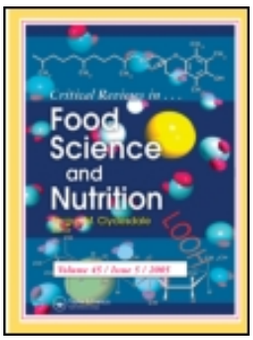

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Ochratoxin A and Human Health Risk: A Review of the Evidence

TRAVIS R. BUI-KLIMKE and FELICIA WU

Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Ochratoxin A (OTA) is a mycotoxin produced by several fungal species including Aspergillus ochraceus, A. carbonarius, A. niger, *and* Penicillium verrucosum. *OTA causes nephrotoxicity and renal tumors in a variety of animal species; however, human health effects are less well-characterized. Various studies have linked OTA exposure with the human diseases Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN), as well as other renal diseases. This study reviews the epidemiological literature on OTA exposure and adverse health effects in different populations worldwide, and assesses the potential human health risks of OTA exposure. Epidemiological studies identified in a systematic review were used to calculate unadjusted odds ratios for OTA associated with various health endpoints. With one exception, there appears to be no statistically significant evidence for human health risks associated with OTA exposure. One Egyptian study showed a significantly higher risk of nephritic syndrome in those with very high urinary OTA levels compared with relatively unexposed individuals; however, other potential risk factors were not controlled for in the study. Larger cohort or case–control studies are needed in the future to better establish potential OTA-related human health effects, and further duplicate-diet studies are needed to validate biomarkers of OTA exposure in humans.*

Keywords Ochratoxin A, human health risks, odds ratios, risk assessment, systematic review

INTRODUCTION

Ochratoxin A (OTA) is a naturally occurring foodborne mycotoxin found in a wide variety of agricultural commodities worldwide, ranging from cereal grains to dried fruits to wine and coffee. It is produced by several different fungi including *Aspergillus ochraceus, A. carbonarius, A. niger*, and *Penicillium verrucosum*. These fungi vary in their optimal growing temperatures and water activity, and contaminate various commodities as summarized in Table 1. Contamination generally occurs as a result of poor storage of commodities and suboptimal agricultural practices during the drying of foods (Moss, 1996). Ingestion is the main source of exposure to OTA, through the foods and beverages listed in Table 1.

OTA is a chemically stable compound; hence, ordinary food processing measures fail to substantially reduce its presence in foods and beverages. OTA has been shown to be toxic and carcinogenic in animals. The kidney is the main target organ for OTA; OTA is a potent renal carcinogen in several animal species (Krogh, 1974; Hagelberg et al., 1989; Kuiper-Goodman and Scott, 1989; IARC, 1993; World Health Organization (WHO), 2002; Duarte et al., 2011). Other adverse effects of OTA include immunotoxicity (Pestka and Bondy, 1994; Bondy and Pestka, 2000), inhibition of macromolecular synthesis, increased lipid peroxidation, and inhibition of mitochondrial respiration (Kuiper-Goodman and Scott, 1989; Marquardt and Frohlich, 1992). OTA has been suspected as a cause of various human nephropathies since the 1970s including Balkan Endemic Nephropathy (BEN) (Barnes et al., 1977; Elling and Krogh, 1977; Sattler et al., 1977; Pfohl-Leszkowicz et al., 2002; Castegnaro et al., 2006) and chronic interstitial nephropathy (CIN) (Abid et al., 2003). The International Agency for Research on Cancer (IARC) has classified OTA as a Group 2B possible human carcinogen, based on demonstrated carcinogenicity in animal studies (IARC, 1993; Fazekas et al., 2005), although OTA-related carcinogenicity has not been conclusively determined in humans. A recent risk assessment on OTA (Haighton et al., 2012) states that OTA was negative in genotoxicity assays with high specificity, and that OTA-DNA adduct levels were low and not typical of genotoxic carcinogens.

OTA has become an important topic in recent years, as Health Canada has proposed maximum limits (MLs) for OTA in a variety of foods and drinks that could have consequences for the marketability of these commodities in Canada, and

Address correspondence to Felicia Wu, Department of Environmental and Occupational Health, 100 Technology Dr., University of Pittsburgh, Pittsburgh PA 15219, USA. E-mail: few8@pitt.edu

Color versions of one or more figures in this article can be found online at www.tandfonline.com/bfsn.

