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ORIGINAL ARTICLE

Mycotoxins as human carcinogens—the *IARC Monographs* classification

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Abstract Humans are constantly exposed to mycotoxins (e.g. aflatoxins, ochratoxins), mainly via food intake of plant and animal origin. The health risks stemming from mycotoxins may result from their toxicity, in particular their carcinogenicity. In order to prevent these risks, the International Agency for Research on Cancer (IARC) in Lyon (France)—through its *IARC Monographs* programme—has performed the carcinogenic hazard assessment of some mycotoxins in humans, on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies. The present article summarizes the carcinogenic hazard assessments of those mycotoxins, especially aflatoxins (aflatoxin B_1, B_2, G_1, G_2 and M_1), fumonisins (fumonisin B_1 and B_2) and ochratoxin A (OTA). New information regarding the genotoxicity of OTA (formation of OTA-DNA adducts), the role of OTA in oxidative stress and the identification of epigenetic factors involved in OTA carcinogenesis–should they indeed provide strong evidence that OTA carcinogenicity is mediated by a mechanism that also operates in humans–could lead to the reclassification of OTA.

Keywords Mycotoxins · Aflatoxins · Fumonisins · Ochratoxin A · IARC · Carcinogenicity

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Introduction

Mycotoxins are naturally occurring secondary metabolites of several toxigenic microfungi which contaminate the whole food chain (differing from year to year), from the agricultural cultures to the plate of consumers. Mycotoxins have been implicated in a range of human diseases and occur in foodstuffs of plant and animal origin. Mycotoxicosis (e.g. ergotism, alimentary toxic aleukia, aflatoxicosis) have historically been described since antiquity, although mycotoxins were not identified as the causative agents at that time (Bennett and Klich 2003; Malir et al. 2006; Richard 2007).

Mycotoxins ingestion can produce both acute and chronic toxicities: (i) acute—characterized by a rapid onset and an obvious toxic response including rapid death or (ii) chronic—resulting from low-dose exposure to mycotoxins over a long period of time, with toxic responses including cancers such as hepatocellular carcinoma (Malir et al. 2006; Kensler et al. 2011). Indeed, mycotoxins have a variety of toxic effects, e.g. haemorrhagic, hepatotoxic, nephrotoxic, neurotoxic, oestrogenic, teratogenic, immunosuppressive, mutagenic and carcinogenic (Newberne 1974; Stark 1980; Malir et al. 2006; Wild and Gong 2010; Kensler et al. 2011).

Though exposure to mycotoxins is mostly by ingestion, toxicity also occurs following inhalation or dermal exposure (Dvorackova 1976; Dvorackova and Pichova 1986; Di Paolo et al. 1993; Johanning et al. 1996; Robbins et al. 2000; Kelman et al. 2004; Degen 2011; Boonen et al. 2012).

Therefore, mycotoxins have attracted worldwide attention not only because of their perceived impact on human health but also because of the economic losses accruing from contaminated foods.

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The aim of this article is to inform nutritionists, food hygienists, risk managers and PhD students, who work in the area of mycotoxins, food safety, nutrition and public health protection and promotion.

Modern history of mycotoxin carcinogenicity

A change in the perception of mycotoxins came in the late 1950s and the early 1960s when aflatoxins were identified as causal agents of turkey "X" disease (Blount 1961). This was the beginning of the modern phase of the research of mycotoxins and their role. Subsequent studies confirmed that these potent liver toxins were capable of inducing acute liver disease in ducklings and liver cancer in rats (Lancaster et al. 1961; Sargeant et al. 1961). Lancaster et al. (1961) showed that rats developed hepatomas when fed the same peanut meal implicated in turkey "X" disease. Later on it was discovered that sublethal doses of aflatoxins could result into chronic toxicity, and low levels of chronic exposure could result in cancer (Wogan and Newberne 1967), primarily liver cancer in some species (Wogan 1973). Epidemiological studies conducted in Africa suggested that aflatoxins were human hepatocarcinogens, and many other reports confirmed their implication in the occurrence of toxicity in exposed humans (Peers et al. 1976; Van Rensburg et al. 1985; Van Rensburg et al. 1990).

