chemistry

MDPI

Review

Modified Mycotoxins, a Still Unresolved Issue

Alberto Angioni [1,*], Mariateresa Russo [2,*], Cinzia La Rocca [3], Ornella Pinto [4] and Alberto Mantovani [3]

[1] Department of Life and Environmental Science, University of Cagliari, 09126 Cagliari, Italy
[2] Department of Agricultural Science, Mediterranean University of Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria, Italy
[3] Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[4] Directorate General of Collegial Bodies for Health Protection, Ministry of Health, Viale Giorgio Ribotta, 5, 00144 Rome, Italy
* Correspondence: aangioni@unica.it (A.A.); mariateresa.russo@unirc.it (M.R.)

Abstract: Mycotoxins are toxic secondary metabolites produced by filamentous microfungi on almost every agricultural commodity worldwide. After the infection of crop plants, mycotoxins are modified by plant enzymes or other fungi and often conjugated to more polar substances, like sugars. The formed—often less toxic—metabolites are stored in the vacuole in soluble form or bound to macromolecules. As these substances are usually not detected during routine analysis and no maximum limits are in force, they are called modified mycotoxins. While, in most cases, modified mycotoxins have lower intrinsic toxicity, they might be reactivated during mammalian metabolism. In particular, the polar group might be cleaved off (e.g., by intestinal bacteria), releasing the native mycotoxin. This review aims to provide an overview of the critical issues related to modified mycotoxins. The main conclusion is that analytical aspects, toxicological evaluation, and exposure assessment merit more investigation.

Keywords: modified mycotoxins; metabolites; risk assessment; dose additivity; toxicity; exposure

Citation: Angioni, A.; Russo, M.; La Rocca, C.; Pinto, O.; Mantovani, A. Modified Mycotoxins, a Still Unresolved Issue. *Chemistry* **2022**, *4*, 1498–1514. https://doi.org/10.3390/chemistry4040099

Academic Editor: Bartolo Gabriele

Received: 19 September 2022
Accepted: 4 November 2022
Published: 9 November 2022

1. Introduction

Mycotoxins are toxic secondary metabolites produced by fungi [1–3]; however, the biological meaning is more restrictive and indicates all the toxic substances produced by food "molds" that cause intoxication in animals, including livestock and humans, after ingestion (mycotoxicosis) [4]. In many cases, mycotoxins can be produced by fungi directly on the plants during vegetation and are already present in agricultural commodities at the time of their collection. Others are produced during storage or technological processes.

A renewed interest in fungal metabolites began after the turkey X disease in the 1960s, leading to the finding of other compounds called mycotoxins in various plant products and reconsidering compounds isolated and characterized since the 1930s and 1940s during research on antibiotics. Currently, several hundred mycotoxins are known; however, most of them are toxic metabolites that form in laboratory conditions with a very low probability of being found as natural contaminants. Indeed, many toxigenic fungi are not food colonizers or show limited synthetic capabilities. Therefore, only a few mycotoxins have been found in foods, and only a few have been irrefutably associated with mycotoxicosis [5].

Nevertheless, the widespread occurrence and remarkable intrinsic toxicity (including carcinogenicity—aflatoxins and ochratoxin A and endocrine disruption—zearalenone) of the main mycotoxins make them a severe global issue for public health and food security. Mycotoxicosis was considered more common in developing world areas; however, climate change is increasingly bringing mycotoxin contamination to industrialized countries, including the European Union [6].

During the mid-1980s, several animals' mycotoxicosis was not correlated to the feed mycotoxin content [7,8]. These new findings have highlighted that regulated mycotoxins

are not the primary source of exposure to these toxins [9]. These new-found toxins are metabolites of mycotoxins and deserve consideration as well. Metabolites can have different origins; they may be biosynthetic precursors (i.e., upstream of the formation of the main mycotoxin), metabolites, or degradation products of the "parent" compound through different pathways: (a) by the fungus itself that spreads them in the host [10]; (b) as a by-product of the metabolism of the hosts (by metabolism in plants, animals, and humans) [10], including the action of the host's microbiome [11]; and (c) they may have been formed abiotically through the chemical reaction of the parent toxin with the matrix during food processing [12,13]. In many cases, the metabolite exists as a conjugated (extractable) derivative or as a non-extractable bound association (covalently or not) with polymeric carbohydrate or protein matrices [12]. These latter ones justify the former denomination of "masked" (or also "hidden") mycotoxins since they are difficult to screen in food and feed samples [8,13–15]. Modified mycotoxins include both extractable conjugated forms and bound (non-extractable) forms. Bound mycotoxins are covalently or non-covalently tied to polymeric carbohydrate or protein matrices [12]. After possible decomposition during digestion, the free toxin can be released, resulting in mycotoxicosis [16]. The domain of modified mycotoxins includes a large and diverse array of free, conjugated, and matrix-associated metabolites. According to different authors [10,14] and in agreement with international and national food safety bodies, including the Italian National Food Safety Committee, we will use the general term "modified" mycotoxins in this paper.

Notwithstanding the growing attention toward modified mycotoxins, limited eligible data is currently available on modified mycotoxins, their stability, properties, and conversion from one form to another during processing [17]. Based on the analysis of state-of-the-art knowledge on their origin, occurrence, and toxicity, our paper intends to highlight critical issues for risk assessment and further research on the occurrence of masked mycotoxins, their stability, properties, and conversion from one form to another during processing.

2. Modified Mycotoxin Origin

Modified mycotoxins can be generated by plants in defense of the parasitic fungus that produces mycotoxins or by the fungus that spreads them in the host [10]. Some studies have shown the possibility that modified mycotoxins are produced during metabolic processes in animals and humans and during food processing [12,18–21]. Therefore, a distinction should be made between the origin and the modifications encountered by modified mycotoxins. The primary source is related to plant and fungus metabolism, whereas the secondary source is related to fungi and animals (including humans), mammalian metabolism, and food processing.

Plants can metabolize xenobiotic compounds, including mycotoxins, as part of their defense against pathogens [22].

Modified mycotoxins arising from plant host activity are the most widespread. They are produced via enzymatic detoxification processes, converting mycotoxins into more polar metabolites, which are transported into vacuoles for further storage or conjugated to biopolymers such as cell wall components [13,14]. *Fusarium* infection usually occurs in the field (in contrast to *Aspergillus* or *Penicillium* infections); the *Fusarium* mycotoxins deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1), T-2 Toxin, HT-2 Toxin, and nivalenol (NIV) are the most prominent targets for conjugation.

Plants can convert trichothecenes and zearalenone into polar derivatives after conjugation with sugars, amino acids, or sulphate groups [22].

Phase I reactions consist mainly of hydrolysis, reduction, and oxidation. The most prevalent reactions involve the cytochrome P450 monooxygenases (P450), which convert lipophilic toxins by oxidation to hydrophilic (excretable) metabolites [23]. Hydrolysis is catalyzed by esterases and amidases and can serve as a detoxification or activation mechanism governing mycotoxin selectivity or resistance [24]. The plant specificity of

esterase varies dramatically among species and biotypes. Moreover, the compounds from phase I reactions could have even higher toxicity [25].

Phase II is mainly characterized by conjugation; the enzymes involved can act on phase I compounds with covalent binding [19]. The main enzymes are glucosyl-, malonyl-, and glutathione-S-transferases (GSTs) [25]. These reactions led to non-toxic or less-toxic chemicals than the parent compound.

GSH (γ-glutamyl-cysteinyl-glycine) is plants' second primary detoxification mechanism [26]. The conjugation compounds are highly polar and hydrophilic and contain a side group with two carboxyls, an amine group, two peptide bonds, and a thiol.

The conjugated mycotoxins follow different metabolic patterns. GSH conjugates lead to products that differ from the parent toxicant; epoxides, lactones, or aldehyde groups form irreversible derivatives, whereas glucosyl compounds can be reversed by numerous glycosidases in plants and the digestive systems of animals.

It was observed that the level of modified mycotoxins after GSH conjugation in plants could increase after herbicide treatments stimulated the biochemical reaction [13].

After conjugation, the compounds are stored in the vacuoles (Phase III) or are irreversibly bound to the cell wall. Thus, detoxification products are concentrated and stored in the plant tissue.

Fusarium mycotoxins (DON, ZEA, T2, HT2, and NIV) are the primary targets for phase II conjugation reactions with monosaccharides, glutathione, or sulphates [19].

Zearalenone-4-glucoside (ZEA4G) and deoxynivalenol-3-glucoside (DON3G) have been detected in naturally contaminated wheat [27,28]. Oligoglycosylated DON was reported in beer, malt, and bread [29]. T-2-glucoside (T-2-G) and HT-2-glucoside (HT-2-G) were detected in naturally contaminated wheat, oats, and maize [28,30].

Glucoside conjugates of fusarenon-X (FUSX-G) and nivalenol (NIV-G) were found in artificial wheat with Fusarium spp. [10,31]. Type A trichotecene glucosides, neosolaniol-glucoside (NEO-G), and diacetoxyscirpenol-glucoside (DAS-G), were found in maize powder [32]. Contaminated wheat showed the presence of DONGSH [33]. DON3S and DON15S are obtained by sulphatase conjugation or glutathione S-transferase [34,35].

Ochratoxin A (OTA) metabolism in plants studied in in vitro trials revealed two principal metabolites, (4R)- and (4S)-4-hydroxy-OTA; moreover, β-glucosides were characterised for both isomers [36,37].

Among fungal conjugates, 3-acetyl-deoxynivalenol (3A-DON) and 15-acetyldeoxynivalenol (15A-DON) have been characterised in cereals contaminated with *F. graminearum* [19,38]. These compounds are biosynthetic precursors of DON, and subsequently a UDP-glucosyltransferase trichothecene specific converts DON into DON3G [19,39]. Fusarenon-X conjugate, a precursor of NIV, was found in infected maize [19]. The saprobic *Rhizopus* fungus can metabolize ZEA to ZEA14S [19].

Mammals can conjugate mycotoxins before excretion. DON and ZEA glucuronides and sulphate have been reported recently [40–43]. Urine samples and human and animal liver microsomes showed the presence of D3GlcA and D15GlcA [44,45]. Other authors reported the presence of OTA-acyl-GlcA, OTA-phenol-GlcA, and OTA-amino-GlcA glucuronides excreted via urine as OTα-acyl-GlcA and OTα-phenol-GlcA in urine samples [46].