Table 1 Ochratoxin-producing fungi, optimal growth conditions, and commodities affected

OTA-producing species	Optimal temperature range (Min-Max) °C	Water activity	Commodities affected
A. ochraceus	24-31 (8–37)	0.95–0.99	Smoked and salted dried fish, dried beans, biltong, soya beans, chickpeas, rapeseed, pepper, dried fruit, and sesame seeds, nuts, cereals rice, barley, maize, wheat, flour, and bran, coffee beans
A. carbonarius	32-25 (N/A−40)	0.82	Grapes and grape products, including table grapes, wines, and dried vine fruits
A. niger	35–37 (6–47)	0.77	Nuts, apples, pears, peaches, citrus, grapes, figs, strawberries, mangoes, tomatoes, melons, onions, garlic, and yams
P. verrucosum	20 (0–30)	0.80	Cereal crops; cheese, meat products

Source: JECFA (Joint FAO/WHO Committee On Food Additivies (JECFA), 2001).

could also affect nations that attempt to export food to Canada (Health Canada, 2009). Yet, little is known about population health impacts of dietary OTA exposure. Thus far, risk assessments on OTA, including those that have guided Health Canada's recently proposed MLs, have largely been based on animal and cell culture assay studies, with relatively less focus on human studies. The goal of this study was to systematically review the epidemiological literature linking OTA exposure with adverse health effects in diverse human populations worldwide. In a discussion of risk assessments conducted on OTA in the past, we compare the state of known data with what is still missing in terms of assessing human health effects. We collected available human studies linking OTA exposure with a variety of health outcomes, and selected those studies that met predefined criteria for inclusion in the review. Odds ratios were estimated for these different studies where the data permitted these calculations. The current state of exposure assessment for OTA is discussed as well. We characterize the risk of OTA to human populations and describe limitations in the available data.

BACKGROUND: RISK ASSESSMENT OF OCHRATOXIN A

Risk assessment, the process of estimating the magnitude and the probability of a harmful effect to populations from certain agents or activities, consists of four main steps: hazard identification, hazard characterization or dose–response assessment, exposure assessment, and risk characterization (National Research Council, 1983). The four steps involved in the estimation of risk are outlined below (Liu and Wu, 2010).

Hazard identification, determining whether exposure to an agent can increase the incidence of a particular health condition, has been carried out for OTA in assessments conducted by multiple institutions; including the International Agency for Research on Cancer (IARC), Health Canada, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA) (Joint FAO/WHO Committee On Food Additivies (JECFA), 1991; IARC, 1993; European Food Safety Authority (EFSA), 2006; Health Canada, 2009). Ochratoxin is identified as a renal carcinogen to particular animal species (Kuiper-Goodman and Scott, 1989) and can cause nephrotoxic, teratogenic, and immunosuppressive effects in multiple animal species (Kuiper-Goodman and Scott, 1989; O'Brien and Dietrich, 2005).

For humans, however, hazard identification has been more difficult. Several adverse human health effects, including the kidney diseases BEN and CIN, have been associated with exposure to OTA; but these associations have thus far been less conclusive than those for OTA-associated adverse effects in laboratory animal studies. The hallmark features of BEN include a familial but not inherited pattern of disease, initial manifestation after living in an endemic village for 15 years or more, and an association with upper urothelial tract cancer (Grollman et al., 2007). However, aristolochic acid (AA), a toxin produced in *Aristolochia* weeds commonly found in Balkan grain fields, has emerged as the most likely causative agent of BEN; as aristolactam-DNA adducts have been found in the renal cortex of BEN patients but not in patients with other chronic renal diseases (26). CIN does not appear to have the familial pattern of BEN, and may be acute or chronic with cases presenting anywhere from a few days up to five months. The etiology of CIN has been postulated to include infections, toxins such as OTA, or reactions to medications (Baker and Pusey, 2004).

Hazard characterization or *dose–response assessment* describes the relationship between different levels of exposure to a substance and associated incidence of disease in a population of animals or humans. Dose–response data from animal studies of a particular toxin are used to extrapolate an acceptable daily or weekly exposure to humans, below which no adverse effects are expected. This step usually involves a critical review of toxicological studies to set appropriate exposure metrics (Kuiper-Goodman et al., 2010), such as tolerable daily or weekly intake or negligible cancer risk intake. In the case of OTA, diverse regulatory and advisory bodies have assessed dose–response data on OTA and have set exposure metrics for tolerable exposure to OTA in humans. These are summarized in Table 2.

Various dose–response studies in animals were the basis for advisory groups' determinations of safe weekly or daily OTA

Table 2 Summary of calculated tolerable human intakes of OTA by international organization

Organization	Tolerable intake metric*	Limit	Reference
European Food Safety Authority (EFSA)	PTWI	120 ng/kg bw/week	EFSA, 2006
Health Canada	PTDI	3 ng/kg bw/day	Kuiper-Goodman et al., 2010
Health Canada	NCRI	4 ng/kg bw/day	Kuiper-Goodman et al., 2010
Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2007	PTWI	100 ng/kg bw/week	JECFA, 2007
Nordic Expert Group on Food Safety	TDI	5 ng/kg bw/day	Olson[CE: Please check spelling in Olson or Olsen] et al., 1991
Scientific Committee of Food (SCF) of the European Union	PTDI	5 ng/kg bw/day	EFSA, 2006

*TDI, tolerable daily intake; PTDI, provisional tolerable daily intake; PTWI, provisional tolerable weekly intake; NCRI, negligible cancer risk intake.

intakes for humans. JECFA first evaluated OTA at its 37th meeting (JECFA, 1991), setting a provisional tolerable weekly intake (PTWI) at 112 ng OTA per kg bodyweight (bw) per week based on a dose–response study of renal function deterioration in pigs, for which the lowest observed adverse effect level (LOAEL) was 8 μg/kg bw/day (Krogh, 1974; Elling, 1979). A combined uncertainty factor (UF) of 500 was applied in the calculation. JECFA re-evaluated OTA at its 44th meeting, taking into account new toxicological data. The PTWI was confirmed, but rounded down to 100 ng/kg bw/week. The most recent assessment of OTA at the 68th meeting in 2008 resulted in retaining the PTWI previously found. JECFA currently estimates OTA exposure from cereals, based on European data, to be about 8–17 ng/kg bw/week: well below the PTWI.