Carcinogenesis

Carcinogenesis is a multi-factorial phenomenon (i.e. with many factors involved), which explains why the evidence of carcinogenic hazard in humans is often difficult to assess (Luch 2005; Loeb and Harris 2008; Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).

In order to prevent cancer risks stemming from exposure to mycotoxins, the International Agency for Research on Cancer (IARC) in Lyon, France has performed the carcinogenic hazard assessment of some mycotoxins in humans, on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies (IARC 2015).

The *IARC Monographs* programme

In 1969, the IARC initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce reviews (called *Monograph*) on the evaluated chemicals. The *IARC Monographs* programme has since been expanded to include consideration of exposures to complex mixtures of chemicals (which occur, for example, in some occupational settings and as the result of human habits) and of exposures to other agents, such as radiations and viruses.

The evaluations are carried out by Working Groups of independent international expert scientists. This programme actually represents the first step in carcinogenic risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that certain exposures could alter the incidence of cancer in humans. The second step is quantitative risk assessment (IARC 2015).

The *IARC Monographs* classification of human carcinogens

The agents evaluated by the *IARC Monographs* programme are classified into five groups (Group 1, 2A, 2B, 3 and 4) defined by the existing scientific evidence for their carcinogenicity (IARC 2015; IARC 2016a) (see Fig. 1).

In short, Group 1 (*Carcinogenic to humans*) applies to agents for which there is sufficient evidence to conclude that they can cause cancer in humans; Group 2A (*Probably carcinogenic to humans*) applies to agents for which there is strong evidence from human, experimental animals and/or mechanistic data that they can cause cancer in humans, but the data remain insufficient; Group 2B (*Possibly carcinogenic to humans*) applies to agents for which there is some evidence from human, experimental animals and/or mechanistic data that they can cause cancer in humans, but the data are still far from being conclusive; Group 3 (*Not classifiable as to its carcinogenicity to humans*) applies to agents for which there are too limited, inadequate, or no data to classify these; and Group 4 (*Probably not carcinogenic to humans*) applies to agents for which there is strong evidence that they do not cause cancer in humans (IARC 2016a).

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures (IARC 2016b). The *IARC Monographs* classification is fully acceptable. Mycotoxins have been (as of October 10, 2016) classified (see Table 1) (IARC 2016b).

The development of the evaluations of the carcinogenic hazard of aflatoxins, fumonisins and ochratoxin A

Carcinogenicity of aflatoxins

In 1987, a first *IARC Monographs* Working Group concluded that there was *sufficient evidence* in humans for the carcinogenicity of naturally occurring aflatoxins (IARC 1987). This led to their classification in Group 1 (*Carcinogenic to humans*) (see Fig. 2).

This conclusion was re-affirmed in two subsequent re-evaluations (IARC 1993, 2002). In 1993, the Working Group also concluded that—in experimental animals—there was *sufficient evidence* for the carcinogenicity of naturally

