Elements in Human Placentae in Jamaica

C Grant[1], G Lalor[1], H Fletcher[2], T Potter[2], M Vutchkov[3], M Reid[4]

ABSTRACT

Aim: *To investigate the relationships, if any, between elemental content of the placenta with age of mother, birthweight and the Apgar scores of a neonate.*

Methods: *Placental samples were collected, stored at -20ºC and then dried and analysed using neutron activation with the SLOWPOKE II reactor at the International Centre for Environmental and Nuclear Sciences (ICENS). A questionnaire was administered at the time of delivery to determine the level of fish consumption, numbers of dental amalgam fillings and use of cough syrups. Placental concentrations of bromine, calcium, chlorine, iron, mercury, potassium, rubidium, selenium, sodium and zinc were determined.*

Results: *The placentae of 52 Jamaican mothers with a mean age 29 years (range 18–42 years) delivering singleton neonates with a mean birthweight of 3.1 kg (1.3 – 5.5 kg) at term were collected. With the exception of iron and bromine, all results for elemental determinations are very similar to those found elsewhere. Correlation was observed for Apgar 2 (5 minutes), calcium and zinc with birthweight, with p-values of 0.002, 0.007 and 0.07, respectively. Negative correlation was observed for the Apgar 2 and potassium (*$p = 0.056$*) and age of mother at birth and bromine (*$p = 0.02$*).*

The mercury concentration in the measured placentae (7.29 ± 9.1 µg/kg w/w) was slightly lower than the mean concentration found in the literature (8 µg/kg w/w). Approximately 93% of the measured placentae in this study are below the derived placentae upper limit of 22 µg/kg. Of the 7% above the upper limit none exceeded the conservative estimated limit of 115 µg/kg at which neural developmental problems start.

Conclusion: *The significant associations noted are of unknown clinical relevance and need further study.*

Keywords: Apgar score, birthweight, instrumental neutron activation analysis, mercury, metals

Elementos en las Placentas Humanas en Jamaica

C Grant[1], G Lalor[1], H Fletcher[2], T Potter[2], M Vutchkov[3], M Reid[4]

RESUMEN

Objetivo: *Investigar las relaciones que pudieran existir entre el contenido elemental de la placenta y la edad de la madre, el peso al nacer, y la puntuación Apgar del neonato.*

Métodos: *Muestras de placenta fueron recogidas, almacenadas a -20ºC y entonces secadas y analizadas usando la activación neutrónica con el reactor SLOWPOKE II del Centro Internacional de Ciencias Medioambientales y Nucleares (ICENS). A la hora del parto se aplicó una encuesta para determinar el nivel de consumo de pescado, el número de amalgamas dentales y el uso de jarabes para la tos. Se determinaron las concentraciones placentales de bromo, calcio, cloro, hierro, mercurio, potasio, rubidio, selenio, sodio y cinc.*

Resultados: *Se recogieron las placentas de 52 madres jamaicanas con una edad promedio de 29 años (rango 18–42 años) que parieron neonatos únicos con un peso promedio de 3.1 kg (1.3–5.5 kg) a término. Con la excepción del hierro y el bromo, todos los resultados para las determinaciones elementales son muy similares a los hallados en otras partes. Se observó una correlación de Apgar 2 (5 minutos), el calcio y el cinc con el peso al nacer, los valores p de 0.002, 0.007 y 0.07 respectivamente.*

From: [1]International Centre for Environmental and Nuclear Sciences, [2]Department of Obstetrics and Gynaecology [3]Department of Physics and [4]Tropical Medicine Research Institute, The University of the West Indies, Kingston 7, Jamaica, West Indies.

Correspondence: Mr C Grant, International Centre for Environment and Nuclear Sciences, The University of the West Indies, Kingston 7, Jamaica, West Indies, E-mail: charles.grant @uwimona.edu.jm

*Se observó una correlación negativa de Apgar 2 y el potasio (*p *= 0.056) y la edad de la madre a la hora del alumbramiento, con el bromo (*p *= 0.02). La concentración de mercurio en las placentas medidas (7.29 ± 9.1 w/w de µg/kg) fue ligeramente más baja que la concentración promedio hallada en la literatura (8 µg/kg w/w). Aproximadamente 93% de las placentas evaluadas en este estudio están por debajo del límite superior de 22 µg/kg. Del 7% por encima del límite superior, ninguna excedió el límite conservador estimado de 115 µg/kg en el cual comienzan los problemas del desarrollo neural.*
Conclusión: *Se desconoce la importancia clínica de las asociaciones significativas observadas y se requiere más estudio.*

Palabras claves: Puntuación Apgar, peso al nacer, análisis instrumental mediante activación neutrónica, mercurio, metales

West Indian Med J 2010; 59 (5): 480

INTRODUCTION

The very high concentrations of both essential and potentially toxic elements in some Jamaican soils could have consequences for agriculture, nutrition and human health. Previous studies on foods and autopsy samples have shown that there are transfers through food (1, 2) resulting in some significant accumulations in human kidneys and livers (3). This, together with the availability of accurate multi-elemental techniques such as Instrumental Neutron Activation Analysis (INAA), encouraged an examination of the elemental contents of other human tissues. In this, the placenta, which has been found to be a significant biomarker for environmental pollution (4), has the advantage that it is effectively waste material despite its vital role in pregnancy and is available non-invasively after birth.