The European Food Safety Authority (EFSA) derived a PTWI for OTA of 120 ng/kg bw/week, based on the 8 μg/kg bw/day LOAEL used in the JECFA evaluation (European Food Safety Authority, 2006). An uncertainty factor of 450, rather than 500 used in JECFA, was applied to the LOAEL. This composite uncertainty factor was based on an intraspecies factor of 10, interspecies factor of 15, and a factor of 3 for use of a LOAEL instead of a no observed adverse effect level (NOAEL). The interspecies factor of 15 was based on the longer OTA half-life in humans and monkeys rather than pigs as determined by Hagelberg et al. (1989).

A Health Canada risk assessment team (Kuiper-Goodman et al., 2010) chose to reevaluate EFSA's PTWI for OTA, positing that the use of LOAEL rather than a NOAEL was not appropriate given the small number of animals per group, and the fact that four out of nine pigs in the lowest dose group showed functional kidney changes. Rather than use a NOAEL or LOAEL, a benchmark dose corresponding to a response of 10% above background (BD_{10}) was derived. Uncertainty factors of 10 for intraspecies variability, 25 for interspecies variability, and 2 for use of a subchronic rather than chronic study were combined in a composite uncertainty factor of 500. Applying this composite uncertainty factor to the BD_{10} of 1.56 μg/kg bw/day resulted in a TDI of 3.0 ng/kg bw/day after rounding (Kuiper-Goodman et al., 2010), which in practice is considerably stricter than the JECFA or EFSA tolerable limits.

In addition, Health Canada derived a negligible cancer risk intake (NCRI) for OTA: the exposure associated with an increased cancer risk of 1:100,000 and equivalent in units to the TDI. The tumorigenic dose at which 5% of the animals are likely to have tumors (TD_{05}) was used to derive the NCRI for OTA (NTP, 1989). This dose was determined to be 19.6 μg/kg bw/day. The TD_{05} was then divided by 5000, the linear extrapolation to zero exposure, resulting in a NCRI of 4 ng/kg bw/day (Kuiper-Goodman et al., 2010).

Exposure assessment involves estimating the intensity, frequency, and duration of human exposures to toxic substances. Ochratoxin exposure is a function of the concentration of ochratoxin in foodstuffs, as well as the amount of these foodstuffs that are consumed in different populations. Depending on location, seasons, and amount of time food is kept in storage, both the amount and contamination levels of food may vary greatly even for the same population or individual (Scott, 2005). Estimating human OTA exposure may be done using food surveys combined with OTA surveillance in commodities, or biomarkers of exposure.

By determining ranges of OTA contamination of foods, OTA exposure can be estimated from the known intake levels of the given commodities (Jørgensen et al., 1996). However, the accuracy of this estimation is limited due to the large variability in OTA content in commodities, as well as variation in dietary habits (Fazekas et al., 2005). Surveillance data on OTA concentrations in different regions of the world is limited. JECFA provides extensive data on OTA exposure concentrations in commodities (JECFA, 2001); however, 85% of sampled commodities including wheat, rye, barley, oats, dried vegetables, olives, and milk came from Europe. However, it was noted that OTA occurs in coffee in countries including Brazil, Canada, Dubai, Europe, Japan, and the USA (Joint FAO/WHO Committee On Food Additivies (JECFA), 2001). In Denmark, Norway, and the UK, OTA was found in oat samples. In Germany, high OTA levels were found in unprocessed cereals, rye and buckwheat, with levels ranging from 95.6–125 μg/kg. In Africa, OTA was found in wheat, barley, cereals, dried vegetables, and olives. Specifically, Maaroufi et al. (1995) found high OTA levels, with a maximum of 33,000 μg/kg in wheat, barley, mixed cereals, dried