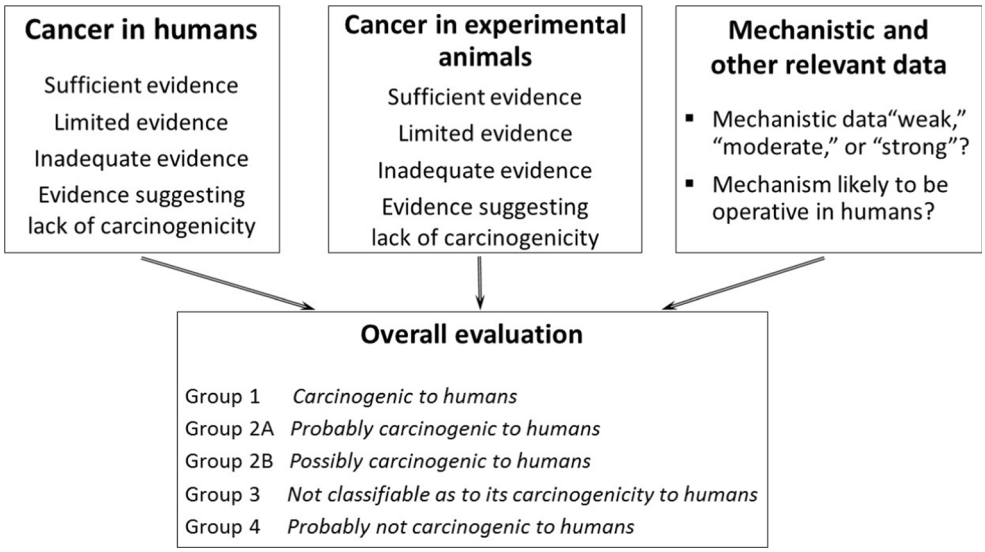

Fig. 1 The *IARC Monographs* classification of carcinogenic hazard

occurring mixtures of aflatoxins and aflatoxins B_1, G_1 and M_1; *limited evidence* for the carcinogenicity of aflatoxin B_2; and *inadequate evidence* for the carcinogenicity of aflatoxin G_2. This led to the classification of aflatoxin M_1 in group 2B (*Possibly carcinogenic to humans*) (IARC 1993).

In 2002, the Working Group reported on several more recent studies which demonstrated that experimental animals infected with hepatitis B virus (woodchucks, tree shrews and transgenic mice heterozygous for the *p53* tumour-suppressor gene) were more sensitive to the carcinogenic effects of aflatoxin B_1 than uninfected animals; it was concluded that these studies confirmed the carcinogenicity of aflatoxins in experimental animals (IARC 2002).

In 2012, a Working Group finally concluded that there was *sufficient evidence* in humans for the carcinogenicity of aflatoxins. It was also stated that aflatoxins cause hepatocellular carcinoma, with strong evidence that aflatoxins carcinogenicity results from a genotoxic mechanism of action that involves metabolite activation to a genotoxic epoxide metabolite, formation of DNA adducts and modification of the *TP53* gene. In addition, in human hepatocellular carcinoma reported from regions where exposure to aflatoxins is high, up to 50% of tumours have been shown to harbour a specific point mutation in the *TP53* tumour-suppressor gene. Therefore, aflatoxins (including aflatoxin B_1, B_2, G_1, G_2 and M_1) were classified as *carcinogenic to humans* (Group 1) (IARC 2012).

Table 1 The *IARC Monographs* evaluations of the carcinogenic hazard of some mycotoxins

Mycotoxins	Group	Volume	References
Aflatoxins (including aflatoxin B_1, B_2, G_1, G_2 and M_1)	1	100 F	IARC 2012
Citrinin	3	Suppl. 7	IARC 1987
Cyclochlorotine	3	Suppl. 7	IARC 1987
Deoxynivalenol	3	56	IARC 1993
Fumonisin B_1	2B	82	IARC 2002
Fumonisin B_2	2B	56	IARC 1993
Fusarenone X	3	56	IARC 1993
Fusarin C	2B	56	IARC 1993
Kojic acid	3	79	IARC 2001
Luteoskyrin	3	Suppl. 7	IARC 1987
Nivalenol	3	56	IARC 1993
Ochratoxin A	2B	56	IARC 1993
Patulin	3	Suppl. 7	IARC 1987
Penicillic acid	3	Suppl. 7	IARC 1987
Rugulosin	3	Suppl. 7	IARC 1987
Sterigmatocystin	2B	Suppl. 7	IARC 1987
T-2 toxin	3	56	IARC 1993
Zearalenone	3	56	IARC 1993

