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An effective self-control strategy for the reduction of aflatoxin M1 content in milk and to decrease the exposure of consumers

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Abstract

The study reports on the results of testing the sensitivity of an early warning sampling plan in detecting milk batches with high AFM1 concentration. The effectiveness of the method was investigated by the analysis of 9017 milk samples collected in Italian milk processing plants that applied control plans with different action limits (AL). In the case of milk processing plants, where 30 ng kg^{-1} AL has been applied, the AFM_1 contamination was significantly lower at or above the 95th percentile of the milk samples compared to plants that used 40 ng kg^{-1} AL. The results show that the control plan can be used effectively for early warning of occurrence of high AFM_1 contamination of milk and to carry out pro-active measures to limit the level of contamination. Estimation of dietary exposure was also carried out, based on the aflatoxin M_1 content of the milk samples and on Italian food consumption data. Estimated Daily Intakes (EDI) and Hazard Indices (HI) were calculated for different age groups of the population. HIs show that in case of adult population no adverse effects are expected, but in case of children under age three, the approximate HI values were considerably higher, which underlines the importance of the careful monitoring and control of aflatoxin M_1 in milk and dairy products.

Keywords: Aflatoxins; Milk; Statistical analysis; Exposure assessment

Introduction

Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus* under favourable growing and storage conditions (WHO 1997; Giorni et al. 2007). They may contaminate feed, especially in tropical and subtropical climatic environment, but they have also been detected in maize grown in other areas such as Italy (Prandini et al. 2009) and Spain (Cano-Sancho et al. 2013). According to the 2013 RASFF Annual Report, most of the notifications related to mycotoxins in feed (21 out of 37) concerned maize products, mostly from south-eastern Europe (EC 2014; RASFF 2013 annual report). As an impact of climate change, the infected areas may further increase (EFSA 2012a). Aflatoxin M_1 and M_2, the metabolites of aflatoxins B_1 and B_2 can be found in milk of animals fed with feed contaminated with aflatoxins, and AFM_1 contamination in milk was reported from several European countries (EFSA 2004; Cano-Sancho et al. 2013; Duarte et al. 2013; Tsakiris et al. 2013; Trevisani et al. 2014). According to Prandini and his co-workers, if contaminated feed has been used, AFM_1 appears in the milk after two or three days, and the depuration interval is about the same after the animals are again fed with AFB_1-free feed. Once milk becomes contaminated with AFM_1 neither pasteurization nor sterilization makes a significant change in the concentration of AFM_1 in it. Moreover, since AFM_1 associates with the casein fraction of milk, processes like cheese-making carries over AFM_1 to the final products (Prandini et al. 2009). Considering these facts and also that there is no procedure at the moment for complete elimination of AFM_1 from milk (Ismail et al. 2015), preventing or limiting contamination of milk with aflatoxins should have priority among the reduction strategies.

Milk is a basic food item for all age groups and gender of the human population, especially for children, due to its nutritional value. Milk contains high-quality protein, which includes all essential amino acids and has high antioxidant capacity - especially its casein fraction (Zeluta et al. 2009). Milk contributes significantly to the intake of calcium, magnesium, selenium, riboflavin, vitamin B_{12} and pantothenic acid. Milk lipids serve as a carrier for fat-soluble vitamins, and the complex fat content of milk contains around 400 types of fatty acids (Weaver et al. 2013). According to EFSA's Comprehensive Food Consumption Database, the mean daily consumption of cow milk is in the range of 42.3 to 292.9 g/day among adults in Europe. The large portion size represented by the 95th percentile average consumption is 592 g/day for toddlers and 483 g/day for the adult population.

Aflatoxins are carcinogenic, mutagenic and genotoxic substances. AFB_1 becomes activated during the metabolism and its epoxide form binds strongly to the DNA, causing damages in the helix. The metabolites of AFB_1 - such as AFM_1 - are poorer substrates for epoxidation and therefore, are less toxic (Wild & Turner 2002). Although the carcinogenicity of AFM_1 is considered 10 times lower than that of AFB_1. It has been classified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (WHO and IARC, 1993), therefore their level in milk should be kept as low as reasonably achievable (WHO 1997; EFSA 2007). In order to reach this goal, the European Commission introduced the principle of establishment of early warning system and taking pro-active measures for reducing the contamination (Directive 2002/32/EC of the EP and the Council). The maximum level for AFM_1 in raw milk, heat-treated milk and milk for the manufacture of milk-based products set by EC No 1881/2006 is 50 ng kg^{-1}. Consequently, the Italian Ministry of Health defined an 'attention limit' of 40 ng kg^{-1} (Ministero della Salute 2013), which accommodates the uncertainty of analytical results.