3. Occurrence of Modified Mycotoxin

Modified mycotoxins have been found in food and feed, predominantly those that are cereal-based. The most frequently identified modified mycotoxin belongs to the family of the fusariotoxins and are β-linked glucose-conjugates of deoxynivalenol, nivalenol, HT-2 (DON3G, NIV3Glc, HT2Glc), zearalenone (ZEA14G, α-ZEL14G, β-ZEL14G), zearalenone-14-sulphate (ZEA14S), and fumonisins-esters (Table 1) [19,47–50].

Table 1. Commonly modified mycotoxins.

Toxin Family	Free Form	Modified Mycotoxin	Food and Feed Occurrence
Fusarium	Zearalenone (ZEA)	ZEA14G; ZEN14ßDG; ZEA 4-β-D-glucopyranoside; ZEA 2,4-O-β-diglucoside; ZEA14ßDGp; ZEA14S; palmitoyl ZEA	maize, wheat bran, grains, grain-based food (breakfast cereals, bread, bakery wares); and vegetable oils
	Deoxynivalenol (DON)	DON3G; DON, 3-β-D-glucopiranoside	wheat, maize, oats, barley, beer, breakfast cereals, and snacks
		DON 3-acetyl; DON 15-acetyl	corn, wheat, and rye grain,
		DON glutathione (DON-GSH); sulpho-conjugates	wheat and oats
	Nivalenol (NIV)	NIV3G; NIV 3-acetyldeoxy; NIV 15-acetyldeoxy; NIV 4-acetyl; NIV 4,7-dideoxy	wheat and corn
	Fumonisin (FB)	HFB1, N-acyl-HFB1	corn
	Trichothecenes	T-2-3α-glucoside; HT-2-3-glucoside; palmitoyl tricotecolone	wheat and oats
		NEO-G; DAS-G	maize
Aspergillus/Penicillium	Ochratoxin A	(4R)- and (4S)-4-hydroxy-OTA; and β-glucosides	tomato, potato, maize, carrots, wheat, soybean, and paprika

ZEA-16-β-D-glucopyranoside (ZEA16G), a novel modified ZEA metabolite, together with α- and β-ZEL14G conjugates (20–100% of free ZEA), were found in bread and breakfast cereals [22,51–53].

Animal feed naturally contaminated with ZEA showed the presence of the sulpho-conjugate ZEA14S with unique trends of mycotoxin occurrence within cultivars and local weather [54]. Other modified mycotoxins conjugated with single-sugars such as di-, tri-, and tetra-glucosides, mixed disaccharides, and malonyl-glucosides have been characterized for DON, T2, HT2, and ZEA [22,29,32,55].

DON3G has been found in wheat, maize, oats, and barley, and the resultant beer, breakfast cereals, and snacks, at relative molar proportions of 20–70% of free DON and at a concentration ranging from 2–1700 mg/kg in naturally contaminated wheat [13,56–59]. Fusarium head blight-resistant wheat produced uncharacterized products from 14C-labelled DON more effectively than susceptible wheat cultivars [60]. A survey from the Czech market showed a higher presence of DON3G than DON [61]. Similar results were found in Belgium [56].

Moreover, DON glutathione (DON-GSH) and sulpho-conjugates were found in in vitro trials on wheat and oats [33,53,54,61,62]. In addition, DON–glutathione and its processing products, DON-S-cysteine, DON-S-cysteinyl–glycine, and DON-malonylglucoside, were found [61]. *Fusarium graminearum*-inoculated or DON-treated wheat showed the presence of DON-3-sulphate and DON-15-sulphate [62]. Afterwards, other biotransformation products such as DON-hexitol were characterised (e.g., mannitol), DON-di-hexoside (e.g., glucose), 15-acetyl-DON-3-β-D-glucoside, and a DON–glutathione derivative missing two protons [35].

Fumonisin protein conjugates have been detected in maize foods; however, conjugates with starch, pectin, hemicellulose, cellulose, and lignin could be theorised, even if their exact composition is still unknown [63].

NIV3G (12–27% of NIV) and fusarenon-X-glucoside (FUSX-3-G) have been reported in wheat [31], as have T2Glc and HT2Glc in wheat and oats [64].

Trichothecenes glucosides type A neosolaniol-glucoside (NEO-G) and diacetoxyscirpenol-glucoside (DAS-G) were detected in maize powder [32].

(4R)- and (4S)-4-hydroxy-OTA and their β-glucosides were found in vegetables contaminated with OTA [27].

The limited data indicate that modified mycotoxins make up a significant fraction of the overall burden of mycotoxin contamination in foods and feeds, particularly concerning cereals' fusariotoxins.

4. Impact of Environmental Conditions on the Development of Modified Mycotoxins

Environmental conditions affect the development of mycotoxins and mycotoxicosis. Environmental changes are related to crop production, seasonality, and climate modifications. Fungi can modify their behavior to adapt to different situations. In particular, other environmental conditions could lead to a different susceptibility of crops to fungi contamination; stressed maize and figs showed more *A. flavus* contamination. Moreover, climate change could affect the ability of fungi to contaminate foods.

The increased global air and water temperatures and the melting of natural snow and ice stocks will affect the food supply chain and possibly create a more comfortable environment for fungal development. *Fusarium* prefers temperate weather ranging from 26–28 °C and water activity (aw) > 0.88, whereas *Aspergillus flavus* needs warm temperatures.

Facing climate changes, humans will have to deal with increased atmosperic CO_2, increased rainfall, desertification, and sudden changes in temperature and humidity, which will affect crop resistance and mycotoxin-producing fungi behavior. The increase in fungi pollution at temperatures and relative humidity ideal for mycotoxin production could increase parent and masked mycotoxins, setting a severe alarm on food contamination and human safety.

5. Fate of Modified Mycotoxins

Emerging knowledge has established that fungi can metabolize mycotoxins to produce modified mycotoxins. Moreover, these compounds can be degraded during food processing or reconverted to the parent compound during digestion [65,66]. During food processing, chemical hydrolysis, fermentation, and germination processes can lead to the release or generation of modified mycotoxins [13].

5.1. Fungi Metabolism

Fungi can have the ability to metabolize mycotoxins produced by mycotoxigenic fungi. The saprobic *Rhizopus* fungus can turn ZEA into zearalenone-14-sulphate (ZEA14S) [19]. In vitro studies showed the ability of *Rhizopus* and *Aspergillus orizae* strains to convert ZEA into ZEA14S, ZEA-O-14- and ZEA-O-16-glucoside. Moreover, α-zearalenol (α-ZEL) and α-ZEL-sulphate were detected [67].

5.2. Food Processing

The wide range of processes at the industrial level involving mechanical or thermal energy can generate unpredictable reactions with sugars, proteins, and lipid molecules or cause the release of the free forms after decomposition. Cereal-based foods are produced via a sequence of operations that depend on the by-products and could lead to enhanced or reduced levels of DON and NIV [68]. The so-called "fumonisin paradox" hypothesises the presence of "bound" or "hidden" fumonisins to explain the toxic effects of low-level contaminated food [69].

Roasting of OTA-contaminated foods leads to conversion to the R stereoisomer 14R-ochratoxin A and 14-decarboxy-ochratoxin, OTA-glucose ester, OTA-methyl-α-D-glucopyranoside ester, and OTA-cellobiose ester [70,71]. FB decreases with reducing sugars during Maillard-type reactions and is consistent with the formation of N-carboxymethyl fumonisin B1 (NCM-FB1) and N-deoxyfructosyl fumonisin B1 (NDFFB1) [72,73]. In contrast, the hydrolyzed form of fumonisin B1 formed by cleavage with carballylic moieties was detected in the production of tortillas in South America [74].

Heat and fermentation are the treatments that most influence the formation of modified mycotoxins. A significant increase of DON3G has been found in fermented malt during malting and brewing, along with DON di-glucosides, tri-glucosides, and acetylated DON forms (A-DONs) [20,75]. Moreover, baking increases DON3G concentration until enzyme inactivation [76,77]. Furthermore, during steamed bread processing, DON3G was reconverted to DON [78].

Coffee roasting causes the formation of OTA degradation products, such as 14R-ochratoxin A and 14-decarboxy-ochratoxin [14,70,79]. The thermal reaction can also lead to coffee polysaccharide esterification, producing OTA-glucose ester, OTA-methyl-α-D-glucopyranoside ester, and OTA-cellobiose ester. Moreover, 2'R-ochratoxin A (2'R-OTA) was detected during the thermal processing of coffee and bread [80].

The ratio HFBs/FB was higher in processed products (cornflakes, snacks) than in flour or raw popcorn grains [49]. FB hydrolysation and stability were heavily influenced during extrusion and nixtamalization, depending on pH and baking conditions [81,82]. During gluten-free bread making, FBs were found physically entrapped in a different macromolecular structure, such as starch [83].

5.3. Insect Metabolism

Insect-derived proteins are increasingly considered potential ingredients in foods and feed [84]; hence, it is relevant to consider the fate of mycotoxins in insects. The relationship between insects and fungi is governed by an optional mutualism, where insects spread the conidia and the fungi enhance the larval diet, improving its growth. Moreover, the damage caused by insects can predispose plants to being colonised by mycotoxin-producing fungi [85]. Herbivorous and helpful insects can be affected by mycotoxins [86,87], but they can also metabolise them [88]. This behavior was reported for *Amyelois transitella* and *Helicoverpa zea* concerning *A. flavus* [89–91].

The insect detoxification system involves mainly the P450s in Clade 3 and is independent of the taxonomic origins of the toxin [92,93]. Moreover, prior induction can enhance their detoxification capacity when larvae are in contact with phytochemicals or pesticides [89,94].

The larvae of *D. melanogaster* showed attraction to and fed on *Aspergillus nidulans* fungal colonies producing sterigmatocystin [95]. The analysis showed that the P450 pathways were related to the metabolism of xenobiotics. *Hermetia illucens* subjected to AFB1 showed an increase in the P450 gene families and converted up to 60% of aflatoxicol and aflatoxin P1, which can have a genotoxic potential [46,96].

5.4. Digestion Metabolism

The behavior of modified mycotoxin after ingestion is still a question with many uncertainties. DON, ZEA, and OTA glucuronides and sulphates conjugates have been found in mammals' biological fluids after contamination [41,44,46,97].

