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Review

Aflatoxins: A silent threat in developing countries

Elias Nortaa Kunedeb Sowley

Department of Agronomy, Faculty of Agriculture, University for Development Studies, P. O. Box TL 1882, Nyankpala Campus, Tamale, Ghana.

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Several mycotoxins are known to contaminate crop produce and processed forms but aflatoxins are the most common. They are mainly produced by fungi belonging to the genera *Aspergillus* and *Penicillium*. Cereals and their products which constitute the staples in most developing countries are particularly vulnerable to attack by aflatoxigenic fungi. Despite the potential health risk posed to animals and humans, many people in developing countries are oblivious of the ability of aflatoxins to cause cancer and other debilitating diseases. This review therefore examines the various types of aflatoxigenic fungi and toxins, their occurrence in foodstuffs, their harmful effects, economic losses caused, regulation including the tolerable limits set by various national and international agencies and how their effects can be minimized or eliminated. Since developing countries are less resourced, there is the need for their developed counterparts and international agencies to offer them financial and technical support, to enable them to embark on education, research and other activities and ultimately minimize contamination in their products.

Key words: Aflatoxicosis, fungi, regulation, standards, toxins.

INTRODUCTION

In developing countries, cereals which constitute the staples are susceptible to fungal infections which result in mycotoxin contamination due to poor agronomic and postharvest practices. Mycotoxins are toxic secondary metabolites produced by fungi in agricultural products that are susceptible to mould infestation and can be classified according to their fungal origin, chemical structure and biological activity (Okello et al., 2010). They are commonly produced by fungi belonging to the genera, *Aspergillus*, *Fusarium* and *Penicillium*. The Food and Agriculture Organisation estimates that one quarter of the world's food crops are affected by mycotoxins (CRA, 2011). Mycotoxin production and contamination are unavoidable and depend on a variety of environmental factors in the field and or during storage, which makes it a unique challenge to food safety (Park and Stoloff, 1989). Their occurrence in food is mainly as a result of direct contamination of agricultural commodity and their survival of food processing to some extent. Over 200 mycotoxins have been reported but only those occurring naturally in foods are of significance in food safety.

E-mail: esowley@gmail.com. Tel: +233248629827.

Aflatoxins are the most widely studied and dangerous mycotoxins (Okello et al., 2010). They were not well known until
the 1960s when turkey poults died in East Anglia after being fed on pelleted feed containing groundnut meal which was shown to be a toxic constituent (Moss, 2002). Although, aflatoxin contamination is a serious problem in developing countries, it is most common in African, Asian and south American countries with warm and humid climates (Dohlman, 2004). Darwish et al. (2014) reported that aflatoxins are the most common mycotoxins (43.75%) in Africa followed by fumonisin (21.87%), ochratoxins (12.5%), zearalenone (9.38%), deoxynevalenol (6.25%) and beauvericin (6.25%). They reported high levels of aflatoxin in samples collected from several African countries including South Africa, Lesotho, Egypt, Tunisia, Morocco, Sudan, Tanzania, Zambia, Uganda, Kenya, Ethiopia, Nigeria, Ghana, Benin, Mali, Togo and Bourkina Faso.

TYPES OF AFLATOXIGENIC FUNGI AND THEIR TOXINS

Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus ochraceoroseus*, *Aspergillus pseudotamarii*, *Aspergillus bombycis*, a species with the imperfect stage, *Emericella venezuelensis* and *Aspergillus niger* (Moss, 2002). Sowley and Baalabong (2013) isolated some aflatoxigenic fungi namely, *A. niger*, *A. flavus* and *A. ochraceus* from grains stored by indigenous methods with *A. flavus* which is the most common producer of aflatoxin (Bankole and Adebanjo, 2003) as the most frequent. According to Ruiqian et al. (2004), *A. flavus* and the closely related species, *A. parasiticus* have a world-wide distribution and normally occur as saprophytes in soil and many kinds of decaying organic matter. Aflatoxigenic fungi produce four major aflatoxins: B1, B2, G1 and, G2 plus two additional metabolic products, M1 and M2, that are of significance as direct contaminants of foods and feeds (Bankole and Adebanjo, 2003; Okello et al., 2010). However, Aflatoxin B1 produced by *A. flavus* and *A. parasiticus* is the major and most common toxin in food; it is among the most potent genotoxic and carcinogenic aflatoxins (EFSA, 2013; Schmalle III and Munkvold, 2015). Aflatoxin M1 is a major metabolite of aflatoxin B1 in humans and animals, which may be present in milk from animals fed with aflatoxin B1 contaminated feed (EFSA, 2013) and may subsequently contaminate other dairy products such as cheese and yogurt (Augusto, 2004).