Table 3 OTA occurrence in human urine

Country	Year collected	Number of positive samples (%) for OTA	Range of urinary OTA levels (ng/ml)	Sampled population	Reference
Bulgaria	1984–1990	44/127 (35)	0.005–0.604	Healthy humans in Balkan Endemic Nephropathy (BEN) areas, Non-BEN areas and BEN patients	(Nikolov et al., 2002)
Bulgaria	2003	16/16 (100)	0.016–0.860	Patients from BEN areas	(Petkova-Bocharova et al., 2003)
Bulgaria	Not Available	61/152 (40)	n.d.-0.03	BEN and Urothelial Tract Tumor (UTT) patients	(Castegnaro et al., 1990)
Croatia	2000	24/63 (38)	0.005–0.086	BEN and Non-BEN areas	(Domijan et al., 2009)
Croatia	2005	9/63 (14)	0.005–0.015	BEN and Non-BEN areas	(Domijan et al., 2009)
Egypt	1998	19/122 (16)	0–8.19	Healthy controls, kidney donors, patients with end-stage renal disease (ESRD), transplant recipients, nephritic syndrome patients, and UTT patients	(Wafa et al., 1998)
Germany	2008	13/13 (100)	0.02–0.13	Healthy volunteers	(Munoz et al., 2010)
Hungary	2003	54/88 (61)	0.006–0.065	Healthy volunteers	(Fazekas et al., 2005)
Italy	2001	25/41 (61)	0.012–0.140	Healthy individuals and karyomegalic interstitial nephritis patients	(Pascale and Visconti, 2000)
Italy	Not Available	10/10 (100)	0.02–0.25	Healthy volunteers	(Solfrizzo et al., 2011)
Korea	Not Available	12/12 (100)	0.012–0.093	Healthy volunteers	(Ahn et al., 2010)
Portugal	2007	174/198 (88)	n.d. −0.071	Healthy volunteers	(Duarte et al., 2010a; Duarte et al., 2009)
Portugal	2004	42/60 (70)	n.d. −0.105	Healthy volunteers	(Pena et al., 2006)
Portugal	2005	13/30 (43)	n.d. −0.208	Healthy volunteers	(Manique et al., 2008)
Sierra Leone	1992–93	63/434 (25)	0.06–148	Healthy child volunteers	(Jonsyn-Ellis, 2001)
Spain	2005	25/31 (81)	n.d. −0.124	Healthy volunteers	(Manique et al., 2008)
Spain	2011	9/72 (13)	0.057–0.562	Healthy volunteers	(Coronel et al., 2011)
Spain	2011	3/27 (11)	n.d. −<1.5	Healthy volunteers	(Rubert et al., 2011)
United Kingdom	2001	46/50 (92)	<0.01–0.058	Healthy volunteers	(Gilbert et al., 2001)

vegetables, and olives in a Tunisian population. In Nigeria, Ghana, and Burkina Faso, OTA was detected in sorghum, maize, and millet.

A more accurate method to estimate exposure, when possible, is through measuring human biomarkers of OTA exposure, as reviewed in Duarte et al. (Duarte et al., 2011). Although neither serum OTA nor urinary OTA has been validated, they have been used in multiple studies to estimate OTA exposure. OTA is found in serum due to its long elimination half-life of about 35 days (Studer-Rohr et al., 2000), and is excreted in urine as both unchanged OTA and its derivatives.

OTA in serum was first detected in 1977 and has been one of the most widely used biomonitoring approaches for human OTA exposure. Renal absorption, enterohepatic circulation, and binding to plasma proteins result in a long half-life for OTA in the body, allowing it to be detected in human blood (Roth et al., 1988; Dahlmann et al., 1998). Epidemiological studies conducted in multiple countries, including Romania (Voo et al., 2002), Spain (Jimenez et al., 1998), the Czech Republic (Malir et al., 2001a; Malir et al., 2001b), Turkey (Ozcelik et al., 2001), Italy (Breitholtz-Emanuelsson et al., 1994), Egypt (Wafa et al., 1998), Algeria (Khalef et al., 1993), and Tunisia (Abid et al., 2003; Hassen et al., 2004), have associated higher serum or plasma OTA levels in patients with kidney and urinary disorders compared to healthy controls, although the associations may not be causal (Scott, 2005).

Although many studies have used serum OTA as a human biomarker of OTA exposure, considerable intrasubject variation has been noted. Levels of serum OTA have been noted to vary up to tenfold in one subject when tested over a 10-year period (Radic et al., 1997) and in repeatedly tested subjects over one year in Tuscany (Palli et al., 1999). Furthermore, studies in Germany (Martlbauer et al., 1996), Switzerland (Studer-Rohr et al., 2000), Czech Republic (Ruprich and Ostry, 1993), Japan (Kawamura et al., 1993), and Bulgaria (Petkova-Bocharova et al., 2003) all showed high intrasubject variability in human subjects tested over time. This variability is likely due to the decreases in plasma concentrations based on the half-life of OTA (Scott, 2005).

Urinary OTA, another potential biomarker of OTA exposure, is often found in very low amounts compared to those in blood; however, new technologies have increase the sensitivity and accuracy of detection (Duarte et al., 2011). Pascale and Visconti (2000) detected OTA in 37 of 55 healthy individuals in Italy with levels ranging from 0.012–0.046 ng/mL. In Hungary, Fazekas et al. (Fazekas et al., 2005) detected OTA in 54 of 88 samples from healthy individuals at levels of 0.006–0.065 mg/mL. Patients with end-stage renal disease or nephritic syndrome in Egypt had significantly higher levels of urinary OTA than two reference groups (Wafa et al., 1998).