Farkas and co-workers elaborated an early warning sampling plan, based on random sampling and analysis of commingled bulk milk collected by the transport tankers typically from 1-6 dairy farms (Farkas et al. 2014). If the AFM_1 concentration exceeded the action limits ranging from 16.7 to 40 ng kg^{-1} - depending on the number of dairy farms -, the milk samples taken from individual farms were consequently analysed and the necessary pro-active actions were recommended to reduce the chance of mixing the contaminated milk with those complying with the legal limit of 50 ng kg^{-1}. The random sampling plan elaborated is applicable only if the number of milk collecting zones remains constant during a given period of time.

The objective of the present study was to develop and test a generally applicable control plan, which can be used effectively for the early warning of occurrence of AFM_1 contamination in milk and making timely pro-active measures to limit the level of contamination. Additionally, the study aimed to use the results of the great number of analyses carried out to estimate the level of exposure of different population groups by the consumption of cow milk.

Materials and methods

AFM_1 data collection

Altogether 9017 milk samples were collected and analysed for AFM_1 between April 2013 and May 2014. 3303 samples were taken during the implementation of a self-control plan of a milk processing plant (called Plant A hereunder) located in Southern Italy, which collects about 40 million litres of milk per year. The milk was collected from 85 farms by eight tankers. In addition, 5714 results of AFM_1 analyses carried out by four dairy plants (indicated as NP hereunder), in the Northern, Central and Southern regions were also collected and used for assessing the efficiency of the control plan developed within this study. Two types of milk were collected separately in both cases: high quality milk (HQM) and normal quality milk (NQM), which are differentiated by Italian law, on the basis of their content in fat, protein, somatic cell count, and total bacterial count. The collection route, including 1-15 farms for one tanker was optimized depending on the production and accessibility of the farms. At each occasion, the quantity and type (HQM or NQM) of milk loaded from a farm were recorded, and a sample of milk was collected and labelled for later investigations if needed.

Analysis of samples for AFM_1

AFM_1 concentration was measured using the ELISA Immunoscreen AFM_1 kit (Tecnas.r.l., Trieste, Italy), which was validated within the range of 5-100 ng kg^{-1} (Rosi et al. 2007). An HPLC method was used for quantitative confirmation of values above 50 ng kg^{-1} (legal limit) (Dragacci & Grosso 2001).

Pro-active measures for reducing AFM_1 contamination of commingled milk

In order to avoid the contamination of huge milk quantities with aflatoxin M_1, dairy plants applied a self-control plan for the monitoring of the AFM_1 content of the consignments. Prior to unloading, samples had been taken from the compartments of each truck, in which milks of different qualities were collected. Depending on the detected AFM_1 levels, the following actions had been carried out:

i) AFM_1 content of a consignment ≤ 30 ng kg^{-1}: milk was unloaded and processed without further actions.

ii) AFM_1 content of a consignment > 30 ng kg^{-1} but ≤ 40 ng kg^{-1}: the milk was unloaded and processed, but each of the samples taken at the dairy farms when the truck had been loaded was also analysed separately.

   a. If the sample collected at a farm contained AFM_1 above 40 ng kg^{-1} but less than 50 ng kg^{-1}, the veterinarian visited the farm on the same day and corrective action was started by changing the feed ration of the cows;

   b. If the sample of a farm contained AFM_1 above 50 ng kg^{-1} (legal limit), the collection of milk from the farm was suspended. Moreover, not only the corrective action had been applied in these cases, but samples were also taken and analysed every three days until the AFM_1 concentration declined below 40 ng kg^{-1}.

iii) AFM_1 content of a consignment > 40 ng kg^{-1} but ≤ 50 ng kg^{-1}, the milk was processed, and the respective authority was notified according to the regulation of the Ministry of Health. Corrective actions at farms providing milk with AFM_1 content above 40 or 50 ng kg^{-1} were applied as reported under points ii)a. and ii)b.

iv) AFM_1 content of a consignment > 50 ng kg^{-1}: the same actions took place as reported under point iii). In addition, the milk consignment was discarded.

