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ARTICLE

Assessment risk to children’s health due to consumption of cow’s milk in polluted areas in Puebla and Tlaxcala, Mexico

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ABSTRACT

This study aimed to determine the heavy metal content in cow’s milk produced in areas irrigated with waste water and to evaluate the health risk with daily consumption of milk for children. The sample consisted of four zones in which small farmers were selected and the milk of 160 cows in two seasons of the year. On average, the metals in the milk in decreasing order were 0.36; 0.046; 0.035; 0.029; 0.015; 0.012, and 0.002 mg kg\(^{-1}\) for Zn, Pb, As, Cu, Cr, Ni, and Cd, respectively. The Pb exceeded the limits allowed by Codex. The values shown in the hazard quotient for the As of more than 1 and HI were higher. On the other hand, the individual risk of cancer showed a descending order As > Cd > Cr > Pb, while the total risk indicated that the combined effect of metals put girls and boys at serious risk.

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KEYWORDS
Cancer risk; hazard index; heavy metals; polluted milk; risk health

Introduction

Cow’s milk is one of the foods of the greatest nutritional value (Soares et al. 2010) and it is recommended by Food and Agriculture Organization of the United Nations (FAO) and United Nations Educational, Scientific and Cultural Organization (UNESCO) as being indispensable in children’s diets. Its consumption per capita in Mexico is of 410 ml day\(^{-1}\) (FAO 2016), but this amount may vary according to its availability in the region, on customs, consumption habits and purchasing power, among other factors.

In certain regions, the use of waste water to produce grain and fodder to feed cows may convert it into a source of the diverse toxic compounds contained by the milk, among which are heavy metals, which are characterised by their bioaccumulation and cause public health problems for being cytotoxic, cancerous diseases, and mutagenic (Sundberg et al. 1999; Dorea & Donangelo 2006; Al-Othman et al. 2012; ATSDR 2013). The milk represents a greater risk to children due to its high degree of absorption, this population being especially vulnerable to the acute, subacute, and chronic effects of the ingestion of heavy metals (European Environment and Health Information System (ENHIS) 2007; Solis et al. 2009).

In Mexico, every year, 18,000 children are reported to be affected by cancer, two-thirds of whom do not receive early diagnosis (Gómez-Fraga et al. 2001; Sistema Nacional de Vigilancia Epidemiológica 2011). Very few studies have been made on this, and it is now of interest to determine the risks of cancer and non-cancerogenous diseases derived from the consumption of milk in regions exposed to this type of pollution. Therefore, this work aimed to determine the concentration of heavy metals in cow’s milk and to evaluate the health risk with respect to daily consumption of milk for children, in the sub-basin of the Alto Balsas in the states of Puebla and Tlaxcala, a region in which industrial waste water has been used for over 100 years to grow fodder.

Material and methods

Sampling zones

The region sampled forms part of the hydrological sub-basin of Alto Balsas and it is located in the Central-South region of the state of Tlaxcala and south-east of the state of Puebla, Mexico. The sub-basin belongs to the number 18 hydrological region, where the rivers Atoyac and Zahuapan are natural collectors of waste water of an industrial and urban origin, which are used at certain times of the year to produce cattle fodder. It is localised between the parallels lat 19°06’ and 19°40’ N
and the meridians long 97°58' and 98°31' W, comprising a surface of 2,031 km². Both rivers join up and flow into the Valsequillo or “Manuel Ávila Camacho” reservoir, which stores 405,000,000 m³ of water, with which is irrigated the zone of Tecamachalco, Puebla, which is situated at parallels lat 18°52'57" N and at lat 97°43'49" W. A mild, wet climate prevails with summer rains, and the main agricultural activities are corn and alfalfa crops associated with the farming of dairy cows.

Four areas of study they established are as follows: (Zone 1) Tepetitla de Lardizábal, (Zone 2) Nativitas, (Zone 3) Santa Isabel Tetlatlahuaca in the state of Tlaxcala, and (Zone 4) in Tecamachalco, Puebla. For each zone, four milk production units, similar in their production systems, were sampled.