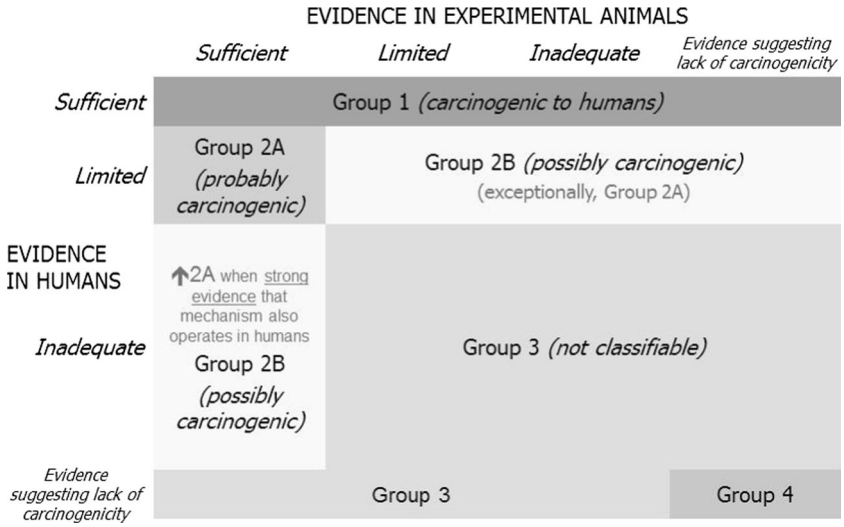

Fig. 2 Scheme of the IARC Monographs evaluation of carcinogenic hazard

Carcinogenicity of fumonisins

In 1993, in the first evaluation by the *IARC Monographs* programme of the carcinogenic hazard to humans of fumonisin B_1 and B_2 (FUM B_1 and FUM B_2) as "toxins derived from *Fusarium moniliforme*", no case reports on cancer or adequate epidemiological studies were available. Only studies on the relationship between *F. moniliforme* (now known as *Fusarium verticillioides*) toxins (of which FUM B_1 and FUM B_2 are the major toxic secondary metabolites) and oesophageal cancer in areas of South Africa and China were summarized. The Working Group concluded that there was *inadequate evidence* in humans for the carcinogenicity of toxins derived from *Fusarium moniliforme*. However, there was *sufficient evidence* in experimental animals for the carcinogenicity of cultures of *F. moniliforme* that contain significant amounts of fumonisins, and there was *limited evidence* in experimental animals for the carcinogenicity of FUM B_1. There was *inadequate evidence* in experimental animals for the carcinogenicity of FUM B_2. Overall, toxins derived from *F. moniliforme* (including FUM B_1 and FUM B_2) were classified as *possibly carcinogenic to humans* (Group 2B) (IARC 1993).

In the second evaluation in 2002, there was *inadequate evidence* in humans for the carcinogenicity of FUM B_1. The reviewed relevant studies provided *sufficient evidence* in experimental animals for the carcinogenicity of FUM B_1, which confirmed the classification of FUM B_1 as *possibly carcinogenic to humans* (Group 2B) (IARC 2002).

Carcinogenicity of ochratoxin A

In the first evaluation of the carcinogenic hazard to humans of ochratoxin A (OTA) by the *IARC Monographs* programme in 1976, no case reports on cancer, epidemiological studies or adequate studies of cancer in experimental animals were available, and no conclusion was made (IARC 1976).

In the second evaluation in 1983, in the absence of adequate epidemiological data, the Working Group stated that no evaluation of the carcinogenicity of OTA in humans could be made, though a single feeding study in mice, showing the occurrence of hepatocellular and renal-cell tumours, provided *limited evidence* of carcinogenicity in experimental animals (IARC 1983). This led to the classification of OTA in Group 3 (*Not classifiable as to its carcinogenicity to humans*) in 1987 (IARC 1987). In 1993, a few studies showing an increase in the incidence of hepatocellular tumours in mice and of renal-cell tumours in mice and rats led to the reclassification in Group 2B (*Possibly carcinogenic to humans*) on the basis of *sufficient evidence* in experimental animals (see Fig. 2) (IARC 1993). Since 1993, OTA has not been re-classified by an *IARC Monographs* Working Group, therefore, new data on OTA carcinogenicity obtained since then are included and summarized in Table 2 (adapted from Malir et al. 2016).

At present, new information regarding the genotoxicity of OTA (formation of OTA-DNA adducts), its role in oxidative stress and the identification of epigenetic factors involved in OTA carcinogenesis—should they indeed provide strong evidence that OTA carcinogenicity is mediated by a mechanism that also operates in humans—could lead to another reclassification of OTA (see Fig. 2). In the light of the previous and of the more recent data published over the last 5 years, it would not be inappropriate to consider an upgrade of its classification from Group 2B to Group 2A (*Probably carcinogenic to humans*) (Pfohl-Leszkowicz and Manderville 2012; Mally 2012; Stachurska et al. 2013; Hennemeier et al. 2014; Heussner and Bingle 2015; Malir et al. 2016). From this aspect, it would be important to develop consensus on research strategies between all interested parties.