The highest incidence of detectable OTA exposures in urine, 100%, were found by Petkova-Bocharova et al. (Petkova-Bocharova et al., 2003) in a BEN-endemic region and in non-endemic regions including Portugal (Duarte et al., 2010b) and Italy (Breitholtz-Emanuelsson et al., 1994). The highest recorded levels of OTA in urine, 367 ng/mL and 1801 ng/mL, were found in two French siblings with renal failure (Godin et al., 1996). Petkova-Bocharova et al. (2003) and Castegnaro et al. (2006) applied similar methodology as Gilbert et al. (2001) when studying 16 human participants (Castegnaro et al., 2006). Increases of OTA intake resulted in an increase of OTA elimination a week after ingestion, not immediately (Castegnaro et al., 2006). Table 3 summarizes urinary OTA levels in populations from different world regions. The table includes OTA levels from Duarte et al. (Duarte et al., 2011) and more recent studies.

A 2001 study on both serum and urinary biomarkers of OTA exposure revealed a stronger correlation between dietary OTA intake and urinary OTA than serum OTA. Gilbert et al. (Gilbert et al., 2001) examined OTA levels in urine and plasma as a function of dietary OTA intake in 50 subjects in the United Kingdom. The volunteers kept a daily food diary and provided blood samples once per week, urine samples daily, and duplicate diet samples daily, for one month. Baseline samples were taken at the beginning of the study. OTA was detected in all but four urine samples, with levels ranging from <0.01–0.058 ng/mL. OTA was detected in all plasma samples with baseline sample levels ranging from 0.15–2.17 ng/mL and composited plasma samples ranging from 0.4–3.11 ng/mL. A statistically significant correlation between urine OTA levels ($R^2 = 0.52$) and dietary OTA consumption was found. However, the authors caution that this relationship is too weak to be used in a predictive manner (Gilbert et al., 2001). In plasma, no significant correlation was found between the two ($R^2 = 0.29$). For the purpose of establishing a valid biomarker of OTA exposure in the future, urinary OTA appears a stronger candidate.

Risk characterization integrates the dose–response and exposure assessments to determine the probability of an adverse effect to human populations by an agent. To estimate risk associated with OTA exposure and various health effects, it was not possible to estimate a population attributable risk due to a lack of available epidemiological data. Instead, a systematic review was performed in this study, and unadjusted odds ratios (ORs) were calculated for various health effects associated with OTA exposure based on data from existing studies.

SYSTEMATIC REVIEW

The purpose of this systematic review was to attempt to reconcile the human and animal study results on OTA toxicity, or lack thereof. A literature search was performed on PubMed until October 4th, 2011. Search terms used without restriction included combinations of: (OTA), (human), (population), (disease), (urinary ochratoxin), (urinary OTA), and (urinary biomarker). In addition, we searched reference lists from retrieved articles and searched ochratoxin review papers for any additional epidemiological studies on adverse effects associated with OTA exposure that may not have been retrieved in the initial search.

Eligibility criteria for inclusion in the review were as follows: (1) epidemiological studies; (2) case–control or cohort study design; (3) OTA as the exposure of interest; (4) OTA exposure measured either in terms of dietary intake or urinary OTA levels; and (5) relative risk (RR) or odds ratio (OR) estimates with 95% confidence intervals (CIs) reported, or data to calculate these. Studies using serum OTA as a marker for exposure were excluded because of the poor correlation between dietary OTA intake and serum OTA as measured in Gilbert et al. (2001).

Data on the following were extracted from each study: authors, publication year, study design and sample size, study location, study period, participants' gender and age, range of ochratoxin exposure, health effect under investigation, and data necessary to calculate ORs for each health effect if the OR was not already calculated. From these data, we calculated unadjusted ORs and 95% CI for each ochratoxin-related health effect examined in each of the studies (several studies examined more than one adverse effect).

RESULTS

The step-by-step process of our literature search is presented in Figure 1. From 2431 results, only those studies that met the criteria listed above were included. Fifteen studies were selected on the basis of information in the title and abstract, and seven more were added on the basis of reference lists in those selected studies. A full-text review of all 22 articles resulted in 19 being excluded because they did not measure urinary OTA or dietary OTA, or did not include both diseased and healthy individuals. Three studies contained the relevant information needed to calculate unadjusted ORs for different OTA health effects.

