando, Pierna, & Baeten, 2017; Xu, Gao, Han, & Zhao, 2017). Also the use of smartphone as readers for biosensors, for example for allergen detection, is gaining interest in the last few years (G. M. S. Ross, Bremer, & Nielen, 2018).

5. Conclusions

In order to discover unknown and known contaminants that are not routinely monitored in our food chain, it is essential to follow-up cases of intoxications in humans and animals. Clear intoxications should be reported to the authorities and follow-up studies should be started, using a combination of effect based assays and targeted/untargeted chemical analysis.

In vitro bioassays, detecting compounds based on their effects, allow the wide screening of samples and are important tools for the identification of novel and emerging contaminants. In the field of non-allowed pharmacologically active substances there is even a higher need for applying bioassays, since it is rather tempting to develop new compounds which will escape detection by chemical analytical methods. In food supplements levels may be rather high to guarantee the claimed effect.

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References

- Aarts, J. M. M. J. G., Denison, M. S., De Haan, L. H. J., Schalk, J. A. C., Cox, M. A., & Brouwer, A. (1993). Ah-receptor mediated luciferase expression: A tool for monitoring dioxin-like toxicity. *Organohalogen Compounds*, 13, 361–364.
- Aartse, A., Scholtens, I. M. J., van der A, H. J. G., Boersma-Greve, M. M., Prins, T. W., van Ginkel, L. A., et al. (2017). Evaluation of a loop-mediated isothermal amplification (LAMP) method for rapid on-site detection of horse meat. *Food Control*, 81, 9–15.
- Anon (1985). Council of the European Communities, Council Directive 85/649/EEC of 31 December 1985. Prohibiting the use in livestock farming of certain substances having a hormonal action. *Off. J. Eur. Commun. L382*, 228–231.
- Anon (1996). Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. *Off. J. Eur. Commun. L125*, 10–32.
- Anon (2002). Commission Regulation (EU) 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Off. J. Eur. Commun. L31*, 1–24.
- Anon (2016). Commission Regulation (EU) 2016/239 of 19 February 2016 amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children. *Off. J. Eur. Commun. L45*, 3–5.
- Aqai, P., Cevik, E., Gerssen, A., Haasnoot, W., & Nielen, M. W. F. (2013). High-throughput bioaffinity mass spectrometry for screening and identification of designer anabolic steroids in dietary supplements. *Analytical Chemistry*, 85(6), 3255–3262.
- Aune, T., Sorby, R., Yasumoto, T., Ramstad, H., & Landsverk, T. (2002). Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. *Toxicol.*, 40(1), 77–82.
- Barnes, J. M., & Butler, W. H. (1964). Carcinogenic activity of aflatoxin to rats. *Nature*, 202(493), 1016–8.
- Benford, D., Ceccatelli, S., Cottrill, B., DiNovi, M., Dogliotti, E., Edler, L., et al. (2015). Scientific Opinion on acrylamide in food. *EFSA Journal*, 13(6).
- Berendsen, B., Stolker, L., de Jong, J., Nielen, M., Tserendorj, E., Sodnomdarjaa, R., et al. (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. *Analytical and Bioanalytical Chemistry*, 397(5), 1955–1963.
- Bernard, A., Hermans, C., Broeckaert, F., De Poorter, G., De Cock, A., & Houins, G. (1999). Food contamination by PCBs and dioxins. *Nature*, 401(6750), 231–232.
- Blount, W. P. (1961). Turkey "X" disease. *Turkey*, 9(7), 52–61.
- Bodero, M., Bovee, T. F. H., Wang, S., Hoogenboom, R. L. A. P., Klijnstra, M. D., Portier,

- L., et al. (2018). Screening for the presence of lipophilic marine biotoxins in shellfish samples using the neuro-2a bioassay. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 35(2), 351–365.
- Bodero, M., Hoogenboom, R. L. A. P., Bovee, T. F. H., Portier, L., de Haan, L., Peijnenburg, A., et al. (2018). Whole genome mRNA transcriptomics analysis reveals different modes of action of the diarrhetic shellfish poisons okadaic acid and dinophysin toxin-1 versus azaspiracid-1 in Caco-2 cells. *Toxicology in Vitro*, 46, 102–112.