The metabolism of mycotoxins in mammals and birds is linked to specific cytochrome P450s, particularly in family CYP3 and subfamilies CYP1A and CYP2A. Mammalian form mycotoxin conjugates in the liver before excretion via urine [13]. Šarkanj et al. established a dose-response relationship in pregnant women from Croatia, and conjugates could be used as biomarkers of exposure to mycotoxins [97]. Glucuronidation and sulphation are the most accepted methods of detoxification for fumonisin. However, it is not entirely demonstrated that these lead to a decrease in the toxic effect [98]. DON-3-glucuronide (DON3GA), and DON-15-glucuronide (DON15GA) have been characterised in urine samples and human and animal liver microsomes [44,96,99]. OTA and OTα undergo glucuronidation, leading to OTA-acyl-GlcA, OTA-phenol-GlcA, OTA-amino-GlcA, OTα-acyl-GlcA, and OTα-phenol-GlcA [100]. OTA ethyl ester (OTC) was rapidly converted to OTA after oral administration to rats [101].

Moreover, liver microsomes from humans, pigs, and rats are capable of metabolising OTA into (4R) and (4S) hydroxy OTA [102–104]. The type of metabolite and the amount of

excretion are influenced by animal species, sex, age, enterohepatic recirculation, and OTA's binding to serum macromolecules. These are different for urine and faeces [104,105]. A significant fraction of OTA is bound to serum macromolecules, representing an internal reserve that can be released over time [106]. Ruminants are much less sensitive to OTA than non-ruminants; the rumen microbiota was found effective in reducing the level of mycotoxins [107,108]. However, a recent study observed that the detoxification process could differ among the various mycotoxins and rumen conditions. The mycotoxins may remain intact without degrading [109]. Biomonitoring studies on the blood pollution of high coffee drinkers showed high exposure to 2'R-OTA; however, no data were found concerning its metabolic pathway [110].

Bioavailability studies on ZEA in swine showed that α- and β-ZEL glucuronides are completely absorbed after oral administration. ZEA14G was less than 61%, suggesting complete pre-systemic hydrolysis; ZEA-4-β-D-glucopyranoside was completely hydrolyzed to zearalenone and α-zearalenol [8].

The microbiome role has yet to be thoroughly investigated but is likely to be important [11]. The human microbiota influences the conjugation binding in DON3G, ZEA14G, and ZEA14S, releasing DON and ZEA in the intestinal tract [111,112].

ZEA-14G, α-ZEL-14G, and glucuronic acid (GlcA) conjugates were observed in plasma after intravenous injection of α-ZEL and α-ZEL-14G. The low oral bioavailability of α-ZEL-14G and ZEN-14G cannot rule out the possibility of causing toxic responses after hydrolysis [113]. The calculated elimination rates of α-ZEL and α-ZEL-14G showed low retention of these compounds in the body [114].

In vitro studies showed the introduction of a hydroxyl group in α-ZEL-14G, enhancing the polarity, promoting the elimination, and decreasing the toxicity [20].

The preferred biochemical pathways of α-ZEL-14G are represented by GlcA conjugation, dehydrogenation, and hydrolysis. In vitro liver microsomes of animals and humans showed a Phase II metabolism that produced ZEN-14G and β-ZEL-14G from α-ZEL-14G obtained from the Phase I system. Moreover, this compound could lose the glucose by hydrolysation, yielding α-ZEL, which could be dehydrogenated into ZEA and, subsequently, GlcA conjugated, forming different metabolites [114].

Dehydrogenation and subsequent GlcA conjugation of α-ZEL-14G can lead to 14-GlcA-α-ZEL-14G, α-ZEL-14G-7-GlcA, and α-ZEL-14G-16-GlcA [115].

Urine and faecal samples collected after oral administration on swine showed the absence of α-ZEL, α-ZEL-14 G, and their metabolites. In faeces, after 24 h, significant glucuronic acid conjugates of α-ZEL and α-ZEL-14G were identified probably related to the enterohepatic circulation [116,117]. The species-specific aspects of metabolism at the ribosomal level led to glycosylated metabolites in monogastric animals (rat, chicken, pig, and humans) that were different from those produced in ruminants, as reported in studies on gastrointestinal enzymes and α-zearalenol [3,20,113]. The favorite binding site for glucuronidation was C-16 in the liver microsomes of rats, chickens, swine, and humans. In ruminants (goats and cows), GlcA bound at C-7 and C-14, forming α-ZEL-14G-7-GlcA and, to a lesser extent, α-ZEL-14G-GlcA [115].

DON3G was stable in in vitro studies with hydrochloric acid and human enzymes. However, lactic acid bacteria showed the ability to partially release DON. Moreover, DON occurs after incubating DON3G with human faeces [49,112].

The metabolism of DON3G in chicken, rats, and swine showed notable differences; in the intestinal tract of swine and rats, it was completely hydrolysed, absorbed as DON, and excreted as glucuronide, whereas in chicken it is less absorbed and is mainly conjugated with sulphate [118–120].

T-2 and T-2-3α-glucoside (T2-G) in chicken showed a greater bioavailability of the glycosylated mycotoxin; no hydrolysed compounds after oral or intravenous administration of T2-3-O-α-Glcp were observed in blood samples [121]. In vitro studies showed the resistance to digestion in the upper gastrointestinal tract of T2-3-O-α-Glcp, whereas the α

and ß anomers were degraded in human colon microbiota conditions [47,122]. The limited data avoids drawing conclusions on the stability of T2-Glcp and HT2- Glcp in humans.

6. Analysis of Modified Mycotoxins

The availability of valid analytical methods for identification and quantification is the basis for the exposure assessment [12,22]. This step is deeply influenced by the absence of analytical standards and a wide variety of modified mycotoxins with changed physicochemical properties leading to modified chromatographic behavior. LC-MS/MS represents the most reliable and widely used analytical technique for mycotoxins; these methods can quantify modified mycotoxins along with their parent forms, which are efficient but very expensive for routine analysis [18,123]. The National Reference Laboratory (LRN) of Mycotoxins has developed and validated an analysis method for the separation and determination of modified forms of DON, namely 3A, 15A-DON, and DON3G in cereal-based products [123]. Fiby et al. reported a new LC/MS method for determining 3A and 15A-DON, and DON3G using a stable isotope dilution assay [124]. Many reviews investigated the analytical methodology for mycotoxin determination in different matrices [125]. However, the methods for detecting modified mycotoxin are still lacking, and the mycotoxin content of samples containing these compounds could lead to their underestimation by HPLC or LC-MS/MS methods (Table S1).

The analytical approach addresses two aspects: the qualitative analysis of the compounds as they are and the quantitative analysis performed after hydrolysis to their parent using enzymes or acidic or alkaline conditions to break the conjugation bond. The quantitative answer is given for the parental compound and the modified mycotoxin through the difference between the two analyses. Most works dedicated to analytical determinations are addressed to DON and ZEA [126,127]. In addition, some authors stated the inconsistency of acid hydrolytic cleavage to determine DON in cereal from DON3G [128]; on the contrary, β-glucosidase was more performant [129].

In addition, mycotoxins are not homogeneously distributed in the matrix, and the sample should be carefully taken to have detailed data [4,130]. Moreover, the extraction and clean-up steps used for mycotoxins have not proved efficient for modified compounds. Modified mycotoxins in the same sample could be soluble in hydrophilic or lipophilic solvents; furthermore, their affinity with the SPE purification stationary phase could be completely different. There are currently no monoclonal antibodies specific to modified mycotoxins.

Immunochemical methods showed a discrete ability to detect modified mycotoxins due to the cross-reactivity of antibodies towards them, even if the epitopes could have been modified or destroyed and were not recognised by the antibodies.

Furthermore, the ELISA methods do not require the clean-up step and seem to account for part of the modified mycotoxin concentration not proving to be fully reliable (high cross-reactivity and lack of reactivity) [131].

7. Toxicology

In order to characterise a mycotoxicosis, a dose-response relationship between the active compound (the parent mycotoxin and metabolites sharing the toxicological effect) and the disease should be defined. When human populations are involved, epidemiological studies are required. Moreover, animal models can confirm suspected human mycotoxicosis [1]. Environmental monitoring in food, air, or other samples and biological monitoring in tissues, fluids, and excreta constitute exposure assessment and can thus integrate mycotoxicosis studies. In particular, biomonitoring studies evaluate aggregate exposure, which takes into account the overall intake through the diet and any non-food environmental exposures. Biomonitoring is especially important in risk assessment as it helps put into relationship indicators of the internal exposure (the dose present in the body) with early indicators of disease, biomarkers of exposure, and biomarkers of effect, respectively [132].

Even if mycotoxin exposure is more likely in developing countries, specific subgroups may be vulnerable to mycotoxin exposure also in industrialised countries, depending on their eating habits; for example, Hispanic populations in the United States consume more maize products than the rest of the population and can be subjected to a higher uptake of mycotoxins [133]. Moreover, exposure to mycotoxin residues is likely to increase in industrialised countries due to climate change. Many modified mycotoxins identified in vivo and in vitro trials are still not corroborated by toxicity studies because a general interest in their toxicological properties is relatively recent. Currently, the evidence mainly involves the reconversion of DON3G to DON and ZEA-S to ZEA by the microbiota of the intestinal tract [112].

It is generally believed that mycotoxin levels and toxicity have been underestimated precisely because of the modified mycotoxins. However, the toxicity of modified mycotoxins is usually considered lower than that of their parent compounds in terms of potency; their toxicity could also be qualitatively similar to the parent compound. For example, DON3G binds to the ribosome to a lesser extent than DON, resulting in a highly diminished inhibition of protein synthesis [134]. Moreover, ZEA14G does not fit entirely with the estrogen receptor, being less estrogenic than ZEA [135]. However, the consideration of Berthiller et al. [13] is still largely valid, asserting that insufficient evidence exists to compare the toxicological potencies of modified mycotoxins with their parent molecules.

Another source of uncertainty is represented by the limited data on modified mycotoxins during digestion and in the intestinal tract. In addition, the microbiota can reconvert it in the parent compound or even into compounds with increased toxicity and/or bioavailability [19,20].

Nevertheless, the reconversion issue is potentially severe, as shown by human biomonitoring data on the fusariotoxins ZEA and DON. High urinary levels of ZEA and its metabolites α-and β-ZEA have been found in heavy consumers of maize, with substantial differences depending on the climatic and environmental conditions that favour fungal contamination of food [136]. Evaluation of the same biomarkers in a cohort of 60 adults in Germany showed urinary levels of α-ZEL, higher than the parental compound [137]. Dellafiora et al. clearly showed high toxicological concerns related to zearalenone-14-glucoside, which is prone to conversion to toxic compounds (i.e., zearalenone and both isomers of zearalenol) when exposed to breast cancer cell cultures [18].