Table 4 provides an overview of the three eligible studies. Based on the data needed to calculate ORs, three studies were included. Due to the lack of similar health endpoints across the different studies, data could not be combined for meta-analysis. The three eligible studies included two in the Balkans (Croatia and Bulgaria) and one in Egypt. All studies measured urinary OTA levels and associated these levels with several different adverse health effects. Each study also had at least one corresponding control group. Domijan et al. (2009) compared individuals in a BEN-endemic village to those in a non-BEN-endemic village, whereas Nikolov et al. (2002) and Wafa et al. (1998) study used healthy human controls. Epidemiological studies were not included in the review if they did not examine both cases (i.e., those with confirmed disease) and controls. For example, Petkova-Bocharova et al. (Petkova-

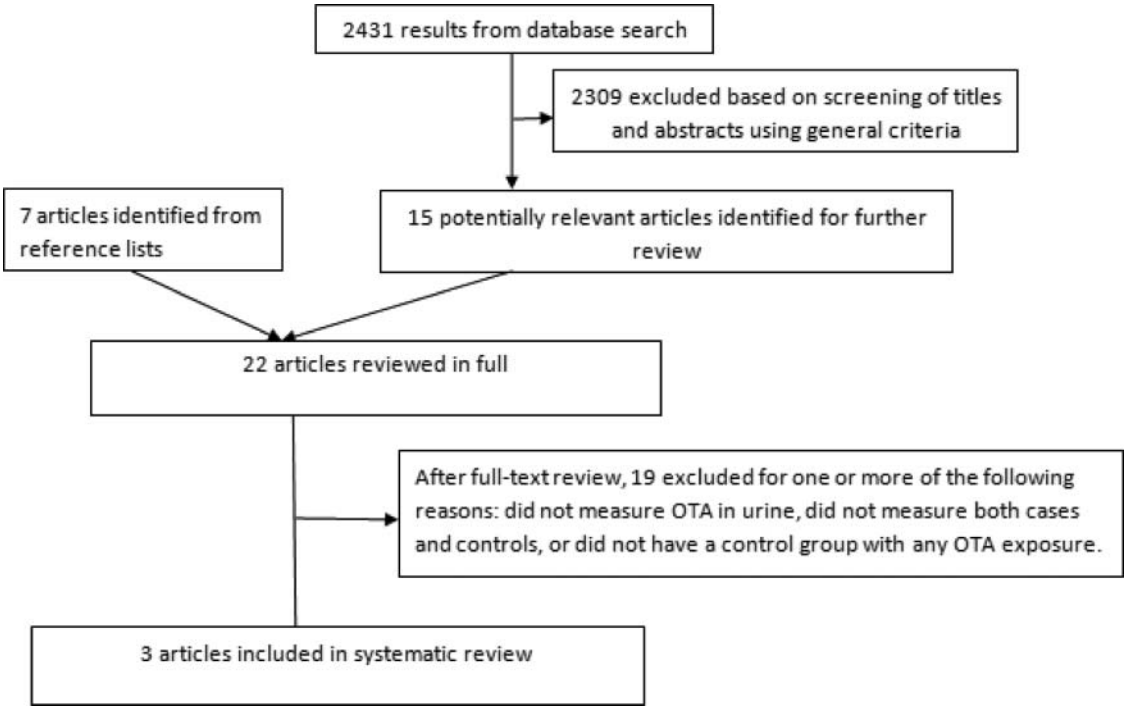

Figure 1 Selection of studies for inclusion in systematic review of adverse health effects associated with OTA exposure as measured by urinary OTA. Format of figure: Liu et al., 2012.

Table 4 Eligible studies and Unadjusted ORs

Location	Population characteristics	Proportion positive for urinary OTA (%)	Mean urinary OTA (ng/mL) (Range)	OR (95% CI)	Reference
Egypt	End-stage renal disease (ESRD) patients w/treatment	4/11 (36.4%)	1.85 (nd*−6.70)	2.74 (0.402–18.69)	(Wafa et al., 1998)
	ESRD dialysis patients	1/11 (9.1%)	0.36 (nd−4.0)	1.23 (0.115–13.14)	
	ESRD Totals	5/22 (22.7%)	Not available	1.94 (0.357–10.52)	
	Renal transplant recipients	2/15 (13.3%)	0.12 (nd−1.36)	1.89 (0.28–12.65)	
	Nephritic Syndrome patients	8/15 (53.3%)	3.09 (nd−8.19)	10.79 (2.28–50.91)	
	Patients with urothelial tract tumors (UTT)	1/15 (6.7%)	0.36 (nd−4.64)	0.88 (0.085–9.14)	
	Potential kidney donors	2/15 (13.3%)	0.26 (nd−3.42)	Not available	
	Controls	3/40 (7.5%)	0.01 (nd−0.31)	Not available	
Bulgaria	BEN/UTT patients	14/36 (38.9%)	Not available (0.005–0.604)	1.29 (0.58–2.87)	(Nikolov et al., 2002)
	Healthy persons from BEN families	12/25 (48%)	Not available (nd-0.033)	Not available	
	Healthy persons from non-BEN families in BEN villages	14/32 (43.8%)	Not available (nd-0.043)	Not available	
	Healthy persons from non-BEN villages in BEN area	4/31 (12.9%)	Not available (nd-0.041)	Not available	
	Healthy persons from non-BEN area	0/3 (0%)	Not available	Not available	
Croatia 2000	Endemic BEN village	19/45 (43%)	0.007 (nd-0.086)	1.90 (0.58–6.23)	(Domijan et al., 2009)
	Healthy control village	5/18 (28%)	0.003 (nd−0.02)	Not available	
Croatia 2005	Endemic BEN village	8/45 (18%)	0.001 (nd−0.015)	3.68 (0.43–31.78)	(Domijan et al., 2009)
	Healthy control village	1/18 (6%)	0.005 (0–0.01)	Not Available	
	Combined BEN Villages	27/90 (30%)	Not available	2.14 (0.80–5.74)	

*nd, non-detect

Note: Mean urinary OTA levels were not provided for all sampled groups and were therefore labeled as “Not Available.” OR labeled “Not Available” were for control groups, hence, no OR could be calculated.