Amongst the many elements, the well known neurotoxin, mercury (Hg), is of particular concern (5). Although all forms of mercury cross the placenta to the fetus, the toxicity of the element is enhanced by formation of methyl mercury (Me Hg) into which it is converted by bacterial action in aquatic environments with trophic transfer up the aquatic food chain. In fact, 95–99 per cent of the mercury found in fish tissue is the highly toxic methyl mercury (6). The non-occupational sources are largely fish and dental amalgams; other sources of Hg to the environment include electrical utilities, incinerators, industrial manufacturing, waste-water treatment plants and improper disposal of consumer products such as batteries, fluorescent light bulbs and Hg switches (7). Other sources of mercury exposure include thimerosal ethyl-mercury, which is used as a stabilizing agent in drugs such as vaccines, eye and nose drops and some cough syrups.

This study presents some preliminary data on the concentrations of mercury in placentae of Jamaicans along with data on the essential elements: calcium (Ca), iron (Fe), chlorine (Cl) potassium (K), sodium (Na), selenium (Se) and zinc (Zn) and the non-essential bromine (Br) and rubidium (Rb). In addition to these analytical data, information collected at delivery, *ie* age of mother, first and second Apgar scores (8), birthweight and results from a questionnaire are also presented. The main objectives of this paper were to determine the mean and range of total Hg concentrations in Jamaican placentae and to investigate the relationships, if any, between elemental content of the placenta, age of mother, birthweight and the Apgar scores of a neonate.

SSUBJECTS AND METHODS

The study was approved by the Ethics Committee of the Faculty of Medical Sciences of The University of the West Indies/University Hospital of the West Indies (UHWI). The study group consisted of fifty-two normal healthy women in the age group 18 to 42 years who delivered at the UHWI. The women were all singleton pregnancies at term (exclusion criteria were therefore preterm births and multiple pregnancies; 77% of the patients were from Kingston and St Andrew with 13% from St Catherine and 3% each from St Thomas, Manchester and Portland. This is in keeping with the usual population of the antenatal clinic. All patients were given iron and folate preparations routinely. In terms of social class, 56% were middle class, 43% lower class and less than 1% upper class, typical of a public clinic. A questionnaire was used to provide information on food intakes (fish), recent use of cough syrups and the subjects were examined for amalgam-based dental fillings.

Fifty-two placental samples were collected between February and March 2003 at delivery and during that period there were a total of 8274 live births for the Island, 98.7% of these were (8166) singleton births (9), providing a confidence interval of 13.6% at 95% confidence level for the questionnaire data (power of 80%). The average weight of a human placenta without cord and membranes is 420g (10); approximately half (4) or about 200g of each sample was stored at -20°C in acid washed pre-rinsed ceramic bowls. The frozen samples were cut into small cubes and any adipose tissue present was removed before drying at 65°C for 3–4 days. These dried samples were again stored at approximately -20°C and ground into a fine powder for analysis as required.

Neutron activation analysis was done using the SLOWPOKE II reactor at ICENS (11). The two irradiation schemes used are shown in Table 1.

Table 1: Irradiation schemes for NAA analysis of placenta samples

Schemes	Sample mass (g)	Flux x 10^{11} $ncm^{-2}s^{-1}$	Irradiation time (mins)	Elements
Short	0.5	5.0	3	Ca, Cl, Na, K
Long	1.0	10.0	240	Br, Fe, Hg, Rb, Se, Zn,

RESULTS

Quality control was maintained by analysis of NIST Bovine liver SRM 1577a, with one sample being irradiated with every batch of seven samples.

As shown in Table 2, the measured results for the SRM agree with the certified values to better than 10% for all elements and all are well within the standard deviation of analytical data, thus validating the methodology.