A pilot study on twenty volunteers subjected to a restricted cereal diet for four days and then to the oral administration of a single dose of 1 μg/kg bw of DON or DON3G showed that the modified forms must be considered together with the parental compound in the exposure assessment [138,139]. A study conducted on 200 healthy volunteers belonging to six groups of consumers (children, adolescents, adults, elderly, vegetarians, and pregnant women) from three European countries (Italy, Norway, and the United Kingdom) [123] showed that deoxynivalenol glucuronide (DON-GlcA) represents up to 80% of total DON, thus representing the primary metabolic form in human urine. The de-epoxide form (DOM-1) was detected at low concentrations in only 12% of the samples of the Norwegian volunteers and 1.5% of the Italian ones. In contrast, it was not present in the UK samples. Hence, modified mycotoxins can make up a significant fraction of the mycotoxin toxicological burden.

8. Modified Mycotoxins and Risk Assessment: Critical Issues and Uncertainties

The consideration of modified mycotoxins in risk assessment suffers from considerable uncertainties. The crucial question is whether and to what extent the modified mycotoxins contribute to the toxicologically relevant exposure associated with the parental mycotoxin in realistic exposure scenarios. In this respect, two pathways can be identified.

First, the toxicity of modified mycotoxins equals that of the parent compound, albeit with a lower potency. In this case, the critical issue is the quantification of modified mycotoxins in foods and organisms to refine the human exposure assessment.

Second, modified mycotoxins can provide a source, or reservoir, of the parent compound; such reconversion to the parent compound is also possible during digestion [13]. The Aflatoxin B1 metabolite, aflatoxicol, is a potential reservoir in the organism of the highly hepatotoxic and carcinogenic parent, Aflatoxin B1 [140]. In this case, the critical issue is the reconversion rate in real-life situations.

Both pathways indicate that consuming contaminated food by humans or animals leads to exposure to both the parental compound and the modified forms, hence a combined exposure that needs to be assessed through an evidence-supported conceptual framework.

Similar considerations motivate the precautionary approach adopted by EFSA, which has considered modified mycotoxins relevant for risk assessment for human and animal health. Two scientific opinions on food and feed have been published: zearalenone derivatives, nivalenol, T-2/HT-2 toxins, and fumonisins [10], deoxynivalenol [141], as well as two on feeds regarding ZEA [142] and fumonisins [143].

Based on the limited data available, EFSA considers an additive effect of the metabolite, assigning the same toxicity as the respective parental compounds [10]. Most of the work on modified mycotoxins is focused on DON3G and ZEA14G, and only a few studies investigate other compounds.

EFSA stated that the additional contribution of modified mycotoxins represents, compared to parental compounds, 10% for trichothecenes, 30% for DON and nivalenol, 60% for fumonisins, and 100% for zearalenone. Hence, the endocrine-disrupting (estrogenic) ZEA, including the modified forms, represents a doubling of the exposure; a significant increase is also estimated for fumonisins.

Therefore, the overall contamination of the food and the consequent exposure to the consumer (compound parental + modified) can increase significantly, especially for ZEA, fumonisin, and DON. EFSA estimated that including modified mycotoxins in exposure would lead to a significant (2–3 times) exceeding of the TDI (Tolerable Daily Intake) values of fumonisins and DON for high consumers (95th percentile) of food-based cereals, especially for children. However, the risk may be overestimated due to the numerous uncertainties addressed with precautionary assumptions [141].

Additionally, when assessing animal health, ZEA should be considered in conjunction with its modified forms [113,143].

Another paper estimated the exposure to modified mycotoxins with an increase of 10% for DON and its metabolites, 36% for ZEA, and 48% for the sum of T-2/HT-2 toxins in infants and children fed with cereal-based products; notwithstanding the increased exposure estimates, values are 2–3 times lower than the tolerable daily intakes [144].

Overall, the combined exposure to multiple modified mycotoxins with possible additive or synergistic effects represents a further aspect of being considered in risk assessment and deserving appropriate studies following the evaluation methodology used in pesticide cocktails [145,146].

9. Conclusions

Beyond the toxicological harms established by known mycotoxins, 'modified mycotoxins' represent a new level of complexity when assessing toxicity and developing an evidence-based risk analysis. Experimental and real-life evidence showed many modified mycotoxins in food and feeds. The number and type of modified mycotoxins are a function of different environmental, biological, chemical, and chemical-physical variables.

The available data, however, indicate that the modified mycotoxins DON, ZEA, and fumonisin B are obvious priorities due to their toxicity and potential exposure to humans and animals.

In consideration of the differences observed between the animal species, including humans, the evaluation of biotransformation and bioavailability is a crucial aspect on which further data and studies are needed, identifying patterns more appropriate as well as considering the possible interactions with the microbiome.

It must be stressed that modified mycotoxins can become a severe problem for farm animals that are often fed cereal-based feeds. Feed ingredients could contain modified forms after harvest. After the material is ingested and broken down in the intestinal tract, the mycotoxin is released from the glucose and can regain its previous toxicity. Such an effect ultimately compromises animal health, performance, ROI (Return on Investment), and financial viability of animal producers. Human nutrition and welfare might also be affected, as animal mycotoxicoses can impair the production and quality of foods of animal origin.

Unfortunately, no sufficient toxicokinetic and toxicodynamic studies are available to reveal possible hazards or risk assessments of modified mycotoxins compared with the parent mycotoxins. Therefore, it is still impossible to perform a proper risk assessment for modified mycotoxins in food due to the lack of data on exposure and toxic properties. Risk assessors should mainly pinpoint the most relevant data gaps and the associated research needs.

One central data gap concerns detection. Although there are numerous LC/MS/MS analytical methods for determining modified mycotoxins in cereals, validated analytical standards are still lacking, making it impossible to carry out routine monitoring of food and feed products.

All these variables and the scarcity of experimental data are among the leading causes of the current uncertainty regarding human exposure.

Dose additivity based on convergence on effects associated with some functional events can be hypothesised, at least for combined exposure to fusariotoxins. The experimental basis is entirely insufficient, but the available data would suggest a scientific approach similar to the one adopted by the EFSA for the evaluation of pesticide cocktails, i.e., considering additivity based on similar toxicological effects. In this sense, combining data on biochemical events and toxic effects at the cellular, organ, and organism level through the description of an Adverse Outcome Pathway (AOP) is a valuable tool for associating effects to exposure and elaborating risk assessment.

Based on the available data, the glycosidic forms of the mycotoxins identified as priorities (e.g., DON, ZEA) should be included in the dietary exposure assessment of these mycotoxins and in judging the fitness of food for consumption. All modified and unmodified mycotoxins should then be evaluated together with their combinations.

Recognising the toxicological relevance of modified mycotoxins in food commodities is a new challenge that should be considered by the respective regulatory bodies, food manufacturers, and monitoring authorities to protect consumers' health.

Efforts should be made to commercialise major modified mycotoxin testing devices so that the mycotoxin analysis in the feed could explain most animal toxicity. Recent research shows that the mycotoxin-binding concept alone will not be able to manage the adverse effects of all mycotoxins. More research is needed to understand if feed additives intended as binders act on modified mycotoxins similarly to the parent mycotoxin.

Finally, scientists, regulators, and policymakers should remember that the mycotoxin issue is a frontline one: research shows climate change to be one of the main drivers of mycotoxin diffusion worldwide.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry4040099/s1, Table S1: Analysis of Modified mycotoxins Par_6.