**Handling of the sample**

One hundred sixty cows were sampled in two seasons; summer (July 2014) and spring (March 2015). The milk was collected in 50-ml Falcon tubes (Fisher scientific, Waltham, MA, USA) each, previously washed with 10% v/v HNO₃ and rinsed three times to remove the acid residues. The milk sample was performed in the morning at the beginning of the milking directly from the udder, transporting it in fridges to the laboratory, where it was frozen –65°C. The samples were then lyophilised in a 4.5-L LABCONCO Freezone (Kansas City, MO, USA).

Next, the milk was digested in a microwave oven (CEM MarsX, CEM Corporation, Mathews, NC, USA). For this, 0.5 g of lyophilised milk was weighed and 10 ml of HNO₃ high purity was added (65%, Suprapur, Merck, Darmstadt, Germany), placing it in the microwave at a power of 1600 W, 15 min on the ramp at a pressure of 800 psi and 200°C of temperature with a 15-min wait. When the digestion had finished, the samples were filtered on Whatman grade 42 paper (GE Healthcare, Little Chalfont, UK), diluted to 50 ml with deionised water, and refrigerated up to analysis.

**Determination of heavy metals**

Cd, Pb, Ni, Cu, Cr, Zn, and As were determined in the milk by inductively coupled plasma optical emission spectroscopy (ICP–OES Varian 730) which has a nebuliser Seaspray 143,164 Ezylok and a spray chamber Glass spantion (Agilent Technologies, Mulgrave, Australia). For the determination of arsenic (As) it was not used-forming hydrides. All the chemical products employed were of an analytical reagent grade. The solutions were prepared in 18.2 MΩ-cm⁻¹ deionised water.

The calibration standards for each metal were prepared using a multi-element standard XVI solution for ICP, composed of 21 elements in HNO₃ Suprapure 6%, with a density of 1.032 g cm⁻³ and 20°C of Merck KGaA, Darmstadt, Germany.

Levels of precision and accuracy were realised with five blanks and with ten repetitions. Quality control was performed using a control standard and control sample, which were used in every 20 samples analysed. Analytical recovery value is determined at 106% on average; the correlation coefficient (r²) was 0.9999. The wavelengths for Cd, Pb, Ni, Cu, Cr, Zn, and As were of 214.439; 220.353; 231.604; 327.395; 267.439; 213.857, and 188.98, respectively. The limits of detection and limits of quantification were calculated with 3 and 10 times the standard deviation of the blank divided by the slope of the analytical curve, respectively. This allows us to determine the minor elements and trace elements in the sample (Khan et al. 2014a).

**Risk determination**

With the analyte values obtained in the milk analysed on a dry basis, it was proceeded to evaluate the risk to health of boys and girls from 3 months to 18 years of age. An intake of 0.547 ± 0.3 kg of milk daily per child was considered; this value was obtained through randomly conducted household surveys of the four areas of interest, where they were asked whether they had children and how much milk they intake daily.

The daily consumption of metal considered to be chronic was calculated using the equation Chronic Daily Intake (CDI) = Cmetal Dmilk intake Baverage weight, which was used by Amin et al. (2013) and Bortey-Sam et al. (2015). In this equation, C is the metal concentration found in the milk (mg kg⁻¹), D is the amount of milk consumed (kg), and B is the body weight (kg), where weights were taken from the Research Institute on Growth and Development (2004).