Table 2: Quality control data (µg/g) for NIST bovine liver

Element	NIST Bovine Liver mean 1577a Measured (Mean ± Stdev)	Certified value
Br	9 ± 1	9
Ca	123 ± 50	120
Cl	2940 ± 167	2800
Fe	194 ± 8	194
K	9533 ± 702	9960
Na	2326 ± 74	2430
Rb	12.6 ± 0.9	12.5
Se	0.67 ± 0.07	0.71
Zn	119 ± 7	123

The ranges and means of the elemental concentrations are summarized in Table 3a along with information obtained from the literature for similar studies conducted in various countries around the world. Table 3b summarizes the mothers' ages, the newly born body weights and Apgar scores after 1 and 5 minutes, similar data from a survey of 106 Swedish women is also presented.

Table 3b: Summary of results and comparisons with literature values

	Present Study (n = 52)		Sweden (n = 106)[14]
Variable	**Range**	**Mean ± Std Dev**	**Mean Literature**
AGE	18 – 42	29 ± 6	31. ± 4
BWT (kg)	1.3 – 5.5	3.14 ± 0.70	3.6 ± 0.48
APGAR1	2.0 – 9.0	8.15 ± 1.6 (88%)[b]	(97%)[b]
APGAR2	7.0 – 10.0	9.00 ± 0.4 (100%)[b]	(99%)[b]
Parity	0 – 9	1.4 ± 1.7 (38%)[c]	1.6 (48%)[c]

[b] percentage above APGAR score of 7
[c] percentage nulliparous

The birthweight and the concentrations of K, Na, Cl, Rb and Se followed normal distributions, the concentration distributions of Ca, Fe, Zn, Hg and Br were log-normal distributions. Some typical distributions are shown in Fig. 1.

The correlation matrix for the dataset is summarized in Table 4, values in bold are significantly different from R= 0 with a significance level alpha = 0.05.

A significant negative correlation was observed for age of mother and Br R=-0.34, p = 0.02). Statistically significant correlation for the second Apgar score and birthweight (R= 0.44, p = 0.002) as well as Ca and birthweight (R = 0.38, p = 0.007) was observed, correlation was also observed for Zn and birthweight (R= 0.26, p = 0.07); no other significant positive correlation was observed with birthweight, however negative correlation was observed for Cl (R =-0.14, p = 0.34), K (R = -0.28, p = 0.06), Na (R = -0.26, p = 0.08), Rb (R = -0.10, p = 0.49) and Se (R = -0.14, p = 0.33). No significant correlation was observed for the first Apgar score and other parameters other than the second Apgar score (R = 0.53, p = 0.0001). Similar trends were observed to that of birthweight for the second Apgar score, with significant positive correlation for Ca (R = 0.41, p = 0.004) and Zn (R = 0.45, p = 0.001) and a significant negative correlation for K (R = -0.47, p = 0.001). Significant inter-elemental correla-

Table 3a: Summary of results and comparisons with literature values, all concentrations in µg/g unless otherwise stated

	Present Study (n = 52)		Compiled Literature		
Element	**Range**	**Mean ± Std Dev**	**Range**	**Mean**	**Countries**
Br	2.8 – 13.2	4.74 ± 1.83	0.4 – 4.5	2.4[12]	UK
Cl (%)	0.16 – 0.26	0.2 ± 0.02	–	0.19[12]	Japan
Ca	188 – 5414	845.7 ± 1086	400 – 6000	1005	UK
Fe	87 – 528	133 ± 63	43 – 115	69[12]	UK, Japan, India, Germany
Hg (µg/kg)	0.3 –52	7.29 ± 9.1	0.18 – 13 (2 ± 3166)[a]	8[13] (185)[a]	Italy, UK, Germany, China, Czechoslovakia, Yugoslav, Japan, Sweden Taiwan
K %	0.13 – 0.22	0.17 ± 0.02	0.13 – 0.20	0.17[12]	Germany, Japan, UK
Na %	0.15 – 0.23	0.19 ± 0.015	–	0.20[12]	Japan, UK
Rb	1.8 – 3.5	2.52 ± 0.37	1.0 – 2.3	2.3[12]	Greece, UK
Se	0.15 – 0.24	0.19 ± 0.02	0.15 – 0.25	0.19[12]	Italy, Germany, France, Greece, Sweden, Yugoslavia
Zn	8.5 – 14.4	10.07 ± 1.1	8.0 – 18	11[12]	UK, Germany, China, Czechoslovakia, Yugoslav, Finland, Estonia, Sweden