Author Contributions: Conceptualisation, A.A., A.M. and C.L.R.; writing—A.A. and M.R.; writing—review and editing, A.A., A.M. and C.L.R.; supervision, O.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516. [CrossRef] [PubMed]
2. Keller, N.P.; Turner, G.; Bennett, J.W. Fungal secondary metabolism from biochemistry to genomics. *Nat. Rev. Microbiol.* **2005**, *3*, 937–947. [CrossRef] [PubMed]
3. De Boevre, M.; Graniczkowska, K.; Saeger, S.D. Metabolism of modified mycotoxins studied through in vitro and in vivo models: An overview. *Toxicol. Lett.* **2015**, *233*, 24–28. [CrossRef] [PubMed]
4. Iqbal, S.Z. Mycotoxins in food, recent development in food analysis and future challenges; a review. *Curr. Opin. Food Sci.* **2021**, *42*, 237–247. [CrossRef]
5. Agriopoulou, S.; Stamatelopoulou, E.; Varzakas, T. Advances in occurrence, importance, and mycotoxin control strategies: Prevention and detoxification in foods. *Foods* **2020**, *9*, 137. [CrossRef] [PubMed]
6. Leggieri, M.C.; Toscano, P.; Battilani, P. Predicted Aflatoxin B_1 Increase in Europe Due to Climate Change: Actions and Reactions at Global Level. *Toxins* **2021**, *13*, 292. [CrossRef]
7. Iqbal, S.Z.; Asi, M.R.; Jinap, S. Detection of aflatoxins and zearalenone contamination in wheat derived products. *Food Control.* **2014**, *35*, 223–226. [CrossRef]
8. Gareis, M.; Bauer, J.; Thiem, J.; Plank, G.; Grabley, S.; Gedek, B. Cleavage of zearalenone-glycoside, a masked mycotoxin, during digestion in swine. *J. Vet. Med. B* **1990**, *37*, 236–240. [CrossRef]
9. Stoev, S.D.; Denev, S.A. Porcine/Chicken or Human Nephropathy as the Result of Joint Mycotoxins Interaction. *Toxins* **2013**, *5*, 1503–1530. [CrossRef]
10. EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on the Risks for Human and Animal Health Related to the Presence of Modified Forms of Certain Mycotoxins in Food and Feed. *EFSA J.* **2014**, *12*, 107. [CrossRef]
11. Daud, N.; Currie, V.; Duncan, G.; Farquharson, F.; Yoshinari, T.; Louis, P.; Gratz, S.W. Prevalent Human Gut Bacteria Hydrolyse and Metabolise Important Food-Derived Mycotoxins and Masked Mycotoxins. *Toxins* **2020**, *12*, 654. [CrossRef]
12. Berthiller, F.; Schuhmacher, R.; Adam, G.; Krska, R. Formation, determination and significance of masked and other conjugated mycotoxins. *Anal. Bioanal. Chem.* **2009**, *395*, 1243–1252. [CrossRef] [PubMed]
13. Berthiller, F.; Crews, C.; Dall'Asta, C.; Saeger, S.D.; Haesaert, G.; Karlovsky, P.; Oswald, I.P.; Seefelder, W.; Speijers, G.; Stroka, J. Masked mycotoxins: A review. *Mol. Nutr. Food Res.* **2013**, *57*, 165–186. [CrossRef] [PubMed]
14. Rychlik, M.; Humpf, H.; Marko, D.; Danicke, S.; Mally, A.; Berthiller, F.; Klaffke, H.; Lorenz, N. Proposal of a comprehensive definition of modified and other forms of mycotoxins including "masked" mycotoxins. *Mycotoxin Res.* **2014**, *30*, 197–205. [CrossRef]
15. Faisal, Z.; Fliszár-Nyúl, E.; Dellafiora, L.; Galaverna, G.; Dall'Asta, C.; Lemli, B.; Kunsági-Máté, S.; Szente, L.; Poór, M. Cyclodextrins Can Entrap Zearalenone-14-Glucoside: Interaction of the Masked Mycotoxin with Cyclodextrins and Cyclodextrin Bead Polymer. *Biomolecules* **2019**, *9*, 354. [CrossRef] [PubMed]
16. Sobral, M.M.C.; Gonçalves, T.; Martins, Z.E.; Bäuerl, C.; Cortés-Macías, E.; Collado, M.C.; Ferreira, I.M.P.L.V.O. Mycotoxin Interactions along the Gastrointestinal Tract: In Vitro Semi-Dynamic Digestion and Static Colonic Fermentation of a Contaminated Meal. *Toxins* **2022**, *14*, 28. [CrossRef] [PubMed]
17. Bryła, M.; Waskiewicz, A.; Ksieniewicz-Wozniak, E.; Szymczyk, K.; Edrzejczak, R.J. Modified fusarium mycotoxins in cereals and their products-metabolism, occurrence, and toxicity: An updated review. *Molecules* **2018**, *23*, 963. [CrossRef] [PubMed]
18. Dellafiora, L.; Perotti, A.; Galaverna, G.; Buschini, A.; Dall'Asta, C. On the masked mycotoxin zearalenone-14-glucoside. Does the mask truly hide? *Toxicon* **2016**, *111*, 139–142. [CrossRef]
19. Broekaert, N.; Devreese, M.; deBaere, S.; DeBacker, P.; Croubels, S. Modified Fusarium mycotoxin sun masked: From occurrence in cereals to animal and human excretion. *Food Chem. Toxicol.* **2015**, *80*, 17–31. [CrossRef]
20. Freire, L.; Sant'Ana, A.S. Modified mycotoxins: An updated review on their formation, detection, occurrence, and toxic effects. *Food Chem. Toxicol.* **2018**, *111*, 189–205. [CrossRef]
21. Kovač, M.; Šubarić, D.; Bulaić, M.; Kovač, T.; Šarkanj, B. Yesterday masked, today modified; what do mycotoxins bring next? *Arh. Hig. Rada Toksikol.* **2018**, *69*, 196–214. [CrossRef]
22. Berthiller, F.; Maragos, C.M.; Dall'Asta, C. Introduction to Masked Mycotoxins Chapter 1. In *Masked Mycotoxins in Food—Formation, Occurrence and Toxicological Relevance*; Issues in Toxicology 24; Dall'Asta, C., Berthiller, F., Eds.; The Royal Society of Chemistry: London, UK, 2016; pp. 1–13.
23. Berenbaum, M.R.; Bush, S.B.; Liao, L.H. Cytochrome P450-mediated mycotoxin metabolism by plant-feeding insects. *Curr. Opin. Insect. Sci.* **2021**, *43*, 85–91. [CrossRef] [PubMed]
24. Hoagland, R.E. Microbial Allelochemicals and Pathogens as Bioherbicidal Agents. *Weed Tech.* **2001**, *15*, 835–857. [CrossRef]
25. Coleman, J.; Blake-Kalff, M.; Davies, E. Detoxification of xenobiotics by plants; chemical modification and vacuolar compartmentation. *Trends Plant Sci.* **1997**, *2*, 144–151. [CrossRef]
26. Cummins, I.; Dixon, D.P.; Freitag-Pohl, S.; Skipsey, M.; Edwards, R. Multiple roles for plant glutathione transferases in xenobiotic detoxification. *Drug Metab. Rev.* **2011**, *43*, 266–280. [CrossRef] [PubMed]

27. Berthiller, F.; Dall'Asta, C.; Schuhmacher, R.; Lemmens, M.; Adam, G.; Krska, R. Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 3421–3425. [CrossRef] [PubMed]
28. Lattanzio, V.M.T.; Visconti, A.; Haidukowski, M.; Pascale, M. Identification and characterization of new Fusarium masked mycotoxins, T2 and HT2 glycosyl derivatives, in naturally contaminated wheat and oats by liquid chromatography-highresolution mass spectrometry. *J. Mass. Spectrom.* **2012**, *47*, 466–475. [CrossRef]
29. Zachariasova, M.; Vaclavikova, M.; Lacina, O.; Vaclavik, L.; Hajslova, J. Deoxynivalenol oligoglycosides: New "masked" Fusarium toxins occurring in malt, beer, and breadstuff. *J. Agric. Food Chem.* **2012**, *60*, 9280–9291. [CrossRef]
30. Nakagawa, H. Research on mycotoxin glucosides (masked mycotoxins). *JSM Mycotoxins* **2016**, *66*, 21–25. [CrossRef]
31. Nakagawa, H.; Ohmichi, K.; Sakamoto, S.; Sago, Y.; Kushiro, M.; Nagashima, H.; Yoshida, M.; Nakajima, T. Detection of a new Fusarium masked mycotoxin in wheat grain by high-resolution LC-OrbitrapTM MS. *Food Addit. Contam. A* **2011**, *28*, 1447–1456. [CrossRef]
32. Nakagawa, H.; Sakamoto, S.; Sago, Y.; Kushiro, M.; Nagashima, H. Detection of masked mycotoxins derived from type A trichothecenes in corn by high-resolution LC-Orbitrap mass spectrometer. *Food Addit. Contam. A* **2013**, *30*, 1407–1414. [CrossRef]
33. Uhlig, S.; Stanic, A.; Hofgaard, I.S.; Kluger, B.; Schuhmacher, R.; Miles, C.O. Glutathione-conjugates of deoxynivalenol in naturally contaminated grain are primarily linked via the epoxide group. *Toxins* **2016**, *8*, 329. [CrossRef] [PubMed]
34. Stanic, A.; Uhlig, S.; Sandvik, M.; Rise, F.; Wilkins, A.L.; Miles, C.O. Characterization of deoxynivalenol-glutathione conjugates using nuclear magnetic resonance spectroscopy and liquid chromatography-high-resolution mass spectrometry. *J. Agric. Food Chem.* **2016**, *64*, 6903–6910. [CrossRef] [PubMed]
35. Kluger, B.; Bueschl, C.; Lemmens, M.; Michlmayr, H.; Malachová, A.; Koutnik, A.; Maloku, I.; Berthiller, F.; Adam, G.; Krska, R.; et al. Biotransformation of the mycotoxin deoxynivalenol in Fusarium resistant and susceptible near isogenic wheat lines. *PLoS ONE* **2015**, *10*, e0119656. [CrossRef] [PubMed]
36. Ruhland, M.; Engelhardt, G.; Wallnöfer, P.R. Transformation of the mycotoxin ochratoxin A in plants. 2. Time course and rates of degradation and metabolite production in cell-suspension cultures of different crop plants. *Mycopathologia* **1996**, *134*, 97–102. [CrossRef] [PubMed]
37. Ruhland, M.; Engelhardt, G.; Wallnoefer, P.R. Transformation of the mycotoxin ochratoxin A in artificially contaminated vegetables and cereals. *Mycotoxin Res.* **1997**, *13*, 54–60. [CrossRef]
38. Alexander, N.J.; McCormick, S.P.; Waalwijk, C.; Van Der Lee, T.; Proctor, R.H. The genetic basis for 3-AC-DON and 15-ACDON trichothecene chemotypes in Fusarium. *Fungal Genet. Biol.* **2011**, *48*, 485–495. [CrossRef]
39. Gardiner, S.A.; Boddu, J.; Berthiller, F.; Hametner, C.; Stupar, R.M.; Adam, G.; Muehlbauer, G.J. Transcriptome analysis of the barley-deoxynivalenol interaction: Evidence for a role of glutathione in deoxynivalenol detoxification. *Mol. Plant Microbe Interact.* **2010**, *23*, 962–976. [CrossRef]
40. Prelusky, D.B.; Veira, D.M.; Trenholm, H.L.; Foster, B.C. Metabolic fate and elimination in milk, urine and bile of deoxynivalenol following administration to the lactating sheep. *J. Environ. Sci. Health* **1987**, *22*, 125–148. [CrossRef]
41. European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission (CONTAM) related to Zearalenone as undesirable substance in animal feed. *EFSA J.* **2004**, *89*, 1–35. [CrossRef]
42. Mirocha, C.J.; Pathre, S.V.; Robison, T.S. Comparative metabolism of zearalenone and transmission into bovine milk. *Food Cosmet. Toxicol.* **1981**, *19*, 25–30. [CrossRef]
43. Olsen, M.; Mirocha, C.J.; Abbas, H.K.; Johansson, B. Metabolism of high concentrations of dietary zearalenone by young male turkey poults. *Poultry Sci.* **1986**, *65*, 1905–1910. [CrossRef]
44. Warth, B.; Sulyok, M.; Berthiller, F.; Schuhmacher, R.; Krska, R. New insights into the human metabolism of the Fusarium mycotoxins deoxynivalenol and zearalenone. *Toxicol. Lett.* **2013**, *220*, 88–94. [CrossRef] [PubMed]
45. Maul, R.; Warth, B.; Kant, J.S.; Schebb, N.H.; Krska, R.; Koch, M.; Sulyok, M. Investigation of the hepatic glucuronidation pattern of the Fusarium mycotoxin deoxynivalenol in various species. *Chem. Res. Toxicol.* **2012**, *25*, 2715–2717. [CrossRef] [PubMed]
46. Klapec, T.; Šarkanj, B.; Banjari, I.; Strelec, I. Urinary ochratoxin A and ochratoxin alpha in pregnant women. *Food Chem. Toxicol.* **2012**, *50*, 4487–4492. [CrossRef] [PubMed]
47. McCormick, S.P.; Kato, T.; Maragos, C.M.; Busman, M.; Lattanzio, V.M.; Galaverna, G.; Dall-Asta, C.; Crich, D.; Price, N.P.; Kurtzman, C.P. Anomericity of T-2 toxin-glucoside: Masked mycotoxin in cereal crops. *J. Agric. Food Chem.* **2015**, *63*, 731–738. [CrossRef]
48. Meng-Reiterer, J.; Varga, E.; Nathanail, A.V.; Bueschl, C.; Rechthaler, J.; McCormick, S.P.; Michlmayr, H.; Malachová, A.; Fruhmann, P.; Adam, G.; et al. Tracing the metabolism of HT-2 toxin and T-2 toxin in barley by isotope-assisted untargeted screening and quantitative LC-HRMS analysis. *Anal. Bioanal. Chem.* **2015**, *407*, 8019–8033. [CrossRef]
49. Bryła, M.; Roszko, M.; Szymczyk, K.; Jędrzejczak, R.; Obiedziński, M.W. Fumonisins and their masked forms in maize products. *Food Cont.* **2016**, *59*, 619–627. [CrossRef]
50. Gratz, S.W.; Duncan, G.; Richardson, A.J. The Human Fecal Microbiota Metabolizes Deoxynivalenol and Deoxynivalenol-3-Glucoside and May Be Responsible for Urinary Deepoxy-Deoxynivalenol. *Appl. Environ. Microbiol.* **2013**, *79*, 1821–1825. [CrossRef]