With the values obtained, the following indicators determined the health risk triggered by heavy metals individually and collectively:

a. *Hazard quotient (HQ)*. HQ = CDI/RfD for which the following values were established for the reference oral doses (RfD); Cd (1.0 × 10⁻³), Cr (3.0 × 10⁻³), Cu (3.7 × 10⁻²), Ni (2.0 × 10⁻²), Pb (3.6 × 10⁻²), As (3.0 × 10⁻²), and Zn (3.0 × 10⁻¹) mg kg⁻¹ day⁻¹ (Huang et al. 2008; Kavcar et al. 2009; Khan et al. 2013; Ferreira-Baptista & De Miguel 2005; Shah et al. 2012; Integrated Risk Information System (IRIS 2015)).

b. *Hazard index (HI)*. The HI was obtained with the summation of HQ (HI = HQ_Cd + HQ_Pb + ... + HQ_n)
and with this, the potential risk of metals was determined collectively (Huang et al. 2008; Cao et al. 2010; Bermudez et al. 2011). An index presenting values of ≥1 (HI > 1) means that it is not safe for human health according to Environmental Protection Agency of the United State (USEPA 2005; Khan et al. 2008, 2013).

c. Cancer risk. The risk of cancer from the consumption of milk produced in the Alto Balsas was calculated using the following equation: Cancer Risk = CDI × SF, where CDI is the daily consumption and SF is the slope factor of the metals considered to be carcinogenic (Cd, Cr, As) and Pb as probably being carcinogenic (Chen et al. 2015; IARC (International Agency for Research of Cancer) 2015). The SF values for oral intake were 0.0085, 1.5, 0.5, and 15 mg kg$^{-1}$ day$^{-1}$ of Pb, As, Cr, and Cd, respectively (USEPA 2002) and the risk of total cancer, which is the sum of the risk of cancer from metal, is given as follows:

$$\text{Cancer risk}_{\text{total}} = \sum \text{cancer risk } \text{Cd} + \text{Pb} + \text{Cr} + \text{As}.$$

### Statistical analysis

The information obtained of each component was analysed under a completely randomised design with a factorial arrangement of 2×2×4, in which the factors include age groups, season of the year and the zones, by means of a general linear model, and for the means comparison, the Tukey’s test was used with the statistical package SAS version 9 (2002).

### Results

#### Metal concentration in milk

The concentration of metal in the milk (Table 1) was significantly different between the zones (p ≤ 0.001), except for As and Ni. The Cd had higher levels in zone 4, in zone 3 the Pb, Cr had the lowest levels, and the Zn showed higher value in zone 1. Regarding the season of the year (Figure 1), there were significant differences between the zones (p ≤ 0.001), where only the As showed values HQ> 1 in all cases of As, Cr, and Ni, it was higher in the spring season.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Girls</th>
<th>SD</th>
<th>Boys</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>$4.59 \times 10^{-2}$</td>
<td>$4.3 \times 10^{-2}$</td>
<td>$4.43 \times 10^{-2b}$</td>
<td>$4.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>Pb</td>
<td>$3.36 \times 10^{-2}$</td>
<td>$2.6 \times 10^{-2}$</td>
<td>$3.24 \times 10^{-2b}$</td>
<td>$2.4 \times 10^{-2}$</td>
</tr>
<tr>
<td>Ni</td>
<td>$1.54 \times 10^{-2}$</td>
<td>$1.1 \times 10^{-2}$</td>
<td>$1.49 \times 10^{-2b}$</td>
<td>$1.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>Cu</td>
<td>$2.04 \times 10^{-4}$</td>
<td>$1.5 \times 10^{-4}$</td>
<td>$1.96 \times 10^{-4b}$</td>
<td>$1.4 \times 10^{-4}$</td>
</tr>
<tr>
<td>Cr</td>
<td>$2.54 \times 10^{-4}$</td>
<td>$1.8 \times 10^{-4}$</td>
<td>$2.45 \times 10^{-4b}$</td>
<td>$1.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>Zn</td>
<td>$3.15 \times 10^{-2}$</td>
<td>$2.4 \times 10^{-2}$</td>
<td>$3.04 \times 10^{-2b}$</td>
<td>$2.2 \times 10^{-2}$</td>
</tr>
<tr>
<td>As</td>
<td>$3.05 \times 10^{-3a}$</td>
<td>$2.3 \times 10^{-3}$</td>
<td>$2.93 \times 10^{-3b}$</td>
<td>$2.1 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Superscript lower-case letters (a, b) represent significant differences p < 0.01 between zones. Values are represented as mean ± standard deviation.
zones, remaining in descending order: Zone 4 (3.36 ± 2.4); Zone 1 (3.27 ± 2.4); Zone 3 (2.84 ± 2.0); and Zone 2 (2.49 ± 1.83). When assessing the HQ according to the children’s ages, the As showed HQ> 1 in all ages, with values of 10.06 (± 1.30) during the first months of life descending to 1.04 (± 0.17) when arriving at 18 years.