[a] Results of survey from industrial region of Japan, known high exposure

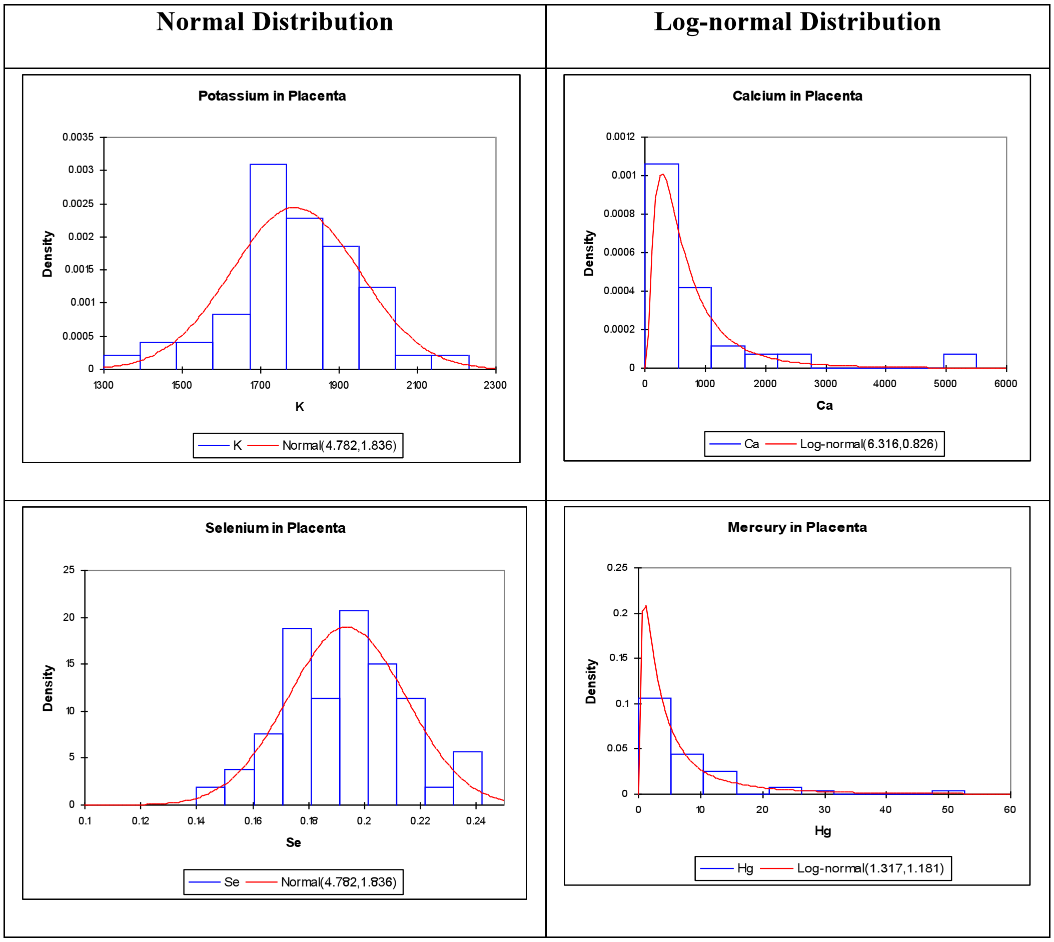

Fig.1: Elemental distributions in placenta

Table 4: Correlation matrix

Variables	AGE	BWT	APGAR1	APGAR2	Br	Cl	Ca	Fe	Hg	K	Na %	Rb	Se	Zn
AGE	1.00													
BWT	0.07	1.00												
APGAR1	-0.20	0.12	1.00											
APGAR2	-0.15	**0.44**	**0.53**	1.00										
Br	**-0.34**	0.03	0.19	0.03	1.00									
Cl	-0.11	-0.14	0.05	-0.16	0.24	1.00								
Ca	-0.10	**0.38**	0.13	**0.41**	-0.07	**-0.32**	1.00							
Fe	0.20	0.00	-0.10	-0.02	0.05	0.03	-0.07	1.00						
Hg	0.06	0.12	0.14	0.21	-0.14	-0.13	0.18	-0.04	1.00					
K	0.01	-0.28	-0.18	**-0.47**	-0.02	**0.40**	**-0.47**	-0.03	**-0.47**	1.00				
Na %	-0.16	-0.26	0.09	-0.02	0.17	**0.69**	-0.21	-0.03	-0.03	0.09	1.00			
Rb	0.06	-0.10	-0.14	-0.07	-0.13	0.10	-0.26	0.05	-0.28	**0.58**	-0.02	1.00		
Se	0.05	-0.14	0.01	-0.05	-0.17	-0.08	-0.02	-0.13	-0.03	0.04	-0.11	0.12	1.00	
Zn	0.04	0.26	0.18	**0.45**	-0.17	-0.19	**0.64**	-0.09	0.01	-0.14	-0.18	0.15	0.20	1.00

tions were also observed for Cl with Ca (R= -0.32, p = 0.028), K (R = 0.41, p = 0.004) and Na (R = 0.69, $p < 0.0001$); Ca with K (R = -0.47, p = 0.001) and Zn (R = 0.65, $p < 0.0001$); Hg and K (R = 0.47, p = 0.001) and Rb and K (R = 0.58, $p < 0.0001$).