51. Yoshinari, T.; Sakuda, S.; Furihata, K.; Furusawa, H.; Ohnishi, T.; Sugita-Konishi, Y.; Ishizaki, N.; Terajima, J. Structural determination of a nivalenol glucoside and development of an analytical method for the simultaneous determination of nivalenol and deoxynivalenol, and their glucosides, in wheat. *J. Agric. Food Chem.* **2014**, *62*, 1174–1180. [CrossRef]
52. EFSA Panel on Contaminants in the Food Chain. Appropriateness to set a group health-based guidance value for zearalenone and its modified forms. *EFSA J.* **2016**, *14*, 4425–4471.
53. Kovalsky Paris, M.P.; Schweiger, W.; Hametner, C.; Stückler, R.; Muehlbauer, G.J.; Varga, E.; Krska, R.; Berthiller, F.; Adam, G. Zearalenone-16-O-glucoside: A new masked mycotoxin. *J. Agric. Food Chem.* **2014**, *62*, 1181–1189. [CrossRef]
54. Kovalsky, P.; Kos, G.; Nahrer, K.; Schwab, C.; Jenkins, T.; Schatzmayr, G.; Sulyok, M.; Krska, R. Cooccurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize—Anextensive survey. *Toxins* **2016**, *8*, 363. [CrossRef]
55. Nathanail, A.V.; Varga, E.; Meng-Reiterer, J.; Bueschl, C.; Michlmayr, H.; Malachova, A.; Fruhmann, P.; Jestoi, M.; Peltonen, K.; Adam, G.; et al. Metabolism of the Fusarium mycotoxins T-2 toxin and HT-2 toxin in wheat. *J. Agric. Food Chem.* **2015**, *63*, 7862–7872. [CrossRef] [PubMed]
56. De Boevre, M.; Di Mavungu, J.D.; Maene, P.; Audenaert, K.; Deforce, D.; Haesaert, G.; Eeckhout, M.; Callebaut, A.; Berthiller, F.; Van Peteghem, C.; et al. Development and validation of an LC-MS/MS method for the simultaneous determination of deoxynivalenol, zearalenone, T-2-toxin and some masked metabolites in different cereals and cereal-derived food. *Food Addit. Contam. A* **2012**, *29*, 819–835. [CrossRef] [PubMed]
57. Varga, E.; Malachova, A.; Schwartz, H.; Krska, R.; Berthiller, F. Survey of deoxynivalenol and its conjugates deoxynivalenol-3-glucoside and 3-acetyl- deoxynivalenol in 374 beer samples. *Food Addit. Contam. A* **2013**, *30*, 137–146. [CrossRef] [PubMed]
58. JECFA. *Evaluation of Certain Contaminants in Food: Seventy-Second Report of the Joint FAO/WHO Expert Committee on Food Additives*; WHO Technical Report Series; WHO: Geneva, Switzerland, 2011; p. 959.
59. Simsek, S.; Ovando-Martínez, M.; Ozsisli, B.; Whitney, K.; Ohm, J.B. Occurrence of deoxynivalenol and deoxynivalenol-3-glucoside in hard red spring wheat grown in the USA. *Toxins* **2013**, *5*, 2656–2670. [CrossRef]
60. Miller, J.D.; Arnison, P.G. Degradation of deoxynivalenol by suspension cultures of the Fusarium head blight resistant wheat cultivar Frontana. *Can. J. Plant Pathol.* **1986**, *8*, 147–150. [CrossRef]
61. Malachova, A.; Dzuman, Z.; Veprikova, Z.; Vaclavikova, M.; Zachariasova, M.; Hajslova, J. Deoxynivalenol, deoxynivalenol-3-glucoside, and enniatins: The major mycotoxins found in cereal-based products on the Czech market. *J. Agric. Food Chem.* **2011**, *59*, 12990–12997. [CrossRef]
62. Warth, B.; Fruhmann, P.; Wiesenberger, G.; Kluger, B.; Sarkanj, B.; Lemmens, M.; Hametner, C.; Frohlich, J.; Adam, G.; Krska, R.; et al. Deoxynivalenol-sulfates: Identification and quantification of novel conjugated (masked) mycotoxins in wheat. *Anal. Bioanal. Chem.* **2015**, *407*, 1033–1039. [CrossRef]
63. Dall'Asta, C.; Galaverna, G.; Aureli, G.; Dossena, A.; Marchelli, R. A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize-based products. *World Mycotoxin. J.* **2008**, *1*, 237–246. [CrossRef]
64. Busman, M.; Poling, S.M.; Maragos, C.M. Observation of T-2 toxin and HT-2 toxin glucosides from Fusarium sporotrichioides by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). *Toxins* **2011**, *3*, 1554–1568. [CrossRef]
65. Scarpino, V.; Vanara, F.; Sulyok, M.; Krska, R.; Blandino, M. Fate of regulated, masked, emerging mycotoxins and secondary fungal metabolites during different large-scale maize dry-milling processes. *Food Res. Int.* **2021**, *140*, 109861. [CrossRef]
66. Schollenberger, M.; Müller, H.M.; Rüfle, M.; Suchy, S.; Drochner, W. Redistribution of 16 fusarium toxins during commercial dry milling of maize. *Cereal Chem.* **2008**, *85*, 557–560. [CrossRef]
67. Brodehl, A.; Moller, A.; Kunte, H.J.; Koch, M.; Maul, R. Biotransformation of the mycotoxin zearalenone by fungi of the genera Rhizopus and Aspergillus. *FEMS Microbiol. Lett.* **2014**, *359*, 124–130. [CrossRef] [PubMed]
68. Scudamore, K.A. Fate of fusarium mycotoxins in the cereal industry: Recent UK studies. *World Mycotoxin J.* **2008**, *1*, 315–323. [CrossRef]
69. Galaverna, G.; Dall'Asta, C.; Mangi, M.; Dossena, A.; Marchelli, R. Masked Mycotoxins: An Emerging Issue for Food Safety. *Czech J. Food Sci.* **2009**, *27*, S89–S92. [CrossRef]
70. Bittner, A.; Cramer, B.; Humpf, H.U. Matrix binding of ochratoxin A during roasting. *J. Agric. Food Chem.* **2013**, *61*, 12737–12743. [CrossRef]
71. Cramer, B.; Königs, M.; Humpf, H.U. Identification and in Vitro Cytotoxicity of Ochratoxin a Degradation Products Formed during Coffee Roasting. *J. Agric. Food Chem.* **2008**, *56*, 5673–5681. [CrossRef]
72. Dall'Asta, C.; Battilani, P. Fumonisins and their modified forms, a matter of concern in future scenario? *World Mycotoxin J.* **2016**, *9*, 727–739. [CrossRef]
73. Howard, P.C.; Churchwell, M.I.; Couch, L.H.; Marques, M.M.; Doerge, D.R. Formation of N-(carboxymethyl)fumonisin B1, following the reaction of fumonisin B1 with reducing sugars. *J. Agric. Food Chem.* **1998**, *46*, 3546–3557. [CrossRef]
74. Suman, M.; Generotti, S. Chapter 5: Transformation of Mycotoxins Upon Food Processing: Masking, Binding and Degradation Phenomena. In *Masked Mycotoxins in Food—Formation, Occurrence and Toxicological Relevance*; Dall'Asta, C., Berthiller, F., Eds.; The Royal Society of Chemistry: Cambridge, UK, 2016; pp. 73–96. [CrossRef]
75. Kostelanska, M.; Hajslova, J.; Zachariasova, M.; Malachova, A.; Kalachova, K.; Poustka, J.; Fiala, J.; Scott, P.M.; Berthiller, F.; Krska, R. Occurrence of deoxynivalenol and its major conjugate, deoxynivalenol-3-glucoside, in beer and some brewing intermediates. *J. Agric. Food Chem.* **2009**, *57*, 3187–3194. [CrossRef]