**Hazard index**

This index expresses the cumulative value of the effects of different heavy metal mixtures, and according to the age of the children, the highest HI values are for the youngest children, where the values are in descending order from 11.10 (±1.40) to 1.11 (± 0.18), corresponding to the first 3 months of life and up to 18 years, respectively.

In the comparison between girls and boys, no difference was found ($p ≥ 0.001$), the HI value of the children averaged 3.13 (±0.082). The HI between the zones showed a significant difference ($p ≤ 0.001$), where zone 1 presents a greater possibility of danger, followed by zones 3, 4, and 2. However, the four zones obtained values of HI> 1; this shows that in all sampled areas, there is a possibility of non-cancerous diseases (Figure 2).

**Cancer risk**

The risk of contracting cancer from the consumption of milk produced in the Alto Balsas sub-basin, based on the individual effect of each of the four metals considered most dangerous by the IARC (International Agency for Research of Cancer) (2015), is illustrated in Figure 3.

Showing significant differences ($p ≤ 0.001$) between them, it gives rise to the following descending order of risk: As> Cd> Cr> Pb. As for age (Figure 3), it was shown that the risk values for each metal affect mainly at an early age, although it was observed that it could occur in children of all ages. When analysing cancer risk for each of the metals (Table 3) in terms of age group, there were no significant differences ($p ≥ 0.01$); however, the areas studied showed significant differences ($p ≤ 0.001$) with a permanent cancer risk, with Cd and Cr being higher in zones 1 and 3, while As and Pb were higher in Zone 1 (Table 3).

The analysis of the summation of cancer risk (total cancer) represented by the metals as a group did not show significant differences ($p ≥ 0.001$) between age groups. However, they had an average value of $2.2 \times 10^{-2}$ (±$5.8 \times 10^{-3}$) being the risk is high.

This same variable between the zones showed significant differences ($p ≤ 0.001$), where Zone 1 had the highest value (3.0 $\times 10^{-3}$ ± 1.8 $\times 10^{-3}$) followed by Zone 3 (2.7 $\times 10^{-3}$ ± 1.5 $\times 10^{-3}$), Zone 4 (1.6 $\times 10^{-3}$ ± 1.5 $\times 10^{-3}$), and Zone 2 (1.6 $\times 10^{-3}$ ± 1.4 $\times 10^{-3}$). In the analysis on the ages of the boys and girls (Figure 4), it was demonstrated that at all ages the children are being affected by the effect caused by the mixture of metals in the milk. However, the highest risk occurs between the first months of life and up to the age of 14 years, afterwards there is a decrease in the values of risk by absorption.

**Discussion**

As heavy metals like Cd and Pb are not necessary for human metabolism, it is important to consider that even at the lowest level found, metals will remain...
dangerous when consumed through food. On the other hand, the rest of the metals analysed here are necessary for the metabolic activities of the human organism, but it becomes dangerous when they exceed their levels. We must also consider that the mixture of the metals classified as carcinogens causes a greater risk to human beings.

In the case of Pb, in the four zones it was over the permissible limits established; on the contrary, the Cd showed levels below these permissible limits by European Union and FAO/WHO standard (Codex Alimentarius Commission 2011), which indicate a value of 0.020 mg kg\(^{-1}\) for Pb and 0.01 mg kg\(^{-1}\) for Cd. Ismail et al. (2015) conducted an evaluation in four zones in Multan city, Punjab Province, Pakistan, finding that in the main industrial zone the values for Pb, Cd, Cu, and Ni were, respectively, 0.034, 0.0033, 1.163, and 0.032 mg kg\(^{-1}\) in cow’s milk. The contents of Cd, Cu, and Ni higher than those found in this research and only the level of Pb was lower than the one determined in this work. Kim et al. (2016) reported a content of Cd (0.027 μg kg\(^{-1}\)) and Pb (1.48 μg kg\(^{-1}\)) in milk, both below than those in this work.