- Bodi, D., Ronczka, S., Gottschalk, C., Behr, N., Skibba, A., Wagner, M., et al. (2014). Determination of pyrrolizidine alkaloids in tea, herbal drugs and honey. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 31(11), 1886–1895.
- Boolell, M., Allen, M. J., Ballard, S. A., Gepi-Attee, S., Muirhead, G. J., Naylor, A. M., et al. (1996). Sildenafil: An orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *International Journal of Impotence Research*, 8(2), 47–52.
- Bovee, T. F. H., Bor, G., Heskamp, H. H., Hoogenboom, R. L. A. P., & Nielsen, M. W. F. (2006). Validation and application of a robust yeast estrogen bioassay for the screening of estrogenic activity in animal feed. *Food Additives & Contaminants*, 23(6), 556–568.
- Bovee, T. F. H., Helsdingen, R. J., Hoogenboom, R. L. A. P., de Nijs, M. W., Liu, X., Vrieling, K., et al. (2015). Are effects of common ragwort in the Ames test caused by pyrrolizidine alkaloids? *Mutation Research*, 778, 1–10.
- Bovee, T. F. H., Hendriksen, P. J., Portier, L., Wang, S., Elliott, C. T., van Egmond, H. P., et al. (2011). Tailored microarray platform for the detection of marine toxins. *Environmental Science & Technology*, 45(20), 8965–8973.
- Bovee, T. F. H., Mol, H. G. J., Bienenmann-Ploum, M. E., Heskamp, H. H., Van Bruchem, G. D., Van Ginkel, L. A., et al. (2016). Dietary supplement for energy and reduced appetite containing the beta-agonist isopropylolotopamine leads to heart problems and hospitalisations. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 33(5), 749–759.
- Bovee, T. F. H., & Pikkemaat, M. G. (2009). Bioactivity-based screening of antibiotics and hormones. *Journal of Chromatography A*, 1216(46), 8035–8050.
- Brambilla, G., Cenci, T., Franconi, F., Galarini, R., Macri, A., Rondoni, F., et al. (2000). Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in Italy. *Toxicology Letters*, 114(1–3), 47–53.
- Brambilla, G., Loizzo, A., Fontana, L., Strozzi, M., Guarino, A., & Soprano, V. (1997). Food poisoning following consumption of clenbuterol-treated veal in Italy. *Journal of the American Medical Association*, 278(8), 635.
- Brouwer, A., Botschuijver, S., Veerhoek, D., Besselink, H. A., Hamm, S., Traag, W., et al. (2005). Observation of an extremely high dioxin level in a human serum sample from Ukraine by DR CALUX® which was confirmed to be 2,3,7,8-Tetrachlorodibenzo-p-dioxin by GC-HRMS. *Organohalogen Compounds*, 67, 1705–1708.
- Caillaud, A., de la Iglesia, P., Darius, H. T., Pauillac, S., Aligizaki, K., Fraga, S., et al. (2010). Update on methodologies available for ciguatera toxin determination: Perspectives to confront the onset of ciguatera fish poisoning in Europe. *Marine Drugs*, 8(6), 1838–1907.
- Canete, E., & Diogene, J. (2008). Comparative study of the use of neuroblastoma cells (Neuro-2a) and neuroblastoma x glioma hybrid cells (NG108-15) for the toxic effect quantification of marine toxins. *Toxicol*, 52(4), 541–550.
- Carter, L. J. (1976). Michigan's PBB incident: Chemical mix-up leads to disaster. *Science*, 192(4236), 240–243.
- Castellani, F. (2004). Yushchenko's acne points to dioxin poisoning. *Nature*. <https://doi.org/10.1038/news041122-041128>.
- Choi, J., Mørck, T. A., Polcher, A., Knudsen, L. E., & Joas, A. (2015). Review of the state of the art of human biomonitoring for chemical substances and its application to human exposure assessment for food safety. *EFSA Supporting Publications*, 12(2), 724E.