In case of NP, points iii) and iv) of the above described action plan were applied.

Statistical evaluation of the distribution of AFM_1 concentrations

The P percentiles of the AFM_1 concentrations were calculated by the NIST procedure (NIST/SEMATECH 2003). The rank numbers of their standard deviations were calculated based on normal approximation (Diem & Seldrup 1982):

$$s = \sqrt{N \times P \times q} \qquad \text{Equation (1)}$$

where N is the number of AFM_1 concentration values in the dataset, P is the selected percentile and q = 1-P.

The 95% confidence intervals are calculated as:

$$Rp_{lower}^{upper} = R_p \pm 1.96s \qquad \text{Equation (2)}$$

where R_p is the rank number of selected percentile.

The AFM_1 concentrations corresponding to the calculated rank numbers of the ordered dataset encompass the 95% concentration range of the AFM_1 concentration corresponding to the R_p.
The confidence intervals for the mean AFM_1 concentration values were calculated as:

$$CI_{0.95} = \bar{C}_{AFM1} \pm 1.96 \times \frac{SD}{\sqrt{N}} \qquad \text{Equation (3)}$$

where SD is the standard deviation of AFM_1 concentrations in N samples and $\bar{C}_{AFM1}$ is the average concentration. No correction was needed for the calculation of SD due to the large number of concentration values.

Dietary risk assessment and risk characterization

Food consumption data were obtained from the EFSA Comprehensive European Food Consumption Database. The original data were reported by Italy based on the results of the Italian National Food Consumption Survey (INFCS) conducted from October 2005 to December 2006. It involved 3322 consumers from 1329 households located in the four main geographical areas of Italy (North-West, North-East, Centre and South and Islands) (Leclercq et al. 2009; EFSA Comprehensive Database).
The average with standard deviation, median, 95^{th} and 99^{th} percentile consumption data of cow milk were reported for each age class: infants (≤ 11 months); toddlers (from 12 to 35 months); children (36 months to 9 years); adolescents (10-17 years); adults (18-64 years); elderly (65-74 years); very elderly (75 years and older). The consumption data of dairy products and milk based beverages were not included in the exposure assessment.
Left censored data (AFM_1 concentrations < LOD) were calculated with 0.5 LOD as recommended for cases where the non-quantified results were < 60% (EFSA 2010).
As 80^{th} percentile milk consumption values were not available, therefore the 80^{th} percentile AFM_1 concentration was selected as the basis for worst-case scenario calculation as a conservative estimate of long-term intake. For the EDI calculation, the weighted mean AFM_1 concentrations unloaded from each tanker and the average consumption were used. The weighted monthly mean AFM_1 values for each month were computed by multiplying the quantities of milk and their AFM_1 concentrations (including left censored data) in the sampled tanker and the sum of these values were divided by the sum of total milk quantities of the given month. For the conservative intake estimation, the same method was used, but the weighted 80^{th} percentile AFM_1 concentrations were inserted into the equation instead of the weighted means.
The estimated daily intakes (EDI: ng kg^{-1} bw day^{-1}) for the population groups were calculated with a deterministic method:

$$EDI = \frac{\sum C_{AFM_1} * AMC}{bw * 365} \qquad \text{Equation (4)}$$

where C_{AFM_1} is the aflatoxin M_1 concentration (ng kg^{-1}), *AMC* is the average milk consumption (kg/year) and *bw* is the average body weight (kg) of the given population group.

In view of the facts that aflatoxins are genotoxic and carcinogenic, it is not possible to identify an intake without risk (EFSA, 2004). Kuiper-Goodmann (1990) proposed to derive a safe dose of 0.2 ng kg^{-1} for AFM_1 from the dose causing 50% of the animals developing tumour (TD_{50}) divided by a safety factor of 50 000. The Hazard Index (HI) was calculated by dividing the estimated daily intake with 0.2. The same approach was also used in other studies (Shundo et al. 2009, Duarte et al. 2013; Tsakiris et al. 2013;).

Following the EFSA advice, to carry out risk assessment of substances, which are both genotoxic, and carcinogenic, the Margin of Exposure (MoE) was calculated as follows:

$$\text{MoE} = \frac{BMDL_{10}}{EDI} \quad \text{Equation (5)}$$

where $BMDL_{10}$ is the benchmark dose for a 10% increase in Hepatocellular carcinoma (HCC) incidence compared to the control group. In the case of AFB_1 (used as a conservative value) $BMDL_{10}$ is 870 ng kg^{-1} bw day^{-1} (EFSA, 2007), which was divided by the mean and 80^{th} percentile EDI values for each age category of consumers. The calculation was carried out for each month from April 2013 to May 2014.