The level of Pb found in milk in this study was low than that reported by Bilandžić et al. (2011) in Croatia, reporting values of 58.7 μg l\(^{-1}\), and the same author reported Cd levels of 1.76 μg l\(^{-1}\) similar value to this research. In As, the values obtained in this work are lower than those reported by Licata et al. (2004) in Italy, reporting values of 56.43 μg kg\(^{-1}\).

Several authors have reported lower levels than those found in this research, such as the case of Rahimi (2013) in Iran, who reported very low values of \(9.2 \times 10^{-7}\) mg kg\(^{-1}\) for Cd and of \(9.99 \times 10^{-6}\) mg kg\(^{-1}\) for Pb, equally Najamezhad and Akbarabadi (2013) who found values of \(3.0 \times 10^{-4}\) mg kg\(^{-1}\) for Cd and of \(1.29 \times 10^{-2}\) mg kg\(^{-1}\) for Pb, Zheng et al. (2007) in China, who reported 0.012 mg kg\(^{-1}\) for Pb value below that found in this investigation and 0.002 mg kg\(^{-1}\) of Cd similar to that reported in this work. Similarly in Spain, Sola-Larrañaga and Navarro-Blasco (2009) detected values of 0.005 mg kg\(^{-1}\) for Pb and 0.0004 mg kg\(^{-1}\) for Cd that were lower than those determined in this study and 0.24, 0.36, and 7.74 mg kg\(^{-1}\) for Cr, Cu, and Zn, respectively, which were higher than those found in this work. In Mexico, Solis et al. (2009) reported values of 0.065 mg kg\(^{-1}\) for Pb and 0.27 mg kg\(^{-1}\) for Cu in milk produced in irrigated areas with domestic and industrial waste water of higher than those found in this study. These authors also reported a value of 24 mg kg\(^{-1}\) for Zn, which was higher than that detected in this research, and only this value was used in the chemical treatment coupled to proton-induced X-ray emission. Comparing the Official Mexican Regulation Norma Oficial Mexicana NOM-184-SSA1-2002, which considers permissible limit for Pb as 0.1 mg kg\(^{-1}\) and as 0.2 mg kg\(^{-1}\) for As. Therefore, the values found in this research are below that limit. The HQ As for children was higher than that reported by Shaheen et al. (2016) in Bangladesh and the rest of the metals had a similar HQ to it, which did not pose any risk to the population. However, they determined that the risk to the children was due to the consumption of cow’s milk containing Cd and As. Therefore, it is considered that the danger

### Table 3. Cancer risk index in children and per zone in sub-basin Alto Balsas in states of Puebla and Tlaxcala, Mexico.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Children</th>
<th>Pb</th>
<th>Cr</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zones</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
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<td>2</td>
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<td>3</td>
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<td></td>
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<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Superscript lower-case letters (a b c) represent significant differences p < 0.01 by zones and children. SD: standard deviation.

Figure 4. Cancer risk per age of children in sub-basin Alto Balsas in the states of Puebla and Tlaxcala, Mexico.
found for arsenic in this work could influence a greater predisposition to the diseases caused by the accumulation of this metal. Children are more susceptible to adverse effects after exposures to arsenic. The behaviour and physiology of children may result in higher doses absorbed with respect to body weight than adults for a given set of exposure conditions. Because the diets consumed by the children are little varied, it could generate at high doses (Ramasamy & Lee 2015). As can act as a modulator of the immune defence system of the body that could make immunocompromised the guest, leaving it predisposed to non-cancerous conditions in the short term and chronically could suffer some form of cancer (Islam 2015).