76. Vidal, A.; Morales, H.; Sanchis, V.; Ramos, A.J.; Marín, S. Stability of DON and OTA during the breadmaking process and determination of process and performance criteria. *Food Cont.* **2014**, *40*, 234–242. [CrossRef]
77. Generotti, S.; Cirlini, M.; Šarkanj, B.; Sulyok, M.; Berthiller, F.; Dall'Asta, C.; Suman, M. Formulation and processing factors affecting trichothecene mycotoxins within industrial biscuitmaking. *Food Chem.* **2017**, *229*, 597–603. [CrossRef] [PubMed]
78. Zhang, H.; Wu, L.; Li, W.; Zhang, Y.; Li, J.; Hu, X.; Sun, L.; Du, W.; Wang, B. Conversion of Deoxynivalenol-3-Glucoside to Deoxynivalenol during Chinese Steamed Bread Processing. *Toxins* **2020**, *12*, 225. [CrossRef] [PubMed]
79. Crews, C.; Jane, S.; Donald, M.A.C. Chapter 2: Natural Occurrence of Masked Mycotoxins. In *Masked Mycotoxins in Food—Formation, Occurrence and Toxicological Relevance*; Dall'Asta, C., Berthiller, F., Eds.; The Royal Society of Chemistry: Cambridge, UK, 2016; pp. 14–31. [CrossRef]
80. Sueck, F.; Hemp, V.; Specht, J.; Torres, O.; Cramer, B.; Humpf, H.U. Occurrence of the Ochratoxin A Degradation Product 2′R-Ochratoxin A in Coffee and Other Food: An Update. *Toxins* **2019**, *11*, 329. [CrossRef] [PubMed]
81. Bryła, M.; Waśkiewicz, A.; Szymczyk, K.; Jędrzejczak, R. Effects of pH and temperature on the stability of fumonisins in maize products. *Toxins* **2017**, *9*, 88. [CrossRef] [PubMed]
82. Voss, K.; Ryu, D.; Jackson, L.; Riley, R.; Gelineau-Van Waes, J. Reduction of fumonisin toxicity by extrusion and nixtamalization (Alkaline Cooking). *J. Agric. Food Chem.* **2017**, *65*, 7088–7096. [CrossRef] [PubMed]
83. Bryła, M.; Roszko, M.; Szymczyk, K.; Jędrzejczak, R.; Bieta Słowik, E.; Obiedzinki, M.W. Effect of baking on reduction of free and hidden fumonisins in gluten-free bread. *J. Agric. Food Chem.* **2014**, *62*, 10341–10347. [CrossRef]
84. EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA). Safety of frozen and freeze-dried formulations of the lesser mealworm (*Alphitobius diaperinus* larva) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA J.* **2022**, *20*, e07325. [CrossRef]
85. Dowd, P.F. Insect management to facilitate preharvest mycotoxin management. *J. Toxicol. Toxin. Rev.* **2003**, *22*, 327–350. [CrossRef]
86. Lopes, L.Q.S.; Quatrin, P.M.; De Souza, M.E.; De Almeida Vaucher, R.; Vianna Santos, R.C. Fungal infections in honey bees. *Fungal Genom. Biol.* **2015**, *4*, 118.
87. Nardoni, S.; D'Ascenzi, C.; Rocchigiani, G.; Papini, R.A.; Pistelli, L.; Formato, G.; Najar, B.; Mancianti, F. Stonebrood and chalkbrood in Apis mellifera causing fungi: In vitro sensitivity to some essential oils. *Nat. Prod. Res.* **2018**, *32*, 385–390. [CrossRef]
88. Camenzuli, L.; van Dam, R.; de Rijk, T.; Andriessen, R.; van Schelt, J.; van der Fels-Klerx, H. Tolerance and excretion of the mycotoxins aflatoxin B1, zearalenone, deoxynivalenol, and ochratoxin A by *Alphitobius diaperinus* and *Hermetia illucens* from contaminated substrates. *Toxins* **2018**, *10*, 91. [CrossRef]
89. Niu, G.; Wen, Z.; Rupasinghe, S.G.; Zeng, R.S.; Berenbaum, M.R.; Schuler, M.A. Aflatoxin B1 detoxification by CYP321A1 in *Helicoverpa zea*. *Arch. Insect Biochem. Physiol.* **2008**, *69*, 32–45. [CrossRef] [PubMed]
90. Ampt, E.A.; Bush, D.S.; Siegel, J.P.; Berenbaum, M.R. Larval preference and performance of *Amyelois transitella* (navel orangeworm, Lepidoptera: Pyralidae) in relation to the fungus Aspergillus flavus. *Environ. Entomol.* **2015**, *45*, 155–162. [CrossRef] [PubMed]
91. Bush, D.S.; Siegel, J.P.; Berenbaum, M.R. Accelerated development and toxin tolerance of the navel orangeworm Amyelois transitella (Lepidoptera: Pyralidae) in the presence of *Aspergillus flavus*. *J. Chem. Ecol.* **2018**, *44*, 1170–1177. [CrossRef] [PubMed]
92. Feyereisen, R. Insect CYP Genes and P450 Enzymes. In *Insect Molecular Biology and Biochemistry*; Gilbert, L.I., Ed.; Elsevier Science Publishers: San Diego, CA, USA, 2012; pp. 236–316.
93. Zhan, S.; Fang, G.; Cai, M.; Kou, Z.; Xu, J.; Cao, Y.; Bai, L.; Zhang, Y.; Jiang, Y.; Luo, X.; et al. Genomic landscape and genetic manipulation of the black soldier fly *Hermetia illucens*, a natural waste recycler. *Cell Res.* **2020**, *30*, 50–60. [CrossRef]
94. Johnson, R.M.; Mao, W.; Pollock, H.S.; Niu, G.; Schuler, M.A.; Berenbaum, M.R. Ecologically appropriate xenobiotics induce cytochrome P450s in Apis mellifera. *PLoS ONE* **2012**, *7*, e31051. [CrossRef]
95. Trienens, M.; Kraaijeveld, K.; Wertheim, B. Defensive repertoire of Drosophila larvae in response to toxic fungi. *Mol. Ecol.* **2017**, *26*, 5043–5057. [CrossRef]
96. Bosch, G.; Fels-Klerx, H.; Rijk, T.; Oonincx, D. Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (*Hermetia illucens*) and yellow mealworms (*Tenebrio molitor*). *Toxins* **2017**, *9*, 185. [CrossRef]
97. Šarkanj, B.; Warth, B.; Uhlig, S.; Abia, W.A.; Sulyok, M.; Klapec, T.; Krska, R.; Banjari, I. Urinary analysis reveals high deoxynivalenol exposure in pregnant women from Croatia. *Food Chem. Toxicol.* **2013**, *62*, 231–237. [CrossRef]
98. Woelflingseder, L.; Warth, B.; Vierheilig, I.; SchwartzZimmermann, H.; Hametner, C.; Nagl, V.; Novak, B.; Sarkanj, B.; Berthiller, F.; Adam, G.; et al. The Fusarium metabolite culmorin suppresses the in vitro glucuronidation of deoxynivalenol. *Arch. Toxicol.* **2019**, *93*, 1729–1743. [CrossRef]
99. Meky, F.A.; Turner, P.C.; Ashcroft, A.E.; Miller, J.D.; Qiao, Y.L.; Roth, M.J.; Wild, C.P. Development of a urinary biomarker of human exposure to deoxynivalenol. *Food Chem. Toxicol.* **2003**, *41*, 265–273. [CrossRef]
100. De Boevre, M.; Njumbe Ediage, E.; Van Pouckea, C.; De Saeger, S. Chapter 4: Untargeted Analysis of Modified Mycotoxins Using High-Resolution Mass Spectrometry. In *Masked Mycotoxins in Food—Formation, Occurrence and Toxicological Relevance*; Dall'Asta, C., Berthiller, F., Eds.; The Royal Society of Chemistry: Cambridge, UK, 2016; pp. 50–72. [CrossRef]
101. Fuchs, R.; Hult, K.; Peraica, M.; Radic, B.; Plestina, R. Conversion of ochratoxin C into ochratoxin A in vivo. *Appl. Environ. Microbiol.* **1984**, *48*, 41–42. [CrossRef] [PubMed]
102. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain); Schrenk, D.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.; Leblanc, J.-C. Scientific Opinion on the Risk Assessment of Ochratoxin A in Food. *EFSA J.* **2020**, *18*, 150. [CrossRef]