Risk Potency (RP) was calculated assuming that 2% of the population is HBV^{+} (considering that the prevalence of carriers of hepatitis B (HBV) in the Italian population is between 1.2% and 2%). Because the carcinogenic potency of aflatoxin is higher in carriers of hepatitis B virus surface antigen than in individuals not being carriers, the potency values suggested by JECFA: 0.3 and 0.01 in HBV^{+} and HBV^{-} population respectively (JECFA 2001) were used as follows (Cano-Sancho et al. 2013):

$$RP = (0.01 \times 0.98) + (0.3 \times 0.02) = 0.016\ HCC\ per\ year\ per\ 100{,}000\ person$$

Equation (6)

The Risk Potency value was then used to calculate the fraction of incidence of liver cancer (LCI) in the Italian population attributable to the intake of milk contaminated with AFM_1 in the following way:

$$LCI = \frac{RP \times BMDL_{10}}{MoE} = \frac{0.016 \times 870}{\frac{870}{EDI}} = 0.016 \times EDI \quad \text{Equation (7)}$$

The fraction of incidence of HCC attributable to intake of AFM_1 was calculated on the basis of MoE considering the mean and the 80^{th} percentile of exposure estimation (MoE_{mean} and $MoE_{P0.8}$).

Results and discussion

Distribution of AFM_1 concentrations and comparison of milk contaminations in plants A and NP

Distributions of AFM_1 concentrations in commingled milk discharged by the tankers are illustrated in Table 1. In Plant A, where the more stringent control plan was applied, even the highest values were below the legal limit, unlike in the case of NP, where some values were twice or three times higher.

The selected percentile values of AFM_1 concentrations with their 95% confidence limits are given in Table 2. The results show that there was no significant difference between the AFM_1 concentration of high and normal quality milks, either in Plant A or in NP. The applied stricter sampling plan in Plant A mainly influenced the frequency of occurrence of high concentration values in the 95^{th}-99^{th} percentile range.

The number of samples taken from Plant A and from NP is presented in Table 3. The table shows the percentages of samples in each plant that resulted in 30 ng kg^{-1} AFM_1 concentration or above. The measured values are aggregated in the table in order to show the distribution of the high values in both plants. The table shows that in 2013 as well as in 2014 less samples resulted in AFM_1 concentration values at or above 30 ng kg^{-1} in the case of Plant A, where the action limit was set at 30 ng kg^{-1} compared to NP, which applied the higher (40 ng kg^{-1}) action limit. In the NP 31 milk samples (0.54%) resulted in AFM_1 concentration values at or above the legal limit (50 ng kg^{-1}), but in Plant A, none of the samples resulted in AFM_1 concentration above 50 ng kg^{-1} during the examined period.

Figure 2 shows the monthly distribution of AFM_1 contamination of the milk sampled in plants A and NP. Figure 1 as well as Tables 2 and 3 demonstrate the advantage of the action plan used by Plant A, where the stricter (30 ng kg^{-1}) action limit was applied compared to 40 ng kg^{-1} used in NP. Between the 50^{th} and 90^{th} percentile, there was no significant difference between the AFM_1 concentrations of milk processed in the different plants. On the contrary, the AFM_1 contamination in NP was higher at or above 95^{th} percentile of the milk samples (Table 2). This indicates that the difference observed above the 90^{th} percentile is attributable to the different control plans applied.

As it was demonstrated by Trevisani et al. (2014), the AFM_1 contamination in milk has a substantial seasonal variation due to the change of the AFB_1 contamination of feed. The action limit of 30 ng kg^{-1} enables the early detection of the beginning of the increase of the AFM_1 contamination of milk. Table 4 gives some examples of the results of the control plans applied in Plant A. The milk collected from various farms by the tanker was sampled at the time of discharge at the processing plants and the samples taken at individual farms were analysed for AFM_1, if the discharged commingled milk contained AFM_1 above 30 ng kg^{-1}. The example shows that in case the aflatoxin levels of feed are not controlled properly, it is very important to screen the individual batches of milk at farm level. This way it is possible to prevent the contamination of milk with high levels of AFM_1, which could otherwise result in the disposal of entire commingled milk causing great economic losses for the producers.