- Cole, R. J. (1986). Etiology of Turkey "X" disease in retrospect: A case for the involvement of cyclopiazonic acid. *Mycotoxin Research*, 2(1), 3–7.
- Crews, C., & Anderson, W. A. (2009). Detection of ragwort alkaloids in toxic hay by liquid chromatography/time-of-flight mass spectrometry. *The Veterinary Record*, 165(19), 568–569.
- De Paola, E. L., Montevecchi, G., Masino, F., Garbini, D., Barbanera, M., & Antonelli, A. (2017). Determination of acrylamide in dried fruits and edible seeds using QuEChERS extraction and LC separation with MS detection. *Food Chemistry*, 217, 191–195.
- Dearfield, K. L., Abernathy, C. O., Otley, M. S., Brantner, J. H., & Hayes, P. F. (1988). Acrylamide - its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. *Mutation Research*, 195(1), 45–77.
- Denison, M. S., Zhao, B., Baston, D. S., Clark, G. C., Murata, H., & Han, D. (2004). Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. *Talanta*, 63(5), 1123–1133.
- Desmarchelier, A., Cuadra, M. G., Delatour, T., & Mottier, P. (2009). Simultaneous quantitative determination of melamine and cyanuric acid in cow's milk and milk-based infant formula by liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 57(16), 7186–7193.
- van Ede, K., Li, A., Antunes-Fernandes, E., Mulder, P., Peijnenburg, A., & Hoogenboom, R. (2008). Bioassay directed identification of natural aryl hydrocarbon-receptor agonists in marmalade. *Analytica Chimica Acta*, 617(1–2), 238–245.
- EFSA BIOHAZ Panel (2013). Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). *EFSA Journal*, 11(6), 3266.
- EFSA BIOHAZ Panel and CONTAM Panel and AHAW Panel (2011). Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). *EFSA Journal*, 9(10), 2351.
- EFSA BIOHAZ Panel and CONTAM Panel and AHAW Panel (2012). Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry). *EFSA Journal*, 10(6), 2741.
- EFSA CONTAM Panel (2013). Scientific Opinion on Tropane alkaloids in food and feed. *EFSA Journal*, 11(10), 3386 n/a.
- EFSA CONTAM Panel and EFSA CEF Panel (2010). Scientific opinion on melamine in food and feed. *EFSA Journal*, 8(4), 1573.
- Firestone, D. (1973). Etiology of chick edema disease. *Environmental Health Perspectives*, 5, 59–66.
- Frank, N., Bessaire, T., Tarres, A., Goyon, A., & Delatour, T. (2017). Development of a quantitative multi-compound method for the detection of 14 nitrogen-rich adulterants by LC-MS/MS in food materials. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 34(11), 1842–1852.
- Fries, G. F. (1985). The PBB episode in Michigan - an overall appraisal. *Crc Critical Reviews in Toxicology*, 16(2), 105–156.
- Fry, H., Mietle, K., Mahnert, E., Zinke, S., Schwieters, M., Pydde, E., et al. (2017). Interlaboratory validation of an LC-MS/MS method for the determination of melamine and cyanuric acid in animal feed. *Food Additives & Contaminants Part a Chem Anal Control Expo Risk Assess*, 34(8), 1320–1332.
- Gallagher, R. T., Richard, J. L., Stahr, H. M., & Cole, R. J. (1978). Cyclopiazonic acid production by aflatoxigenic and non-aflatoxigenic strains of *aspergillus flavus*. *Mycopathologia*, 66(1–2), 31–36.
- van Ginkel, L. A., van Blitterswijk, H., Zootjes, P. W., van den Bosch, D., & Stephany, R. W. (1988). Assay for trenbolone and its metabolite 17 alpha-trenbolone in bovine urine based on immunoaffinity chromatographic clean-up and off-line high-performance liquid chromatography-thin-layer chromatography. *Journal of Chromatography*, 445(2), 385–392.
- Haasnoot, W., Streppel, L., Cazemier, G., Salden, M., Stouten, P., Essers, M., et al. (1996). Development of a tube enzyme immunoassay for 'on-site' screening of urine samples in the presence of beta-agonists. *Analyst*, 121(8), 1111–1114.