Ten (20%) patients had dental amalgam range of 1–9 teeth, median of 3. Comparison of amalgam restoration with placenta Hg levels was not significant (R = 0.27, p = 0.45). Five (10%) patients used cough syrup during the pregnancy. All used diphenhydramine hydrochloride (DPH) expectorant which contains no thimerosal. Comparison use of cough syrup and placental Hg level was not significant (R = -0.03, p = 0.83). Seventeen (34%) patients consumed fish throughout the pregnancy with the median frequency per week being two. Approximately 94% of the fish consumed was sea fish, with snapper being the most popular. No large predatory fish such as marlin, tilefish, swordfish, king mackerel or shark which exhibit trophic bio-magnification of Hg were consumed by the participating population. Comparison of fish consumption with placenta Hg levels were not significant (R = 0.06, p = 0.83).

Seventeen per cent of patients had Hg concentration at or near the detection limit. Seventy-five per cent of these patients had no fish throughout the pregnancy. The patient with the maximum placental Hg concentration, 52 µg/kg, had no dental amalgam restoration, used no cough syrup during the pregnancy but had a dietary intake of fish once per week. There was one patient who had 9 dental amalgam restorations performed, who admitted using cough syrup during the pregnancy and ate fish at least once per week and had placenta Hg levels of 5 µg/kg, below the mean of the measured population.

DISCUSSION

Although no physiological reason was determined for the decrease in placental Br with age of mother, a strong correlation was found (Fig. 2). It should be noted however that the Br concentrations reported here are almost twice that found in the UK, the only country for which reported placental Br data were found. Bromine is found throughout the human body and usually concentrates in the intracellular fluids and saliva (15). A small but statistically significant increase in risk of birth defects was associated with bromine exposure in one study from Australia (16).

The birthweights observed in this work are slightly less than the average birthweight reported in the literature (14, 17) for developed countries. The distribution of birthweight follows a normal distribution with approximately 16% of the population having low birthweight (LBW), < 2.500 g, with a further 4% with very low birthweight (VLBW), 1500 g, more than double that of the USA at 7.6% (18). It is estimated that 11% of births in developing countries are term LBW (17). Unlike developed countries, the majority of LBWs are full-term infants who have experienced growth retardation *in utero* (19). Birthweight is a strong predictor of an infant's chances of survival and LBW has also been associated with profound later life health effects, even after recovering from low weight at birth (20).

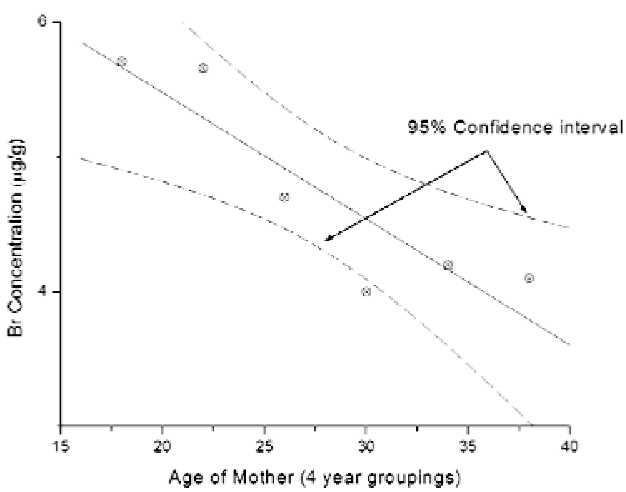

Fig. 2: Placenta Br and Age of mother at delivery.

The significant correlation (R = 0.38, p = 0.007) between Ca and birthweight (Fig. 3) is most likely due to Ca

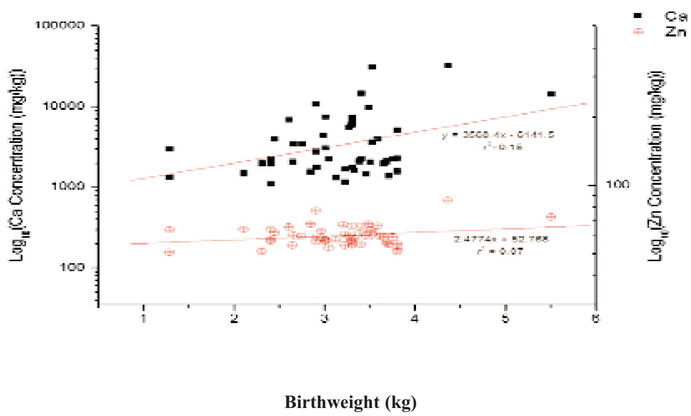

Fig. 3: Birthweight *vs* Placental Ca and Zn Concentrations

being an essential nutrient which is required for the proper development of the fetus (12).