OCCURRENCE OF AFLATOXINS IN FOODSTUFFS

Aflatoxins have been detected in several foodstuffs of plant and animal origin. They can occur in foods, such as groundnuts, treenuts, maize, rice, figs and other dried foods, spices and crude vegetable oils and cocoa beans, as a result of fungal contamination before and after harvest (EFSA, 2013). Milk, eggs and meat products are sometimes contaminated because of the animal consumption of aflatoxin-contaminated feed. However, the commodities with the highest risk of aflatoxin contamination are maize, peanuts and cotton seed (Anonymous, 2014a).

Maize which is a major staple in most developing countries can easily be contaminated with aflatoxins which have been detected at varying levels. For instance in Ethiopia, Ayalew (2010) detected aflatoxin in 88% of maize samples with concentrations below 5 µg kg^{-1}, except in one sample which had 27 µg kg^{-1}. In Benin, aflatoxin B1 level up to 14 g kg^{-1} and aflatoxin G1 level up to 58 g kg^{-1} were detected in stored maize (Bankole and Adebanjo, 2003). Kpodo (1996) reported that all the maize samples collected from silos and warehouses in Ghana contained aflatoxins at levels ranging from 20 to 355 g kg^{-1}, while fermented maize dough collected from major processing sites contained aflatoxin levels of 0.7 to 313 g kg^{-1}. Hennigen and Dick (1995) detected aflatoxins B1 and G1 in concentrations that varied from 12 to 906 µg kg^{-1} in 34.8% of samples collected from silos and 10 to 14 µg kg^{-1} in 23% of samples collected from maize farms in Rio Grande do Sul, Brazil. Sekiyama (2005) also detected aflatoxins in 3.2% of maize-based food samples in Brazil. Maize samples from Indian communities in which there was an outbreak of aflatoxicosis were contaminated with aflatoxin (0.000625 to 0.0015.6 g kg^{-1}) and the affected people were suspected to have consumed between 2000 and 6000 µg kg^{-1} of aflatoxins daily for a month (Reddy and Raghavender, 2007).

Apart from maize, groundnut is another crop which is widely cultivated, consumed locally and also exported by most developing countries. Just like maize, it is vulnerable to attack by aflatoxigenic fungi. Bankole and Adebanjo (2003) reported that groundnuts cultivated in Northern Nigeria were contaminated with aflatoxin levels up to 2000 g kg^{-1}. Philips et al. (1996) reported that a contaminated groundnut meal used to feed dairy cattle had aflatoxin as high as 3000 µg kg^{-1}. All weanimix samples collected from the Ejura-Sekyedumase district of Ghana, were contaminated with 83.34% above the 20 µg kg^{-1} limit for aflatoxin set by the U.S. Food and Drug Administration (Kumi et al., 2014).

Fresh milk which is often consumed in developing countries without treatment poses a high risk to consumers. Iqbal et al. (2014) reported that aflatoxin M1 was found above the measurable level (0.004 µg l^{-1}) in 64 and 52% of milk samples from urban and rural farmhouses, respectively, in Pakistan. According to them, 99.4% of all samples analysed exceeded the EU limit of 0.05 µg l^{-1}. In Turkey, Polat and Gul (2014) also detected a mean level of 0.03 µg l^{-1} of aflatoxin in milk produced by animals which were fed with contaminated feed.

Nonconventional food sources can also be contaminated with aflatoxin. For instance, in Nigeria, aflatoxins were detected in bush mango seed samples from which *A. flavus* was isolated (Adebayo-Tayo et al., 2006). The concentration of aflatoxins B1 and G1 which were detected in the bush mango samples ranged between 0.2 and 4.0 µg kg^{-1}, and 0.30 and 4.20 µg kg^{-1}, respectively.