- Hagmar, L., Tornqvist, M., Nordander, C., Rosen, I., Bruze, M., Kautiainen, A., et al. (2001). Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scandinavian Journal of Work, Environment & Health*, 27(4), 219–226.
- Heres, L., Hoogenboom, L. A. P., Herbes, R., Traag, W., & Urlings, B. (2010). Tracing and analytical results of the dioxin contamination incident in 2008 originating from the Republic of Ireland. *Food Additives & Contaminants Part a Chem Anal Control Expo Risk Assess*, 27(12), 1733–1744.
- Higginbotham, G. R., Huang, A., Firestone, D., Verrett, J., Ress, J., & Campbell, A. D. (1968). Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-p-dioxin. *Nature*, 220(5168), 702–703.
- Hogervorst, J. G., Schouten, L. J., Konings, E. J., Goldbohm, R. A., & van den Brandt, P. A. (2007). A prospective study of dietary acrylamide intake and the risk of endometrial ovarian, and breast cancer. *Cancer Epidemiology Biomarkers & Prevention*, 16(11), 2304–2313.
- Hogervorst, J. G. F., Schouten, L. J., Konings, E. J. M., Goldbohm, R. A., & van den Brandt, P. A. (2009). Dietary acrylamide intake and brain cancer risk. *Cancer Epidemiology Biomarkers & Prevention*, 18(5), 1663–1666.
- Hoogenboom, L. A. P., Bovee, T., Portier, L., Bor, G., van der Weg, G., Onstenk, C., et al. (2004). The German bakery waste incident; use of a combined approach of screening and confirmation for dioxins in feed and food. *Talanta*, 63(5), 1249–1253.
- Hoogenboom, L. A. P., Kan, C. A., Bovee, T. F. H., van der Weg, G., Onstenk, C., & Traag, W. A. (2004). Residues of dioxins and PCBs in fat of growing pigs and broilers fed contaminated feed. *Chemosphere*, 57(1), 35–42.
- Hoogenboom, L. A. P., Traag, W. A., & Mengelers, M. J. B. (1999). The Belgian dioxin crisis; involvement of RIKILT. *Dioxin Venice*, Italy.
- Hoogenboom, L. A. P., Van Eijkeren, J. C. H., Zeilmaker, M. J., Mengelers, M. J. B., Herbes, R., Immerzeel, J., et al. (2007). A novel source for dioxins present in recycled fat from gelatin production. *Chemosphere*, 68(5), 814–823.
- Hoogenboom, L. A. P., Zeilmaker, M., van Eijkeren, J., Kan, K., Mengelers, M., Luyckx, D., et al. (2010). Kaolin clay derived PCDD/Fs in the feed chain from a sorting process for potatoes. *Chemosphere*, 78(2), 99–105.
- Hsu, S. T., Ma, C. I., Hsu, S. K., Wu, S. S., Hsu, N. H., Yeh, C. C., et al. (1985). Discovery and epidemiology of PCB poisoning in Taiwan: A four-year followup. *Environmental Health Perspectives*, 59, 5–10.
- Kakar, F., Akbarian, Z., Leslie, T., Mustafa, M. L., Watson, J., van Egmond, H. P., et al. (2010). An outbreak of hepatic veno-occlusive disease in Western Afghanistan associated with exposure to wheat flour contaminated with pyrrolizidine alkaloids. *Journal of Toxicology*, 313280 2010.
- de Kok, J. J., de Kok, A., Brinkman, U. A., & Kok, R. M. (1977). Analysis of poly-brominated biphenyls. *Journal of Chromatography*, 142, 367–383.
- Kuratsune, M., Yoshimura, T., Matsuzaka, J., & Yamaguchi, A. (1972). Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environmental Health Perspectives*, 1, 119–128.
- van Larebeke, N., Hens, L., Schepens, P., Covaci, A., Baeyens, J., Everaert, K., et al. (2001). The Belgian PCB and dioxin incident of January-June 1999: Exposure data and potential impact on health. *Environmental Health Perspectives*, 109(3), 265–273.