According to one of the latest reviews on this subject (Flores-Flores et al. 2015), studies conducted in Italy and in other countries show the occurrence of AFM_1 levels in milk within the same order of magnitude as it was during our study period. Nonetheless the results cannot be directly used as a basis for the evaluation of the control plan, because the aflatoxin concentrations vary within the year and depend on the geographical location and climatic conditions.

The AFM_1 concentration values of milk samples show the effectiveness of the applied self-control plans. The continuous monitoring of consignments makes possible the early detection of elevated AFM_1 levels in milk and the localization of farms, where cows are being fed by contaminated feed. The results show that the 30 ng kg^{-1} action limit applied in the self-control plans is effective in keeping the AFM_1 concentration below the legal limit.

Risk characterization and exposure assessment

Because of the carcinogenic potential of aflatoxins, JECFA (2001) did not specify a numerical tolerable daily intake (TDI) for aflatoxins and concluded that daily exposure, even <1 ng kg^{-1} bw, contributed to the risk of liver cancer.

Figure 2 shows the seasonal variation of estimated daily intakes (EDI) for NQM during the study period (from April 2013 till May 2014) in the populations of adults and toddlers. The aflatoxin concentrations of the two different milk qualities were similar, therefore the HQM values are not shown in the figures. Two different EDI values were calculated using equation (4) in the case of both milk qualities. EDI_{mean} values were computed by multiplying the weighted mean AFM_1 concentration of the given month. The $EDI_{P0.8}$ values were calculated in a similar way but using the 80^{th} percentile of AFM_1 concentration instead of the weighted mean. For the adult population, EDI values were calculated with a mean body weight of 70 kg, and mean milk consumption of 0.102 kg day^{-1}. For infants, a mean body weight of 5 kg and the mean milk consumption of 0.086 kg day^{-1}; for toddlers, a mean body weight of 12 kg and the mean milk consumption of 0.246 kg day^{-1} were used (EFSA Comprehensive Database, EFSA 2012b).

Figure 2. clearly shows that the estimated intakes (ng kg^{-1} bw day^{-1}) were significantly higher in the case of toddlers.

The seasonal variations of EDI values are in line with the variation of aflatoxin levels in feed reported by other studies (Farkas et al. 2014; Bilandžić et al. 2015).

Figure 3 shows the average EDI values of the different population groups in case of normal quality milk consumption. The lowest values were observed in the group of adults, while the highest values occurred in the groups of infants and toddlers. The intakes of “high consumers” were approximated by the calculation of $EDI_{P0.8}$ values. According to the results, the estimated daily intake of large portion size consumers is about 40-50% higher than the average consumers.

The Hazard Indices (HI) were calculated by dividing the estimated daily intake values with 0.2. In the case of adult and elderly population (Figures 4 and 5), the HIs were less than 0.5, which means a level at which no adverse effects are expected. The average HI values of all age groups were below one, except for infants and toddlers, where the HIs were between 1 and 2.5 in most months of the studied period indicating that the youngest population groups might be exposed to AFM_1 above the safe level. It is important to note that according to the Italian food consumption survey, only 25% of the infant population is being fed with cow milk, but among toddlers, the ratio of cow milk consumers is 91.7%.

Taking into account that the daily consumption values included not all kinds of milk and dairy products, only cow milk, the overall HI values of AFM_1 for this food category might be even higher.
The liver cancer incidence attributable to AFM_1 was calculated using the cancer potency of 0.016 hepatocellular carcinoma/year/100,000 persons. In the study period, the incidence estimated with the mean MoE for the adult population ranged between 0.0001 and 0.0006 cancer cases/year/100,000 persons in the case of HQM and from 0.0002 to 0.0007 cancer cases/year/100,000 persons for NQM. The hepatocellular carcinoma (HCC) incidence estimated with the mean MoE for the population of toddlers ranged between 0.0018 and 0.0067 cancer cases/year/100,000 persons calculated with the HQM results and from 0.0018 to 0.0079 in the case of NQM.