Table 2 OTA carcinogenicity and genotoxicity research after the year 1992

Year	Testing	Source
1993	OTA-DNA adducts are observed in mice and rats tissues after acute and sub-chronic exposure, and in urinary tract tumours (UTT) in exposed Bulgarian subjects.	Pfohl-Leszkowicz et al. 1993a, 1993b, 1993c
1993–2009	OTA-DNA adducts are also detected in tissues of humans presumably exposed to OTA in several countries (Bulgaria, Serbia, Croatia, Germany, Belgium, France, Tunisia).	Pfohl-Leszkowicz et al. 1993b; Maaroufi et al. 1994; Azémar et al. 1997; Arlt et al. 2001; Faucet et al. 2004; Pfohl-Leszkowicz et al. 2007; Pfohl-Leszkowicz 2009
1998–2002	DNA adduction following chronic exposure of rats to OTA is first described; sex differences and dual mechanism—oxidative pathways and DNA adduction—are observed.	Castegnaro et al. 1999; Pfohl-Leszkowicz et al. 1998; Pfohl-Leszkowicz et al. 2002
1998	OTA-DNA adducts are observed in mother and progeny of mice fed OTA: 9 months after birth, male mice developed cancer.	Petkova-Bocharova et al. 1998
2000–2001	In vitro formation of dG-OTA adduct.	Obrecht-Pflumio and Dirheimer 2000; Obrecht-Pflumio and Dirheimer 2001
2001–2002	Other studies with radiolabeled OTA are unable to detect any DNA binding of OTA, but an explanation of this discrepancy is given by Pfohl-Leszkowicz and Castegnaro in 2005 (Pfohl-Leszkowicz and Castegnaro 2005).	Gautier et al. 2001; Gross-Steinmeyer et al. 2002
2003	OTA-DNA adduct in pigs sub-chronically exposed to low doses of OTA; a relation with biotransformation is established.	Petkova-Bocharova et al. 2003
2002–2010	OTA may be involved in testicular cancer.	Schwartz 2002; Schwartz et al. 2007; Schwartz et al. 2010; Jennings-Gee et al. 2010; Mantle et al. 2010
2002–2004	MicroRNA represents a new approach to cancer.	Calin et al. 2002, 2004
2003–2008	Citrinin increases the genotoxicity of OTA and modifies OTA metabolism in rats exposed to low doses for 3 weeks.	Molinié and Pfohl-Leszkowicz 2003; Pfohl-Leszkowicz et al. 2008
2004	Evidence for covalent DNA adduction by OTA following chronic exposure of rats and sub-acute exposure of pigs.	Faucet et al. 2004
2004	Another research group, using the highly sensitive accelerator mass spectrometry technique, does not detect DNA adducts after the administration of ^{14}C–labelled OTA to rats.	Mally et al. 2004
2004	In 2004, a review of the NTP carcinogenicity study of rats exposed to OTA, places OTA in the category of "chemicals inducing renal tumours through direct interaction of the parent compound or metabolite with renal DNA" based on the histopathological evidence.	Lock and Hard 2004
2004–2010	The long-term OTA studies confirm the increased incidence of tumours in rats; in male rats, tumours are related to OTA dose.	Lock and Hard 2004; Mantle et al. 2005; Mantle and Kulinskaya 2010
2004–2011	OTA induces the formation of reactive oxygen species and consequent oxidative DNA damage.	Lock and Hard 2004; Mally et al. 2005 Arbillaga et al. 2007; Ali et al. 2011
2004–2012	OTA is a direct genotoxic compound forming covalent DNA adducts in the kidney. OTA can indeed react with DNA via a phenolic radical resulting in C8-deoxyguanosine adduct (synthesized and chemically identified by mass spectrometry).	Faucet et al. 2004; Mantle et al. 2010; Mantle and Kulinskaya 2010; Manderville 2005; Pfohl-Leszkowicz and Manderville 2012
2006	Confirmation of OTA genotoxicity by comet assay in the rat kidney.	Zeljezic et al. 2006
2007		Pfohl-Leszkowicz et al. 2007