HARMFUL EFFECTS OF AFLATOXINS

People in most developing countries are ignorant about the harmful effects of aflatoxins. This is supported by N'dede et al. (2012) report that the majority of the respondents in Benin did not have any information on aflatoxin contamination of peanut and its harmful health effects on human and animals. Awuah et al. (2008) also reported that the menace caused by aflatoxins was not well appreciated by Ghanaians because it has never been considered as a serious enough issue to merit an awareness campaign. Over four billion people in developing countries are repeatedly exposed to aflatoxins, contributing to greater than 40% of the disease burden in these countries (Schmalle III and Munkvold, 2015). The impact of aflatoxin on health has been supported by experiments in China and African countries which have a high incidence of the hepatitis B infection where dietary exposure to aflatoxin was prevalent. Exposure to aflatoxin is widespread in West Africa, probably starting in utero, and blood tests have shown that very high percentage of West Africans are exposed to aflatoxins. In a study carried out in the Gambia, Guinea Conakry, Nigeria and Senegal, over 98% of subjects tested positive to aflatoxin markers (Wild, 1996).

Serious fatalities can result from the consumption of contaminated produce due to the toxic nature of aflatoxins. For instance, a serious fatality occurred in India in 1974 when nearly 1000 people fell ill while over 100 died following the consumption of contaminated maize (Moss, 2002). The health conditions caused by aflatoxins are varied and depend on the level and length of exposure. Some of the health problems posed by aflatoxin contamination include aflatoxicosis, cancer, infertility, hepatocellular carcinoma, liver cirrhosis, nephropathy, immunodeficiency, anaemia, stunting, underweight in humans and nutritional interference (William et al., 2004; Darwish et. al., 2014).

Among the health problems caused by aflatoxin contamination, aflatoxicosis is one of the most serious ones. This is confirmed by Bommakanti and Waliyar (2012) report that the condition has been experienced in many countries including China, India, Thailand and in several African countries, which are all in the developing world. In Kenya, aflatoxicosis accounted for the death of 125 out of several hundred people who became severely ill from the consumption of food contaminated with aflatoxins (Lewis et al., 2005; Strosnider et al., 2006). The Malaysian state of Perak experienced an outbreak of aflatoxicosis in 1988 which killed 13 children who consumed noodles contaminated with up to 3 mg of aflatoxin (Mohd-Redzwan et al., 2013).

Cancer is another serious condition resulting from the consumption of food contaminated with aflatoxins. Among aflatoxins B1, B2, G1, and G2 which have been listed as group I carcinogens and are said to be the cause of hepatotoxicity in developing countries (CRA, 2011), aflatoxin B1 is the most potent and commonly occurring and has also been recognized as a teratogen, mutagen, hepatocarcinogen, immunosupressant and a potent inhibitor of protein synthesis. According to Augusto (2004), aflatoxin M1, just as toxic as aflatoxin B1 is listed as a Group 2B carcinogen by the International Agency for Research on Cancer. Groopman et al. (1988), also reported that epidemiological, clinical and experimental studies have revealed that exposure to large doses (>6000 mg) may cause acute toxicity with lethal effect, whereas exposure to small doses for prolonged periods is carcinogenic.

Apart from humans, aflatoxins are highly toxic to livestock and poultry (Cassel et al., 2012). Consumption of low concentrations by animals sensitive to aflatoxins can lead to death in 72 h and at nonlethal levels, the health and productivity of animals fed contaminated feed are seriously impaired (Cassel et al., 2012). In 1966, the first outbreak of aflatoxicosis in India occurred in the Mysore state resulting in the death of 2219 chicks. A worse incident occurred in the Chittoor district of Andhra Pradesh in 1982 resulting in heavy mortality in chicks with one hundred percent mortality in commercial farms.