Correlation was also observed for Zn and birthweight (R = 0.26, p = 0.07), a low Zn intake has been associated with approximately a two-fold increase in the risk of LBW after controlling for calories and other confounding variables (21), a very significant correlation was also observed for Ca and Zn (R = 0.65, p < 0.0001).

A negative correlation with K was found for both the birthweight (R = -0.28, p = 0.056) and for Apgar score after 5 minutes (R = -0.47, p = 0.001).

The negative correlation of K and birthweight is of interest; it has been shown that an increase in K for adults of 4.2 grams/day (RDA 2 to 3.5 g/day) can lead to an 8–9 mmHg fall in systolic pressure (22). Maternal hypotension in pregnancy has been associated with small babies (23) and might explain the negative correlation between birthweight and K concentration. Although there was an observed correlation of K with the second Apgar score, the survey data were too underpowered (less than 10% with an Apgar score that differ from 9) to detect if this relationship was significant or not (Fig. 4).

The Fe concentrations observed in this work are higher than those in the reported literature and are most likely due to improper blood removal from the placenta. The essential

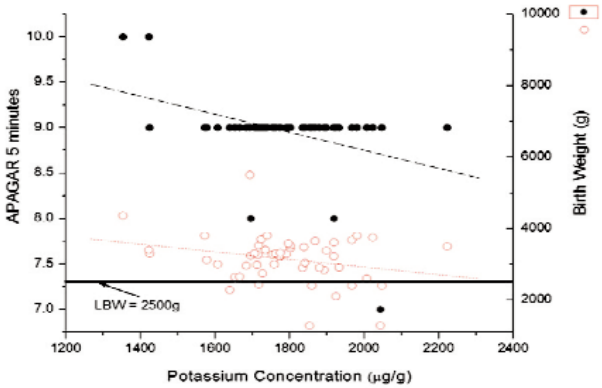

Fig. 4: Concentration of K with Apgar score (5 min) and birthweight.

elements Cl, Na and Se showed no correlation with Apgar score, birthweight or age of mother; all three elemental concentrations are within the ranges found in the literature, similarly for the non-essential element Rb.

The range of total Hg concentration in placenta, 0.3 – 52 µg/kg was greater than that found in the literature review for normally exposed women of 2 – 13 µg/kg but lower than that of exposed populations 2 – 3166 µg/kg; however the mean concentration of 7.3 µg/kg found in this study was less than the 8 µg/kg reported by Iyengar and Rapp (13).

There was no significant difference between the presence of dental amalgam restored teeth and placental mercury level (*p*-value = 0.45). This result was in keeping with the findings of Factor-Litvak and co-workers (24). Mercury from dental amalgam is in the inorganic form,which does not cross the placenta as readily as the organic forms (25).

Only 34% or 17 patients consumed fish on a regular basis, maximum of 4 meals per week and a median of 0.6 for the cohort, these results confirm the findings of a recent survey of 437 Jamaican women showing that fish consumption is relatively low at 15.6 g/day (26) compared to the average for 10 European countries of 35.8 g/day (27). This relatively low rate of fish consumption may explain why no significant correlation was observed for Hg concentration and fish intake. A recent survey in Taiwan (28), a high fish consumption country (median of 3 – 4 meals per week) showed similar findings to this work when correlating fish consumption and placental Hg, but found significant correlation to cord blood Hg and fish consumption during pregnancy. The same Taiwanese study found correlation between fish consumption before pregnancy and placental Hg concentration, confirming the proposal by Iyengars and Rapp of the placenta as long term bio-monitor rather than a real time bio-monitor (4).

In 2000, the US National Research Council (USNRC) concluded that umbilical cord blood mercury concentrations exceeding 58 µg/L influenced neural development of the fetus. To provide for variations in race and individual health, and with an uncertainty factor of 10, the recommended upper limit was placed at 5.8 µg/L (29). A recent study in a Swedish cohort (30) and a separate study in the Taiwanese cohort (28) showed significant correlations between cord blood Hg and placenta Hg concentrations, with ratios of median cord blood Hg (µg/L) to median placenta Hg (µg/kg) varying from 3.8 to 2.0 for the Swedish and Taiwanese respectively. Using these derived ratios of 2.0 and 3.8 for cord blood to placenta, it would be reasonable to assume that the cord blood limit would be exceeded at a placental concentration in the region of 11.5 to 22 µg/kg.