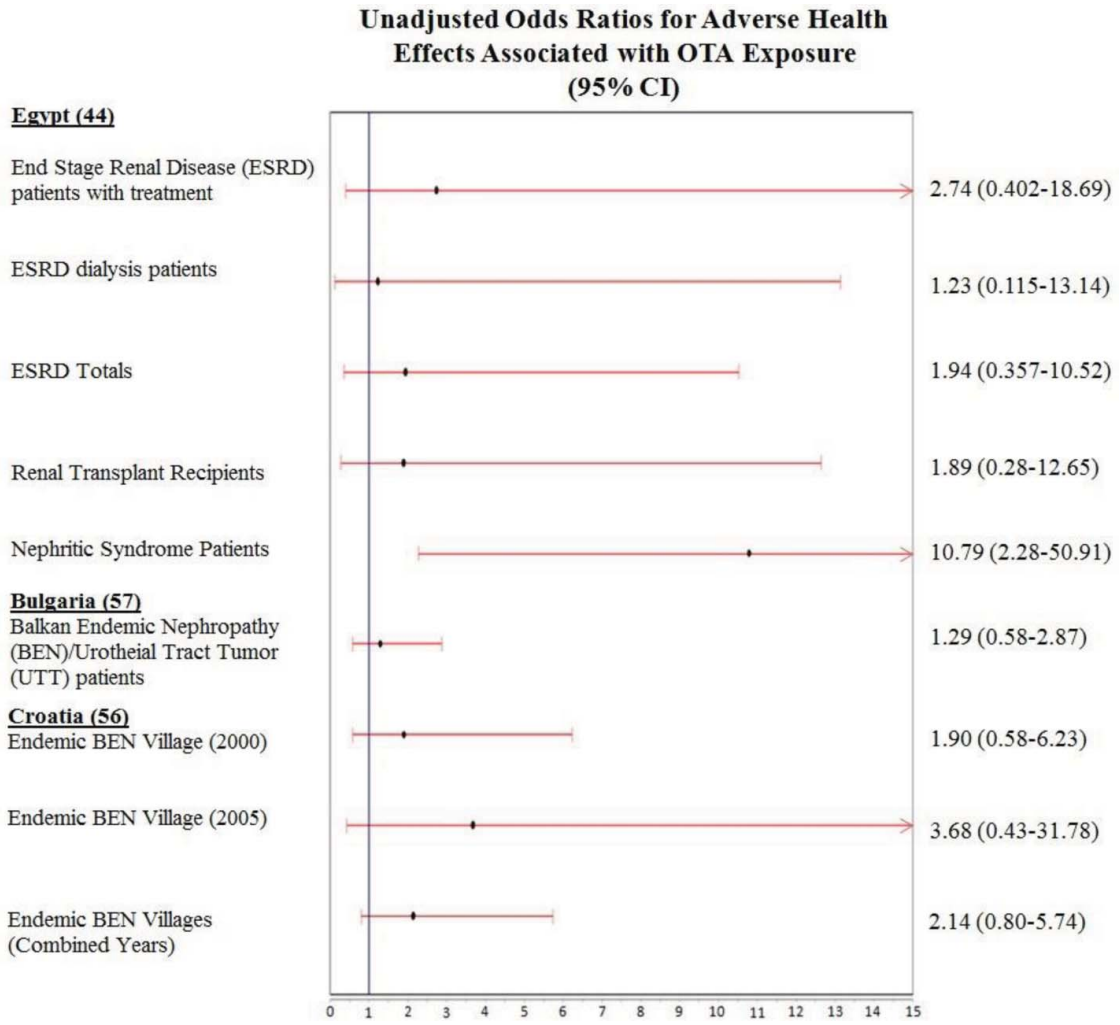

Figure 2 Unadjusted OR and 95% CI for various health endpoints of OTA exposure identified through a systematic review.

Bocharova et al., 2003) and Castegnaro et al. (Castegnaro et al., 2006) examined OTA in serum and urine in human subjects living in BEN-endemic versus non-BEN-endemic villages, but all subjects involved in the study were healthy.

The calculated unadjusted ORs are summarized by study and health effect in Table 4. The table includes the three studies used to calculate unadjusted ORs organized by location. Information on each disease assessed, the proportion of subjects with each disease who had detectable urinary OTA, the levels (mean and range) of measured urinary OTA, and unadjusted odds ratio with 95% CI are included in the table. The highest level of measured urinary OTA was in nephritic syndrome patients in Egypt, followed by patients being treated for ESRD in Egypt. Patients mean levels of urinary OTA were 3.09 ± 3.4 ng/mL and 1.85 ± 2.8 ng/mL, respectively. Unadjusted ORs ranged from 0.88–10.79 for all adverse health endpoints. While these ORs were unadjusted, demographics including age, sex, socio-economic status, and other lifestyle factors, were similar across both cases and controls in each accepted study.