Being the highest HI values in the first 3 months of life and up to 14 years, it can be deduced that there is a certain probability of risk of non-cancerous diseases in the first years until puberty. However, it should be noted that if children have accumulated heavy metals during all that time, the diseases could affect them at later ages, although at that time HI < 1.

Both age groups showed HI> 1, which means that adverse health effects could be generated. And in the case of girls, it can be aggravated because they develop earlier physiologically than children due to the early release of estradiol, which triggers the growth and maturation of bone, coupled with increased accumulation of fatty tissue, which predisposes to be more likely to suffer non-cancerous by the combination of different metals. The values found for HI in all variables were greater than 1 and were in accordance with the work done by Khan et al. (2014a, 2014b) who mentioned that milk contaminated with heavy metals gives HI higher than that obtained by human ingestion of vegetables.

The cancer risk values found in this study were higher than those tolerated $\left(10^{-6} - 10^{-4}\right)$ by USEPA (2002) and higher than those reported by Hu et al. (2012) who at the same time indicate that values that are slightly below $\left(1 \times 10^{-4}\right)$ should be considered as potentially risky. In addition, the cancer risk obtained for the four individual metals was higher than those reported by Islam et al. (2015). When we evaluated the effect of the four metals, we found that boys and girls are at risk of cancer because of the effect of the mixture contained in the milk produced in the four sampling zones.

At any age there is predisposition to accumulate heavy metals (Figure 4); however, the greatest predisposition is in the first 13 years of life, which may be because, as the bodies of children develop, they consume more food per unit of body weight compared to adults. In the first 6 months of life, they drink up to seven times more water per kilogram of body weight, and between 1 and 5 years, they eat three to four times more food per kilogram of body weight than an adult. That is why children are most affected by heavy metals (European Environment and Health Information System (ENHIS) 2007). If we compare the data found in this study with those reported by the Mexican Secretary of Health (SINAVE 2011), in which the highest cancer rates in children were between 5 and 14 years of age, way coincides with what was found in this work.

In this study, risk was calculated based on the same value of milk intake per day for all ages, and the results of this risk estimate (Figure 4) show some similarity to the intake behaviour reported by Ismail et al. (2015), who determined a range of $0.569-0.195$ kg day$^{-1}$ in boys and girls between 1 and 16 years of age. It follows that the older the milk intake decreases and therefore the risk.

All this leads us to assume that as children are exposed to them from an early age, they progressively accumulate toxic metals in different organs, developing the disease when they become older. This is because pollution could begin for them since the intrauterine phase and continue with the consumption of mother’s milk since the metals are mobilised from maternal reserves, transferred from the bones to the mammary gland through the blood, and then excreted into the milk, which is the ideal nutritional source for the baby (Örün et al. 2011; Liu et al. 2013). After that, the next stage is the children’s consumption of cow’s milk from which they absorb a high degree of heavy metals due to the under-development of their digestive tract, thus eliminating very few of them and accumulating them in liver, kidneys, bones, and, mostly in the brain (Dorea & Donangelo 2006; Solis et al. 2009) causing different health disorders.

**Conclusions**

Based on the results obtained, we can conclude that on evaluating the HI and the risk of cancer, it was noted that the health of the children in these study zones is at a serious risk for their intake of different heavy metals present in milk. This indicates that the consumption of small amounts of heavy metals individually or mixed, and chronically, is the cause of the risk in contracting non-cancerogenous and cancerogenous diseases. Therefore, it would be necessary to consider the modification of the permissible limits in the different official regulations for the case of the cow’s milk, and a better control on dairy production in those areas or improve the irrigation systems in the pasture used.

On the other hand, research should be conducted on the heavy metal concentrations in other types of foods produced in the study zones and the risk by consuming these, as well as analysing the hygiene conditions under which the inhabitants of the areas sampled live,
monitoring the milk quality constantly and establishing agricultural soil remediation programmes in order to prevent the risk of diseases.

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Disclosure statement

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