Table 2 (continued)

Year	Testing	Source
	Chronic exposure to low OTA doses can be much more toxic and carcinogenic than acute exposure to a high dose.	
2007	DNA diploidy in OTA-induced rat tumours is associated to genetic damage.	Brown et al. 2007
2007	OTA induces an increase in mutation at two loci: hypoxantine-guanine phophoribosyl transferase and thymidine kinase.	Palma et al. 2007
2008	DNA adduct cannot be confirmed, but an explanation is given by Pfohl-Leszkowicz et al. (2009).	Delatour et al. 2008
2008	Correlation between biotransformation of OTA and direct covalent binding to DNA.	Manderville and Pfohl-Leszkowicz 2008
2009	The kidney DNA adduct pattern of Balkan endemic nephropathy (BEN) patients is similar to the kidney DNA adduct pattern of pigs living in the same farm and pigs co-exposed to OTA, fumonisins and citrinin.	Pfohl-Leszkowicz 2009
2009	OTA increases the expression of inducible nitric oxide synthase and stimulates protein nitration.	Cavin et al. 2009
2009	Propositions of a different mechanism of OTA-mediated renal carcinogenesis and of a threshold model for OTA risk assessment.	Mally and Dekant 2009
2009–2010	Identification by LC-MS/MS of OTA-DNA adduct in the rat kidney.	Pfohl-Leszkowicz et al. 2009
2010	OTA is carcinogenic for poultry.	Stoev 2010
2011	OTA exposure leads to reactive oxygen species/reactive nitrogen species productions, with increased levels of oxidative DNA, lipid and protein damage.	Marin-Kuan et al. 2011
2011	Induction of mutation only in the medulla of kidneys of rats exposed to a carcinogenic dose.	Hibi et al. 2011
2012	Structure-activity relationships studies indicate that ochratoxin hydroxyquinone (OTHQ) is responsible for direct genotoxicity, whereas some other OTA-deriving structures are cytotoxic.	Hadjeba-Medjoub et al. 2012; Pfohl-Leszkowicz and Manderville 2012
2012	OTA is activated to a species that is a directly genotoxic mutagen. OTHQ in the presence of cysteine is also mutagenic.	Akman et al. 2012
2012	Renal OTA carcinogenicity would be due to a combination of genetic instability and increased proliferative drive as consequences of mitotic disruption.	Mally 2012
2013	The induction of miR-132 and miR-200c by OTA elevates reactive oxygen species levels and pro-fibrotic (pro-fibrotic transforming growth factors β, TGFβ) expression.	Stachurska et al. 2013
2014	OTA has the potential to initiate or support the development of fibrotic kidney diseases by involving post-transcriptional regulation mechanisms comprising miR-29b. OTA reduces the impact of miR-29b and thus enhances collagen protein expression.	Hennemeier et al. 2014
2014	A low dose of OTA induces micronuclei and OTA delays the DNA repair kinetics.	González-Arias et al. 2014
2014	OTA increases proliferating cell nuclear antigen after 13 weeks in rat kidney; it causes kidney damage with cell proliferation but limited oxidative stress.	Qi et al. 2014
2015	Dietary exposure to OTA represents a serious health issue, including urinary tract tumours in exposed humans.	Heussner and Bingle 2015
2016	OTA disrupts renal development via microRNA 731.	Wu et al. 2016

Conclusions

Mycotoxins are ubiquitously found all over the world in many foodstuffs and feedstuffs. Carcinogenic effects and related mechanisms of some mycotoxins (e.g. aflatoxins) are well known. However, for some other important mycotoxins (e.g. OTA, FUM B_1 and FUM B_2), there is a need for continued research on understanding these mechanisms, also taking into account co-occurrence in food and synergistic (e.g. potentiating) effects with other mycotoxins, for public health purposes (e.g. evaluations by the *IARC Monographs* programme) and prevention of economic losses.

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Conflict of interest The authors declare that there are no conflicts of interest.

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