103. Stormer, F.C.; Pederson, J.I. Formation of 4-hydroxy-ochratoxin A from ochratoxin A by rat liver microsomes. *Appl. Environ. Microbiol.* **1980**, *39*, 971–975. [CrossRef] [PubMed]
104. Storen, O.; Holm, H.; Stormer, F.C. Metabolism of ochratoxin A by rats. *Appl. Environ. Microbiol.* **1982**, *44*, 785–789. [CrossRef] [PubMed]
105. The Nordic Working Group on Food Toxicology and Risk Evaluation (NNT). *Nordiske Seminar-og Arbeidsrapporter*; Health Evaluation of Ochratoxin A in Food Products; Nordic Council of Ministers: Copenhagen, Denmark, 1991; pp. 1–29.
106. Dai, J.; Park, G.; Perry, J.; Ilichev, Y.; Bow, D.; Pritchard, J.; Faucet, V.; Pfohl-Leszkowicz, A.; Manderville, R.; Simon, J. Molecular aspects of the transport and toxicity of ochratoxin A. *Acc. Chem. Res.* **2004**, *37*, 874–881. [CrossRef]
107. Mobashar, M.; Hummel, J.; Blank, R.; Südekum, K.H. Ochratoxin A in Ruminants–A Review on Its Degradation by Gut Microbes and Effects on Animals. *Toxins* **2010**, *2*, 809–839. [CrossRef]
108. Blank, R.; Rolfs, J.P.; Südekum, K.-H.; Frohlich, A.A.; Wolffram, S. Effects of chronic ingestion of ochratoxin A on blood level and excretion of the mycotoxin in sheep. *J. Agric. Food Chem.* **2003**, *51*, 6899–6905. [CrossRef]
109. Debevere, S.; Cools, A.; Baere, S.; Haesaert, G.; Rychlik, M.; Croubels, S.; Fievez, V. In Vitro Rumen Simulations Show a Reduced Disappearance of Deoxynivalenol, Nivalenol and Enniatin B at Conditions of Rumen Acidosis and Lower Microbial Activity. *Toxins* **2020**, *12*, 101. [CrossRef]
110. Cramer, B.; Osteresch, B.; Muñoz, K.A.; Hillmann, H.; Sibrowski, W.; Humpf, H.U. Biomonitoring using dried blood spots: Detection of ochratoxin A and its degradation product 2'R-ochratoxin A in blood from coffee drinkers. *Mol. Nutr. Food Res.* **2015**, *59*, 1837–1843. [CrossRef]
111. Berthiller, F.; Krska, R.; Domig, K.J.; Kneifel, W.; Juge, N.; Schuhmacher, R.; Adam, G. Hydrolytic fate of deoxynivalenol-3-glucoside during digestion. *Toxicol. Lett.* **2011**, *206*, 264–267. [CrossRef]
112. Dall'Erta, A.; Cirlini, M.; Dall'Asta, M.; Del Rio, D.; Galaverna, G.; Dall'Asta, C. Masked mycotoxins are efficiently hydrolyzed by human colonic microbiota releasing their aglycones. *Chem. Res. Toxicol.* **2013**, *26*, 305–312. [CrossRef] [PubMed]
113. EFSA Contaminants in the Food Chain. Risks for animal health related to the presence of zearalenone and its modified forms in feed. *EFSA J.* **2017**, *15*, 4851. [CrossRef]
114. Yang, S.; Li, Y.; De Boevre, M.; De Saeger, S.; Zhou, J.; Li, Y.; Zhanga, H.; Sun, F. Toxicokinetics of α-zearalenol and its masked form in rats and the comparative biotransformation in liver microsomes from different livestock and humans. *J. Hazard. Mat.* **2020**, *393*, 121403. [CrossRef]
115. Yang, S.; Zhang, H.; Zhang, J.; Li, Y.; Jin, Y.; Zhang, S.; De Saeger, S.; Li, Y.; Zhou, J.; Sun, F.; et al. Deglucosylation of zearalenone-14-glucoside in animals and human liver leads to underestimation of exposure to zearalenone in humans. *Arch. Toxicol.* **2018**, *92*, 2779–2791. [CrossRef] [PubMed]
116. Binder, S.B.; Schwartz-Zimmermann, H.E.; Varga, E.; Bichl, G.; Michlmayr, H.; Adam, G.; Berthiller, F. Metabolism of zearalenone and its major modified forms in pigs. *Toxins* **2017**, *9*, 56. [CrossRef] [PubMed]
117. Ueberschar, K.H.; Brezina, U.; Danicke, S. Zearalenone (ZEN) and ZEN metabolites in feed, urine and bile of sows: Analysis, determination of the metabolic profile and evaluation of the binding forms. *Landbauforschung* **2016**, *66*, 21–28.
118. Nagl, V.; Schwartz, H.; Krska, R.; Moll, W.D.; Knasmüller, S.; Ritzmann, M.; Adam, G.; Berthiller, F. Metabolism of the masked mycotoxin deoxynivalenol-3-glucoside in rats. *Toxicol. Lett.* **2012**, *213*, 367–373. [CrossRef]
119. Nagl, V.; Woechtl, B.; Schwartz-Zimmermann, H.E.; Hennig-Pauka, I.; Moll, W.D.; Adam, G.; Berthiller, F. Metabolism of the masked mycotoxin deoxynivalenol-3-glucoside in pigs. *Toxicol. Lett.* **2014**, *229*, 190–197. [CrossRef]
120. Broekaert, N.; Devreese, M.; van Bergen, T.; Schauvliege, S.; De Boevre, M.; De Saeger, S.; Vanhaecke, L.; Berthiller, F.; Michlmayr, H.; Malachová, A.; et al. In vivo contribution of deoxynivalenol-3-β-D-glucoside to deoxynivalenol exposure in broiler chickens and pigs: Oral bioavailability, hydrolysis and toxicokinetics. *Arch. Toxicol.* **2017**, *91*, 699–712. [CrossRef]
121. Broekaert, N.; Devreese, M.; De Boevre, M.; De Saeger, S.; Croubels, S. T-2 Toxin-3α-glucoside in Broiler Chickens: Toxicokinetics, Absolute Oral Bioavailability, and in Vivo Hydrolysis. *J. Agric. Food Chem.* **2017**, *14*, 4797–4803. [CrossRef]
122. Gratz, S.W. Do Plant-Bound Masked Mycotoxins Contribute to Toxicity? *Toxins* **2017**, *9*, 85. [CrossRef]
123. Brera, C.; de Santis, B.; Debegnach, F.; Miano, B.; Moretti, G.; Lanzone, A.; Del Sordo, G.; Buonsenso, D.; Chiaretti, A.; Hardie, L.; et al. Experimental study of deoxynivalenol biomarkers in urine. GP/EFSA/CONTAM/2013/04. *EFSA Support. Publ.* **2015**, *12*, 818E.
124. Fiby, I.; Sopel, M.M.; Michlmayr, H.; Adam, G.; Berthiller, F. Development and Validation of an LC-MS/MS Based Method for the Determination of Deoxynivalenol and Its Modified Forms in Maize. *Toxins* **2021**, *13*, 600. [CrossRef] [PubMed]
125. Tittlemier, S.A.; Brunkhorst, J.; Cramer, B.; DeRosa, M.C.; Lattanzio, V.M.T.; Malone, R.; Maragos, C.; Stranska, M.; Sumarah, M.W. Developments in mycotoxin analysis: An update for 2019–2020. *World Mycotoxin J.* **2021**, *14*, 3–26. [CrossRef]
126. Schneweis, I.; Meyer, K.; Engelhardt, G.; Bauer, J. Occurrence of zearalenone-4- beta-D-glucopyranoside in wheat. *J. Agric. Food Chem.* **2002**, *50*, 1736–1738. [CrossRef]
127. Lancova, K.; Hajslova, J.; Poustka, J.; Krplova, A.; Zachariasova, M.; Dostalek, P.; Sachambula, L. Transfer of Fusarium mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. *Food Addit. Contam. A* **2008**, *25*, 732–744. [CrossRef]
128. Malachová, A.; Štočková, L.; Wakker, A.; Varga, E.; Krsk, R.; Michlmayr, H.; Adam, G.; Berthiller, F. Critical evaluation of indirect methods for the determination of deoxynivalenol and its conjugated forms in cereals. *Anal. Bioanal. Chem.* **2015**, *407*, 6009–6020. [CrossRef]

129. Michlmayr, H.; Varga, E.; Malachová, A.; Nguyen, N.T.; Lorenz, C.; Haltrich, D.; Berthiller, F.; Adam, G. A Versatile Family 3 Glycoside Hydrolase from Bifidobacterium adolescentis Hydrolyzes β-Glucosides of the Fusarium Mycotoxins Deoxynivalenol, Nivalenol, and HT-2 Toxin in Cereal Matrices. *Appl. Environ. Microbiol.* **2015**, *81*, 4885–4893. [CrossRef]
130. European Commission: Commission Regulation (EC). No. 1525/ 98 of 16 July 1998 Amending Regulation (EC) No. 194/97 of 31 January 1997 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Off. J. Eur. Communities* **1998**, *L201*, 43–45.
131. Gallo, G.; Lobianco, M.; Bognanni, R.; Saimbene, G. Mycotoxins in Durum Wheat Grain:Hygienic-Health Quality of Sicilian ProductionG. *J. Food Sci.* **2008**, *73*, T42–T47. [CrossRef]
132. Zare Jeddi, M.; Hopf, N.B.; Louro, H.; Viegas, S.; Galea, K.S.; Pasanen-Kase, R.; Santonen, T.; Mustieles, V.; Fernandez, M.F.; Verhagen, H.; et al. Developing human biomonitoring as a 21st century toolbox within the European exposure science strategy 2020–2030. *Environ. Int.* **2022**, *168*, 107476. [CrossRef]
133. Milićević, D.R.; Skrinjar, M.; Baltić, T. Real and perceived risks for mycotoxin contamination in foods and feeds: Challenges for food safety control. *Toxins* **2010**, *2*, 572–592. [CrossRef]
134. Poppenberger, B.; Berthiller, F.; Lucyshyn, D.; Sieberer, T.; Schuhmacher, R.; Krska, R.; Kuchler, K.; Glössl, J.; Luschnig, J.; Adam, G. Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. *J. Biol. Chem.* **2003**, *278*, 47905–47914. [CrossRef] [PubMed]
135. Poppenberger, B.; Berthiller, F.; Bachmann, H.; Lucyshyn, D.; Peterbauer, C.; Mitterbauer, R.; Schuhmacher, R.; Krska, R.; Glössl, J.; Adam, G. Heterologous expression of Arabidopsis UDP-glucosyltransferases in Saccharomyces cerevisiae for production of zearalenone-4-O.-glucoside. *Appl. Environ. Microbiol.* **2006**, *72*, 4404–4410. [CrossRef] [PubMed]
136. Lorenz, N.; Dänicke, S.; Edler, L.; Gottschalk, C.; Lassek, E.; Marko, D.; Rychlik, M.; Mally, A. A critical evaluation of health risk assessment of modified mycotoxins with a special focus on zearalenone. *Mycotoxin Res.* **2019**, *35*, 27–46. [CrossRef] [PubMed]
137. Ali, N.; Degen, G.H. Urinary biomarkers of exposure to the mycoestrogen zearalenone and its modified forms in German adults. *Arch Toxicol.* **2018**, *92*, 2691–2700. [CrossRef] [PubMed]
138. Mengelers, M.; Zeilmaker, M.; Vidal, A.; De Boevre, M.; De Saeger, S.; Hoogenveen, R. Biomonitoring of Deoxynivalenol and Deoxynivalenol-3-glucoside in Human Volunteers: Renal Excretion Profiles. *Toxins* **2019**, *11*, 466. [CrossRef]
139. Payros, D.; Alassane-Kpembi, I.; Pierron, A.; Loiseau, N.; Pinton, P.; Oswald, I.P. Toxicology of deoxynivalenol and its acetylated and modified forms. *Arch Toxicol.* **2016**, *90*, 2931–2957. [CrossRef]
140. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain); Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.C.; et al. Scientific opinion–Riskassessment of aflatoxins in food. *EFSA J.* **2020**, *18*, 6040. [CrossRef]
141. EFSA. Contaminants in the Food Chain. Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA J.* **2017**, *15*, 4718. [CrossRef]
142. EFSA. Contaminants in the Food Chain. Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. *EFSA J.* **2018**, *16*, 5242. [CrossRef]
143. Catteuw, A.; Broekaert, N.; De Baere, S.; Lauwers, M.; Gasthuys, E.; Huybrechts, B.; Callebaut, A.; Ivanova, L.; Uhlig, S.; De Boevre, M.; et al. Insights into In Vivo Absolute Oral Bioavailability, Biotransformation, and Toxicokinetics of Zearalenone, α-Zearalenol, β-Zearalenol, Zearalenone-14-glucoside, and Zearalenone-14-sulfate in Pigs. *J. Agric. Food Chem.* **2019**, *27*, 3448–3458. [CrossRef]
144. Postupolski, J.; Starski, A.; Ledzion, E.; Kurpińska-Jaworska, A.; Szczęsna, M. Exposure Assessment of Infants and Young Children on Selected Fusarium Toxins. *Rocz. Panstw. Zakl. Hig.* **2019**, *70*, 5–14. [CrossRef]
145. EFSA. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. *EFSA J.* **2013**, *11*, 3293. [CrossRef]
146. Leist, M.; Ghallab, A.; Graepel, R.; Marchan, R.; Hassan, R.; Bennekou, S.H.; Limonciel, A.; Vinken, M.; Schildknecht, S.; Waldmann, T.; et al. Adverse outcome pathways: Opportunities, limitations and open questions. *Arch. Toxicol.* **2017**, *91*, 3477–3505. [CrossRef]