FACTORS THAT PREDISPOSE CROPS TO AFLATOXIN CONTAMINATION

Field and postharvest practices can predispose crop produce to aflatoxin contamination. The risk of contamination is greater in developing countries where peasant farmers who constitute the majority face financial challenges and have little or no access to improved technology. The factors that influence mycotoxin production are either biological (biotic), environmental (abiotic) or nutritional (Diener and Davis, 1966; Okello et al., 2010). Some of the biotic factors include cultivar susceptibility and growth stage, insect and bird damage and presence of other fungi or microbes and strain variation in the fungus while abiotic factors include mechanical damage, moisture, temperature, pH and other crop stresses such as drought, soil type, suitability of substrate, excessive rainfall, gaseous exchange and gaseous environment and preservatives and crowding of plants (CAST, 1989; CRA, 2011; Suttajit, 1989; Robens, 1990; William et al., 2004). Nitrogen stress is another biotic factor which can also predispose crops to aflatoxin contamination. Most of the factors enumerated above are beyond the control of farmers in developing countries. For instance, unpredictable rainfall which is worsened by climate change makes crops grown in developing countries more prone to water stress and therefore a higher risk of aflatoxin contamination. Also, due to lack of access to improved technology, farmers in developing countries cannot test soils to determine their physicochemical characteristics before cropping.

ECONOMIC LOSSES

Developing countries suffer most from impact of enforcement of regulation by European and international agencies, particularly the former which is a major importer of agricultural commodities from developing countries. The economic losses to developing countries are varied. The losses do not only arise from crop and livestock losses but also from costs associated with regulatory compliances (CRA, 2011). For instance, Bankole and Adebanjo (2003) reported that as a result of regulation, exports of agricultural products particularly groundnuts from developing countries had dropped considerably resulting in major economic losses to producing countries. Losses from rejected shipments and lower prices for inferior quality can devastate developing country export markets (Bhat and Vasanthi, 2003). In 2011, Argentina, China, India and South Africa experienced 37, 60, 136 and 12 rejections, respectively (Codex Alimentarius Commission, 2014). The World Bank predicted that, policy change by the EU will reduce by 64% imports of cereals, dried fruits and nuts from African countries like Chad, Egypt, Gambia, Mali, Nigeria, Senegal, South Africa, Sudan and Zimbabbwe, and thus cost African countries about US$670 million in trade per year (Bankole and Adebanjo, 2003).

Wu et al. (2011) reported that, the magnitude of the economic impacts of the health consequences associated with consumption of aflatoxin-contaminated food in developing countries is not known due to a lack of good data. According to them, the quantification of economic losses and estimation of the effects of aflatoxin on health will encourage Health Ministries to enforce standards and provide crucial advocacy to benefit the rural poor, such as improving their level of education about aflatoxin exposure. The toll of the effects on human health includes the cost of mortality, the cost of productive capacity lost when people die prematurely, the cost of morbidity, losses resulting from hospitalization and the cost of health care services, both public and private. There is intangible cost of pain, suffering, anxiety and reduction of the quality of life (Bhat and Vasanthi, 2003).

According to Otsuki et al. (2001), compliance requirements on exporters impose costs on developing countries, such as the cost of upgrading production systems, processing and storage equipment, and quality control stations. The FAO has also highlighted a number of compliance problems which include lack of funds allocated to research on aflatoxins, scarcity of highly trained and experienced personnel, inadequate facilities for safe aflatoxin research, lack of maintenance of laboratory facilities and inadequate infrastructure (FAO-WHO, 1997). Contamination of maize, a staple in developing countries reduces its economic value which can result in large monetary losses and lead to the removal of large amounts from the market as a result of stringent regulatory limits (Riley and Norred, 1999).

REGULATION AND TOLERANCE LIMITS

Due to the potential health hazards for humans, threshold levels of aflatoxins in commodities have been established worldwide (Augusto, 2004). In 2003, FAO reported that 15 countries were known to have specific mycotoxin regulations but by 2011, the number had risen to 99 with some having specific regulations for aflatoxin B1 or M1 in milk (CRA, 2011). For majority of the African countries, specific mycotoxin regulations seem to be lacking but several of these countries recognize that they have problems due to mycotoxins and that regulations should be developed (FAO, 2003). The maximum limits for aflatoxins in foodstuffs are the single most commonly established mycotoxins limits worldwide. The limits for aflatoxins may be controlled as the total aflatoxins referring to the sum of aflatoxin B1, B2, G1, G2 and or aflatoxin B1 (Kubo, 2012).