- van Leengoed, L., Kluijvers, M., Herbes, R., Langendijk, P., Stephany, R., van den Berg, M., et al. (2002). The weakest link: Medroxyprogesterone acetate in pig feed. *Tijdschrift voor Diergeneeskunde*, 127(17), 516–519.
- Li, N., Xia, Q., Ruan, J., Fu, P. P., & Lin, G. (2011). Hepatotoxicity and tumorigenicity induced by metabolic activation of pyrrolizidine alkaloids in herbs. *Current Drug Metabolism*, 12(9), 823–834.
- Malisch, R. (2000). Increase of the PCDD/F-contamination of milk, butter and meat samples by use of contaminated citrus pulp. *Chemosphere*, 40(9–11), 1041–1053.
- Malisch, R., & Kotz, A. (2014). Dioxins and PCBs in feed and food - review from European perspective. *The Science of the Total Environment*, 491, 2–10.
- Manger, R. M., Woodle, D., Berger, A., & Hungerford, J. M. (2003). Sensitive and rapid detection of marine toxins using mouse neuroblastoma cells and voltage sensitive

- dyes. *Abstracts of Papers American Chemical Society*, 226, U69 U69.
- Martinez-Navarro, J. F. (1990). Food poisoning related to consumption of illicit beta-agonist in liver. *Lancet*, 336(8726), 1311.
- Mastovska, K., & Lehotay, S. J. (2006). Rapid sample preparation method for LC-MS/MS or GC-MS analysis of acrylamide in various food matrices. *Journal of Agricultural and Food Chemistry*, 54(19), 7001–7008.
- Mattocks, A. R. (1967). Spectrophotometric determination of unsaturated pyrrolizidine alkaloids. *Analytical Chemistry*, 39(4), 443–447.
- Mattocks, A. R. (1986). *Chemistry and toxicology of pyrrolizidine alkaloids*. Academic Press.
- Ming, L., Thorgeirsson, S. S., Gail, M. H., Lu, P. X., Harris, C. C., Wang, N. J., et al. (2002). Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. *Hepatology*, 36(5), 1214–1220.
- Mocarelli, P., Marocchi, A., Brambilla, P., Gerthou, P., Young, D. S., & Mantel, N. (1986). Clinical laboratory manifestations of exposure to dioxin in children. A six-year study of the effects of an environmental disaster near Seveso, Italy. *Journal of the American Medical Association*, 256(19), 2687–2695.
- Mucci, L. A. (2006). Dietary exposure to acrylamide and cancer risk: The role of epidemiology. *Epidemiology*, 17(6), S78 S78.
- Muir, L. A. (1988). Effects of beta-adrenergic agonists on growth and carcass characteristics of animals. *Committee on technological options to improve the nutritional attributes of animal products*. Board on Agric. NRC. Washington, DC, USA: Designing foods. National Academy Press.
- Mulder, P. P. J., Lopez, P., Castelari, M., Bodi, D., Ronczka, S., Preiss-Weigert, A., et al. (2018). Occurrence of pyrrolizidine alkaloids in animal- and plant-derived food: Results of a survey across Europe. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 35(1), 118–133.
- Mulder, P. P. J., Pereboom-de Fauw, D. P., Hoogenboom, L. A. P., de Stoppelaar, J., & de Nijs, M. (2015). Tropane and ergot alkaloids in grain-based products for infants and young children in The Netherlands in 2011–2014. *Food Additives & Contaminants Part B Surveill*, 8(4), 284–290.
- Muller, W., Engelhart, G., Herbold, B., Jackh, R., & Jung, R. (1993). Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. *Environmental Health Perspectives*, 101(Suppl 3), 33–36.
- Nicolas, J., Hoogenboom, R. L. A. P., Hendriksen, P. J. M., Boder, M., Bovee, T. F. H., Rietjens, I. M. C. M., et al. (2017). Marine biotoxins and associated outbreaks following seafood consumption: Prevention and surveillance in the 21st century. *Global Food Security-Agriculture Policy Economics and Environment*, 15, 11–21.