No statistically significant associations between OTA exposure and any human disease were found in the Bulgarian or Croatian study populations. Only one adverse health effect, nephritic syndrome, was found to have a statistically significant association with OTA exposure, in the Egyptian study population (Wafa et al., 1998). The OR of OTA-related nephritic syndrome in this population was 10.79 (95% CI: 2.28–50.91). However, it is worth noting that the sample size of this particular study group was 15: relatively small. Figure 2 summarizes ORs and 95% CI for each of the adverse health outcomes associated with OTA exposure as measured by urinary OTA in the three study populations.

Urinary OTA levels obtained by Wafa et al. (1998) were compared to those obtained by Gilbert et al. (Gilbert et al., 2001) and the urinary OTA studies are summarized in Table 3. Upon comparison, the levels of OTA found in urine in the Gilbert study ranged from nondetectable to 0.06 ng/mL: much lower than the 3.09 ng/mL mean urinary OTA level found in the nephritic syndrome patients in Wafa et al. (1998). In both the Wafa et al. (1998) and Gilbert

et al. (2001) studies, as well as the Croatian study in the systematic review (Domijan et al. 2009), OTA presence was determined and analyzed by HPLC. Urinary OTA levels in humans from several different world regions, summarized in Table 3, range from <0.01–148 ng/mL. The extremely high end of this range comes from a study in Sierra Leone (Jonson-Ellis, 2001). When this study and the Egyptian study are excluded, the urinary OTA levels measured in different world regions ranges from nondetectable to 0.860 ng/mL: much lower than the levels found in the study populations in Egypt and Sierra Leone.

CONCLUSIONS

The review of the epidemiological data suggests that, with one exception, there appears to be no statistically significant evidence for human health risks associated with OTA exposure. The one exception concerns an increased risk of nephritic syndrome at very high exposures to OTA, based on case–control studies assessing multiple potential adverse health effects in an Egyptian population (Wafa et al., 1998). However, the sample size of this studied population was very small, and the urinary OTA levels associated with nephritic syndrome were much higher than urinary OTA levels measured in multiple other world regions, with the exception of Sierra Leone.

Nephritic syndrome, also known as glomerulonephritis, is a disorder of the glomeruli characterized by body tissue swelling (edema), high blood pressure, and the presence of red blood cells in the urine. The cause of nephritic syndrome is multifactorial, and the term "nephritic syndrome" itself describes a condition with multiple symptoms. As no other risk factors were controlled for in Wafa et al. (Wafa et al., 1998), it is possible that OTA is not the only etiologic factor in all the cases of nephritic syndrome in this study population. Moreover, the OTA exposures measured in Wafa et al. (Wafa et al., 1998) were over three orders of magnitude higher than the highest exposures measured in Gilbert et al. (Gilbert et al., 2001) and the vast majority of other urinary biomarker studies. Populations in which OTA exposures are extremely high (such as those studied in Egypt and Sierra Leone) may experience a significantly increased risk of nephritic syndrome. However, because this extremely high level of OTA exposure is not expected in most other parts of the world as evidenced by urinary OTA levels collected in multiple other world regions, the risk of OTA-related nephritic syndrome on a global scale is not expected to be significant.

Several limitations exist with our analysis of epidemiological studies on OTA. The main limitation is lack of validated markers of exposure in human populations. While multiple studies examined urinary and serum OTA levels in humans in different regions of the world, none of the studies for which odds ratios were calculated measured actual OTA exposure in the diet. Ideally, the urinary OTA biomarker of exposure should be validated by repeated associations with dietary OTA intake, with reasonable statistical significance, in other populations worldwide. Another limitation of our analysis concerns the small number of studies assessed, and the relatively small sample population sizes in these few case–control studies. It was not feasible to conduct a meta-analysis of OTA-related health disease, because each study assessed measured a different health endpoint. Finally, it was not possible to calculate adjusted odds ratios, because the studies did not provide sufficient data on potential confounders; instead, unadjusted odds ratios were calculated.

For the purposes of establishing appropriate regulatory policies regarding human exposure to OTA, it is critical to gain a better understanding of OTA's impacts on human health. To improve our understanding of possible effects of OTA exposure on human health, two types of further studies would be useful. First, larger cohort or case–control studies in different parts of the world, which control for sociodemographic and other potential risk factors, are needed to better establish potential OTA-related human health effects. Second, further duplicate-diet studies are needed to validate biomarkers of OTA exposure in humans. Ideally, these studies would be replicated in different parts of the world; and, similar to (56), would assess OTA intake and biomarker levels over at least one month to account for the long serum half-life and renal elimination of OTA. Such studies would allow for improved exposure assessment, as well as improved correlation with human diseases and conditions, to better inform human health risk assessment of OTA.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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