The limits or standards set by various national and international agencies are varied as shown by the ensuing examples, some of which consider only total aflatoxins while others consider both total and aflatoxin B1. The European Union aflatoxin tolerance standards are 2 μg kg^{-1} aflatoxin B1 and 4 μg kg^{-1} total aflatoxins (B1, B2, G1 and G2) for peanuts, nuts, dried fruits and cereals for direct human consumption (Augusto, 2004). It appears European standards are more stringent than those of the United States which has an action level of 20 μg kg^{-1} for human food except milk (FAO, 1996; Schmalle III and Munkvold, 2015). In Europe, the maximum levels of aflatoxin M1 in milk meant for adult consumption and milk meant for infants or baby food production are 0.050 and 0.025 μg kg^{-1}, respectively (Iqbad et al., 2014) while that for the United States is 0.05 μg kg^{-1} (Augusto, 2004).

Food production systems in developing countries do not favour the implementation of international regulations such as those set by Codex Alimentarius Commission to regulate the amounts of aflatoxin in food (William et al., 2004). As a result, there is a higher risk of exposure in developing countries because where there is trade, the least contaminated foods and feeds are exported and the more highly contaminated products are retained at home for consumption. It is therefore not surprising that African countries are greatly concerned about the standards imposed on their exports. This situation was aggravated by the use of different regulations by various developed countries, but these concerns were partly addressed through the harmonization of aflatoxin standards by the European Union, which eventually took effect in April 2002 (EU Commission Regulation No. 466/2001, 2001). As part of the harmonisation process, in 1997, the maximum acceptable limit of 10 μg kg^{-1} for groundnuts subject to further processing and 4 μg kg^{-1} in groundnuts intended for direct consumption were amended in 1998 to 15 μg kg^{-1} (8 μg kg^{-1} for aflatoxin B1) for the groundnut subject to further processing and 4 μg kg^{-1} (2 μg kg^{-1} for aflatoxin B1) for groundnuts intended for direct consumption (EU Commission Regulation No. 1525/98, 1998a). Although, the harmonization of aflatoxin standards in EU member countries seemed to have alleviated the situation, developing countries faced greater challenges because the new EU standards were more stringent than those set by Codex Alimentarius Commission, the United States and other countries such as Australia. For instance, while EU standards for total aflatoxins in groundnuts for processing and consumption are 15 and 4 μg kg^{-1}, respectively, those set by Australia are 15 and 5 μg kg^{-1}. The USA has an even lower standard of 20 μg kg^{-1}. The Codex Alimentarius standard is more considerate because it has no separate standard for aflatoxin B1 based on the assumption that 70% or about 10 μg kg^{-1} of the total aflatoxin level of 15 μg kg^{-1} is accounted for by aflatoxin B1. Fortunately, the EU regulation on aflatoxins agrees with existing Codex Alimentarius maximum aflatoxin level, but the EU standards cover more products and have separate maximum levels for aflatoxin B1 (Anonymous, 2010).

PREVENTION AND MANAGEMENT OF AFLATOXIN CONTAMINATION

According to Suttajit (1989), prevention of aflatoxin contamination can be primary, secondary or tertiary. The primary prevention is considered as the most important and most effective for reducing fungal growth and mycotoxin production. Some of the primary prevention practices include development of plant varieties resistant to fungi, lowering moisture content of seed after harvest and during storage, storing commodities at low temperature, application of fungicides and preservatives and control of insect infestation in stored bulk grains with approved insecticides. Secondary prevention includes re-drying of products, removal of contaminated seeds, inactivation or detoxification. Tertiary prevention involves complete destruction of contaminated products and detoxification or destruction of mycotoxins to the minimum level. Some methods of preventing aflatoxin contamination include education and extension, rapid drying, physical separation, smoking, use of plant products, biological control, detoxification, seminars and workshops, adoption of good agronomic practices, early harvesting, sanitation, use of improved storage structures, synthetic chemicals, resistant varieties and fumigation (Bankole and Adebanjo, 2003). Bhat and Vasanthi (2003) proposed good agricultural practices such as rotating crops, irrigating to eliminate drought stress, controlling weeds, cultivating mould-resistant stocks and introducing biocontrols such as non-mycotoxigenic fungal strains. They also suggested that drying rapidly by mechanical means and keeping crops dry, sorting out contaminated nuts by physical means; sorting by color, and washing with water, the use of chemical methods of detoxification such as ammoniation, application of chemicals like oltipraz and chlorophyllin, physical sorting of contaminated grains or nuts and change of diets by individuals to avoid risky foods such as maize could reduce exposure to aflatoxins. Cassel et al. (2012) recommended that aflatoxin contamination can also be prevented by keeping storage and feeding facilities clean. According to them, aflatoxin contaminated feed can be tolerated by some livestock particularly older animals but the risk becomes greater with increasing levels of contamination. They maintained that feed additives including organic acids like propionic, sorbic and benzoic acids and their salts such as calcium propionate, potassium sorbate and copper sulphate inhibit mould growth in feed. Minerals such as zeolite and bentonite as well as hydrated sodium calcium aluminosilicate (HSCAS) can protect animals by binding to any aflatoxin that may be present in feed. The Codex Alimentarius Commission (2014) also recommended the implementation of good agricultural practices (GAP) and good manufacturing practices (GMP) by producers.