The cancer incidence estimated with the 80th percentile MoE for the adult population ranged between 0.0001 and 0.0006 cancer cases/year/100,000 persons in the case of HQM and from 0.0001 to 0.0007 cancer cases/year/100,000 persons in the case of NQM. The HCC incidence estimated with the 80th percentile MoE for the population of toddlers ranged between 0.0008 and 0.0089 cancer cases/year/100,000 persons in the case of HQM and from 0.0008 to 0.0105 cancer cases/year/100,000 persons in the case of NQM. The average liver cancer incidence (LCI) values with standard deviations are indicated in Figure 6 for each population groups. The average LCI values are the highest in the groups of infants and toddlers.

According to our results, the most exposed Italian population groups are the infants ($\leq$ 11 months) and toddlers (from 12 to 35 months), but especially toddlers, because, while only 25% of the infants consume cow milk, this ratio among toddlers is over 90%.

In order to reduce the exposure of children, especially of the ones less than 3 years old, the most sensitive population group - specifically selected milk batches should be used for producing milk and milk products for young children. For each population group, an average EDI less than 0.2 ng kg^{-1} bw day^{-1} is necessary to reach in order to get a HI below 1, a level at which no adverse effects are expected. Considering the average body weights and consumption levels in the Italian population groups, the AFM_1 concentration below 11.7 ng kg^{-1} for infants and a concentration below 9.7 ng kg^{-1} in the case of toddlers would result an EDI less than 0.2 ng kg^{-1} bw $day^{-1}$and consequently a HI below 1. Therefore, milk containing $AFM_1 \leq 10$ ng kg^{-1} should be used for producing milk and milk-based products specifically for young children. Considering the uncertainty of analytical measurements (~18%), it means that those commingled milk batches discharged from the tankers which contain $\leq$ 8 ng kg^{-1} AFM_1 should be stored and processed separately for the youngest population groups.

The results underline the importance of regular control of produced milk, applying appropriate action limit in combination with immediate corrective actions at farm level. Our study has shown that the applied monitoring plan is sensitive enough to reveal milk batches with high AFM_1 concentration and keep the AFM_1 concentration below 50 ng kg^{-1} in commingled milk for general use and selection of batches with low ($\leq$ 8 ng kg^{-1}) AFM1 contamination for preparing milk and milk products for infants and toddlers.

Abbreviations used

AFB_1	Aflatoxin B_1
AFM_1	Aflatoxin M_1
AMC	Average milk consumption
$BMDL_{10}$	Benchmark dose lower confidence limit for a 10% response
BW	Body weight
CL	Confidence level
$CI_{0.95}$	95% confidence intervals
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HQM	High quality milk
IARC	International Agency for Research on Cancer
INFCS	Italian National Food Consumption Survey
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LCI	Liver cancer incidence
LCL	Lower confidence limit
LOD	Limit of detection
MoE	Margin of Exposure
MoE_{mean}	Margin of Exposure considering the mean of exposure estimation
$MoE_{P0.8}$	Margin of Exposure considering the 80^{th} percentile of exposure estimation
NIST	National Institute of Standards and Technology
NP	Dairy plants in the Northern, Central and Southern regions of Italy
NQM	Normal quality milk
SD	Standard deviation
TD_{50}	Dose causing 50% of the animals developing tumour
TDI	Tolerable daily intake
UCL	Upper confidence limit
WHO	World Health Organization

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Figure Captions

Figure 1: AFM_1 contamination [ng kg^{-1}] found in milk samples from the collection tankers in Plants NP and A in a monthly distribution. Note: AFM_1 concentration values below 30 ng kg^{-1} and ranging from 60 to 150 ng kg^{-1} are not shown in the figure.

Figure 2: Monthly variation of the mean and 80^{th} percentile Estimated Daily Intake (EDI) values of the adult population (Adults) and the children between 1 and 3 years of age (Toddlers) from normal quality milk (NQM). Note: the months are indicated with their first character e.g. A13 corresponds to April 2013.

Figure 3: Average Estimated Daily Intake of AFM_1 attributable to normal quality milk (NQM) consumption in the different population groups of Italy.

Figure 4: Monthly variation of the Hazard Indices (HI) in case of consumption of normal quality milk (NQM) calculated from the AFM_1 contamination measured in Plant A and the mean and 80^{th} percentile consumption of the adult population and the group of toddlers.

Figure 5: Average Hazard Index values attributable to AFM_1 contamination normal quality milk (NQM) consumption in the different population groups of Italy.

Figure 6: Comparison of the average estimated liver cancer incidences (LCI) in the different population groups of Italy attributable to AFM_1 in case of average and high contamination of normal quality milk.

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Note: The authors declare no competing financial interest.