- Nielen, M. W. F., Elliott, C. T., Boyd, S. A., Courtheyn, D., Essers, M. L., Hooijerink, H. H., et al. (2003). Identification of an unknown beta-agonist in feed by liquid chromatography/bioassay/quadrupole time-of-flight tandem mass spectrometry with accurate mass measurement. *Rapid Communications in Mass Spectrometry*, 17(14), 1633–1641.
- Okumura, M., Tsuzuki, H., & Tomita, B. (2005). A rapid detection method for paralytic shellfish poisoning toxins by cell bioassay. *Toxicol*, 46(1), 93–98.
- Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Research*, 33(1), 65–73.
- Pikkemaat, M. G. (2009). Microbial screening methods for detection of antibiotic residues in slaughter animals. *Analytical and Bioanalytical Chemistry*, 395(4), 893–905.
- Piskorska-Pliszczynska, J., Mikolajczyk, S., Warenik-Bany, M., Maszewski, S., & Strucinski, P. (2014). Soil as a source of dioxin contamination in eggs from free-range hens on a Polish farm. *The Science of the Total Environment*, 466, 447–454.
- Qian, G. S., Ross, R. K., Yu, M. C., Yuan, J. M., Gao, Y. T., Henderson, B. E., et al. (1994). A follow-up-study of urinary markers of aflatoxin exposure and liver-cancer risk in Shanghai, Peoples-Republic-of-China. *Cancer Epidemiology Biomarkers & Prevention*, 3(1), 3–10.
- Reeuwijk, N. M., Venhuis, B. J., de Kaste, D., Hoogenboom, L. A., Rietjens, I. M., & Martena, M. J. (2013). Sildenafil and analogous phosphodiesterase type 5 (PDE-5) inhibitors in herbal food supplements sampled on the Dutch market. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess*, 30(12), 2027–2034.
- Reich, M. R. (1983). Environmental politics and science - the case of Pbb contamination in Michigan. *American Journal of Public Health*, 73(3), 302–313.
- Rijk, J. C. W., Bovee, T. F. H., Wang, S., Van Poucke, C., Van Peteghem, C., & Nielen, M. W. F. (2009). Detection of anabolic steroids in dietary supplements: The added value of an androgen yeast bioassay in parallel with a liquid chromatography-tandem mass spectrometry screening method. *Analytica Chimica Acta*, 637(1–2), 305–314.
- Ross, G. M. S., Bremer, M. G. E. G., & Nielen, M. W. F. (2018). Consumer-friendly food allergen detection: Moving towards smartphone-based immunoassays. *Analytical and Bioanalytical Chemistry*, 410(22), 5353–5371.
- Ross, R. K., Yu, M. C., Henderson, B. E., Yuan, J. M., Qian, G. S., Tu, J. T., et al. (1992). Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *The Lancet*, 339(8799), 943–946.
- Sanger, V. L., Scott, L., Hamdy, A., Gale, C., & Pouden, W. D. (1958). Alimentary toxemia in chickens. *Journal of the American Veterinary Medical Association*, 133(3), 172–176.
- Sargeant, K., Sheridan, A., & Okelly, J. (1961). Toxicity associated with certain samples of groundnuts. *Nature*, 192(480), 1096.
- Schmeits, P. C., Katika, M. R., Peijnenburg, A. A., van Loveren, H., & Hendriksen, P. J. (2014). DON shares a similar mode of action as the ribotoxic stress inducer anisomycin while TBTO shares ER stress patterns with the ER stress inducer thapsigargin based on comparative gene expression profiling in Jurkat T cells. *Toxicology Letters*, 224(3), 395–406.
- Schmeits, P. C., Volger, O. L., Zandvliet, E. T., van Loveren, H., Peijnenburg, A. A., & Hendriksen, P. J. (2013). Assessment of the usefulness of the murine cytotoxic T cell line CTLL-2 for immunotoxicity screening by transcriptomics. *Toxicology Letters*, 217(1), 1–13.
- Schmittle, S. C., Edwards, H. M., & Morris, D. (1958). A disorder of chickens probably due to a toxic feed; preliminary report. *Journal of the American Veterinary Medical Association*, 132(5), 216–219.