Control of mycotoxin in Africa is a matter of importance not only for health implications, but also for improvement of the economy in the affected countries. According to Darwish et al. (2014), a number of strategies for reduction and control of mycotoxins have been considered in different African countries. These include prevention of mould growth in crops and other feedstuffs, decontamination of mycotoxin-contaminated foods and continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food. Other control measures that have been tried in some African countries include segregation of contaminated peanuts in Malawi, detoxification of peanut meal for export in Senegal, regulation of mycotoxins in animal feed according to the susceptibility of the animal species in Zimbabwe, selection of peanut varieties less susceptible to aflatoxin contamination in Bourkina Faso and improvement in produce-handling practices during the 1960s in Nigeria and the 1990s in The Gambia (Bhat and Vasanthi, 2003). According to Cassel et al. (2012), time of harvest is important in influencing the occurrence and levels of aflatoxin. For instance, harvesting maize above 20% moisture content followed by rapid drying to at least 14% within 24 to 48 h of harvest checks the growth of *Aspergillus* spp. and minimizes aflatoxin production. Chulze (2010) reported that it is possible to control aflaoxins in stored commodities by controlled atmospheres, preservatives or natural inhibitors; the use of antioxidants and essential oils is possible but the cost can be prohibitive on a large scale.

In recent times, there have been initiatives aimed at controlling aflatoxins in developing countries, especially Africa. One of such initiative is the Partnership for Aflatoxin Control in Africa (PACA), which is based on a Memorandum of Understanding that was signed between the African Union Commission and Mars Incorporated, aimed at sharing food safety resources and expertise to control aflatoxins in food crops which constitutes a significant threat and a major deterrent to use of key African raw materials in global supply chains (African Union Commission, 2015). Another initiative is the aflatoxin control in maize and peanuts project, which is aimed at developing and implementing holistic strategies to address aflatoxin contamination in maize and peanuts including developing and scaling up biological control technology interventions to improve the health and income of farmers and their families and generate wealth in the crop value chain (African Agricultural Technology Foundation, 2015). The project is funded by Bill and Melinda Gates Foundation and African Agricultural Technology Foundation (AATF) through the IITA and UK aid from the UK government, respectively.

CONCLUSION

It is obvious that impoverished and less privileged people of developing countries stand an even greater risk of further impoverishment and starvation, if stringent measures are not applied for the management of aflatoxin contamination. Implementation of recommended prevention and control strategies could make food more expensive and less affordable, since farmers will have to invest in drying and storage equipment among others. Their plight is worsened by the absence of laboratories for testing foods which are economically and financially inaccessible. However, it will be better to ensure that contamination levels are minimal to safeguard the health of people in developing countries whose lifespan is relatively short. The plight of people in developing countries is worsened by the fact that international bodies like the World Health Organisation (WHO) do not consider aflatoxin as a high-priority risk; hence, little attention is paid to the health issues resulting from the consumption of contaminated food.

Developed countries and international agencies such as the FAO and WHO should provide the necessary financial and technical assistance to enable developing countries to carry out research and education. This would ultimately inure to the benefit of developing countries in terms of increased foreign exchange earnings, from the sale of products that meet required standards and better health through the consumption of safer food, devoid of or containing minimal levels of aflatoxins.

Conflict of interests

The author has not declared any conflicts of interests.

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