Approximately 79% (Fig. 5) of the measured placentae in this study were below the lower limit of 11.5 µg/kg with

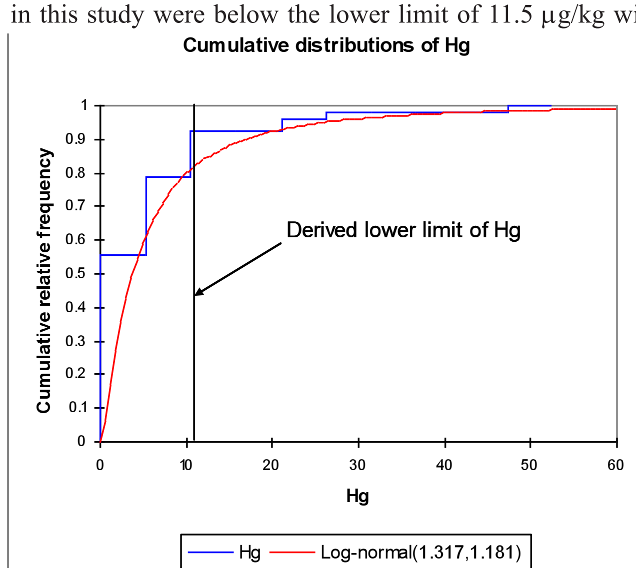

Fig.5: Cumulative distribution of Hg in Jamaican placentae

93% below the upper limit of 22 µg/kg. Of the 7% above the upper limit, none exceeded the conservative estimated limit of 115 µg/kg at which neural developmental problems start.

With the exception of Fe and Br, all placenta data for essential and non-essential elements are close to those observed in similar studies for several countries around the world.

The Hg levels in Jamaican placenta are all below the level at which neural developmental problems become manifest. However, 7% are above the lower limit set by USNRC in 2000. The fact that the fish consumption in Jamaica is relatively low and that there was no apparent correlation between dental fillings and Hg concentration suggests that there are environmental factors other than fish and dental fillings that account for the observed levels in placental total Hg. It must be noted, however, that the small number of patients who had fillings and/or consumed fish has rendered the survey for Hg underpowered to detect potential differences due these sources. Considering this, several samples (n = 8) of local snapper fish, the most frequently consumed fish in this study, obtained recently from fishing beaches along the South coast, have fresh weight Hg contents ranging from

0.05 – 1.56 μg/kg and mean concentration of 0.62 μg/kg. The US FDA has set no limits on the mercury content of commercial fish, however, the USEPA has human health limit of 0.3 mg/kg in fish tissue for consumers of non-commercial freshwater/estuarine fish (31) and a further limit for human consumption of 0.1 μg/kg meHg body weight /day (29), as adults have also been shown to be affected by mercury poisoning from contaminated fish (32). With this in mind, we would recommend that the FDA limit of 340g per week of fish species with known low MeHg levels be adhered to during pregnancy.

Because of chemical similarities between certain essential and toxic elements, the same transport mechanisms which allow the transfer of essential elements may also aid the transfer of toxic elements (12, 33). Moreover, the barrier role of the placenta and mechanisms in allowing the passage of essential elements to the fetus while restricting some heavy metals such as cadmium, may be elaborated as more extensive studies are performed on these and a variety of other elements.