- Schoeters, G., & Hoogenboom, R. (2006). Contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls. *Molecular Nutrition & Food Research*, 50(10), 908–914.
- Shao, J., Katika, M. R., Schmeits, P. C., Hendriksen, P. J., van Loveren, H., Peijnenburg, A. A., et al. (2013). Toxicogenomics-based identification of mechanisms for direct immunotoxicity. *Toxicological Sciences*, 135(2), 328–346.
- Sharma, R. K., Khajuria, G. S., & Atal, C. K. (1965). Thin-layer chromatography of pyrrolizidine alkaloids. *Journal of Chromatography*, 19(2), 433–434.
- Sheu, S. C., Tsou, P. C., Lien, Y. Y., & Lee, M. S. (2018). Development of loop-mediated isothermal amplification (LAMP) assays for the rapid detection of allergic peanut in processed food. *Food Chemistry*, 257, 67–74.
- Sorg, O., Zennegg, M., Schmid, P., Fedosyuk, R., Valikhnovskiy, R., Gaide, O., et al. (2009). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in victor Yushchenko: Identification and measurement of TCDD metabolites. *Lancet*, 374(9696), 1179–1185.
- Toorians, A. W., Bovee, T. F. H., De Rooy, J., Stolker, L. A., & Hoogenboom, R. L. A. P. (2010). Gynaecomastia linked to the intake of a herbal supplement fortified with diethylstilbestrol. *Food Additives & Contaminants Part a Chem Anal Control Expo Risk Assess*, 27(7), 917–925.
- Traag, W., Kotz, A., van der Weg, G., Malisch, R., & Hoogenboom, R. (2009). Bioassay directed detection of brominated dioxins in the feed additive choline chloride. *Organohalogen Compounds*, 71, 2210–2213.
- Turner, A. D., Powell, A., Schofield, A., Lees, D. N., & Baker-Austin, C. (2015). Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014. *Euro Surveillance*, 20(2), 2–8.
- Venhuis, B. J., Blok-Tip, L., & de Kaste, D. (2008). Designer drugs in herbal aphrodisiacs. *Forensic Science International*, 177(2–3), E25–E27.
- Vermeulen, P., Ebene, M. B., Orlando, B., Pierna, J. A. F., & Baeten, V. (2017). Online detection and quantification of particles of ergot bodies in cereal flour using near-infrared hyperspectral imaging. *Food Additives & Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 34(8), 1312–1319.
- Vlams, A., Katikou, P., Rodriguez, I., Rey, V., Alfonso, A., Papazachariou, A., et al. (2015). First detection of tetrodotoxin in Greek shellfish by UPLC-MS/MS potentially linked to the presence of the dinoflagellate *Prorocentrum minimum*. *Toxins*, 7(5), 1779–1807.
- Wegh, R. S., Berendsen, B. J. A., Driessen-Van Lankveld, W. D. M., Pikkemaat, M. G., Zuidema, T., & Van Ginkel, L. A. (2017). Non-targeted workflow for identification of antimicrobial compounds in animal feed using bioassay-directed screening in combination with liquid chromatography-high resolution mass spectrometry. *Food Additives & Contaminants Part a Chem Anal Control Expo Risk Assess*, 34(11), 1935–1947.
- WHO (2009). *Toxicological and health aspects of melamine and cyanuric acid: Report of a WHO expert meeting in collaboration with FAO, supported by health Canada, Ottawa, Canada 1-4 December 2008*.
- WHO-IPCS (1988). *Environmental health criteria 80, Pyrrolizidine alkaloids*.
- Winkler, J. (2015). High levels of dioxin-like PCBs found in organic-farmed eggs caused by coating materials of asbestos-cement fiber plates: A case study. *Environment International*, 80, 72–78.
- Xu, M. L., Gao, Y., Han, X. X., & Zhao, B. (2017). Detection of pesticide residues in food using surface-enhanced Raman spectroscopy: A review. *Journal of Agricultural and Food Chemistry*, 65(32), 6719–6726.