REFERENCES

1. Nriagu J, Boughanen M, Linder A, Howe A, Grant C, Rattray R et al. Levels of As, Cd, Pb, Cu, Se and Zn in bovine kidneys and livers in Jamaica. Ecotoxicol Environ Saf 2009; **72:** 564–71,
2. Howe A, Hoo Fung L, Lalor G, Rattray R, Vutchkov M. Elemental composition of Jamaican foods 1: A survey of five food crop categories. Environ Geochem and Health 2004; **27:** 19–30.
3. Lalor GC, Rattray R, Williams N, Wright P. Cadmium levels in kidney and liver of Jamaicans at autopsy. West Indian Med J 2004; **52:** 76–80.
4. Iyengar GV, Rapp A. Human placenta as a dual biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 1: Physiology, function and sampling of placenta for elemental characterisation 2001; **280:** 195–206.
5. Kim SA, Jeon CK, Paek DM. Hair mercury concentrations of children and mothers in Korea: Implication for exposure and evaluation, Sci Total Environ 2008; **402:** 36–42.
6. Scudder BC, Chasar LC, Wentz DA, Bauch NJ, Brigham ME, Moran, PW et al. 2009, Mercury in fish, bed sediment, and water from streams across the United States, 1998–2005: US Geological Survey Scientific Investigations Report 2009–5109.
7. Driscoll CT, Han YJ, Chen CY, Evers DC, Lambert KF, Holsen TM et al. Mercury contamination in forest and freshwater ecosystems in the Northeastern United States. BioScience 2007; **57:** 17–28.
8. Finster M, Wood M. The Apgar score has survived the test of time. Anesthesiology 2005; **102:** 855–7.
9. Registrar General's Department, 2004 Annual Vital Statistics Report: http://www.rgd.gov.jm/book/print/201.
10. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the task group on reference man. International Commission on Radiological Protection 23 (ICRP-23), Pergamon Press, 1975.
11. Grant C, Lalor GC, Preston J, Rattray R and Vutchkov MK. Neutron Activation Analysis with the Slowpoke Reactor in Jamaica. Jamaican J Sci Technol 1998; **9:** 63.
12. Iyengar GV, Rapp A. Human placenta as a dual biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 2: Physiology, function and sampling of placenta for elemental Characterization. Sci Tot Environ 2001; **280:** 207–19.
13. Iyengar GV, Rapp A. Human placenta as a dual biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as biomarker for these elements. Sci Tot environ 2001; **280:** 221–38.
14. Osman K, Åkesson A, Berglund M, Bremme K, Schutz A, Ask K et al. Toxic and essential elements in placentas of Swedish Women. Clin Biochem 2000; **33:** 131–8.
15. Roza G. Bromine. The Rosen Publishing Group, 2009.
16. Chisholm K, Cook A, Bower C, Weinstein P. Risk of birth defects in Australian communities with high levels of brominated disinfection by-products. Environ Health Perspect. 2008; **116:** 1267–73.
17. Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Psychosocial intervention improves the development of term low-birth-weight infants. J Nutr 2004; **134:** 1417–23.
18. Mbofung CMF, Subbarau VV. Trace element (zinc, copper, iron and magnesium) concentrations in human placenta and their relationship to birth weight of babies. Nutr-Res 1990; **10:** 359–66.
19. Ashworth A, Morris SS, Lira PI. Postnatal growth patterns of full-term low birthweight infants in Northeast Brazil are related to socio-economic status. J Nutr 1997; **127:** 1950–6.
20. Forrester TE, Wilks R, Bennett FI, Simeon D, Osmond C, Allen M et al. Fetal growth and cardiovascular risk factors in Jamaican school-children. BMJ 1996; **312:** 156–60.
21. Scholl TO, Hediger ML, Schall JI, Fisher RL, Khoo C-S. Low zinc intake during pregnancy: its association with preterm and very preterm delivery. Am J Epidemiol 1993; **137:** 1115–24.
22. Kuh D(ed) and Ben-Shlomo Y (ed). A Life Course Approach to Chronic Diseases Epidemiology, Second Edition, Oxford University Press Inc; 1997.
23. Steer PJ, Little MP, Kold-Jensen T, Chapple J, Elliott P. Maternal blood pressure in pregnancy, birthweight, and perinatal mortality in first births: prospective study. BMJ 2004; 329:1312, doi: 10.1136/bmj.38258.566262.7C
24. Factor-Litvak P, Hasselgren G, Jacobs D, Begg M, Kline J, Geier J et al. Mercury derived from dental amalgams and neuropsychologic function. Environ Health Perspect. 2003; **111:** 719–23.
25. Mansour MM, Dyer NC, Hoffman LH, Schulert AR, Brill AB. Maternal-fetal transfer of organic and inorganic mercury via placenta and milk. Environ Res 1973; **6:** 479–84.
26. ICENS work in progress funded by IDRC 2007.
27. Welch AA, Lund E, Amiano P, Dorronsoro M, Brustad M, Kumle M et al. Variability of fish consumption within the 10 European countries participating in the European Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2002; **5:** 1273–85.
28. Hsu CS, Liu PL, Chien LC, Chou SY, Han BC. Mercury concentration and fish consumption in Taiwanese pregnant women. BJOG 2007; **114:** 81–5.
29. National Research Council (US). Board on Environmental Studies and Toxicology. Toxicological Effects of Methylmercury. Washington DC: National Academies Press; 2000.
30. Ask K, Akesson A, Berglund M, Vahter M. Inorganic mercury and methylmercury in placentas of Swedish women, Environ Health Perspect 2002; **110:** 523–6.
31. USEPA 2001. Water Quality Criterion for the Protection of Human Health: Methyl-mercury. Washington (DC): Office of Water. Report no. EPA-823-R-01-001.
32. Hightower JM, Moore D. Mercury levels in high-end consumers of fish. Environ Health Perspec 2003; **111:** 604–8.
33. Rossipal E, Krachler M, Li F, Micetic-Turk D. Investigation of the transport of trace elements across barriers in humans: studies of placental and mammary transfer. Acta Pædiatr 2000; **89:** 1190–5.