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# 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 a biomarker for these elements

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## Abstract

The human placenta as a body component is exposed to several harmful substances, depending upon the environmental conditions encountered. In the case of toxic metals, placental tissue can be regarded as a *dual biomarker* to assess maternal and fetal health. The average range of concentrations for toxic trace elements in placenta based on wet weight are found to be: cadmium 1–6 ng/g; total mercury 2–13 ng/g; methyl mercury 1–14 µg/g; and lead 5–60 ng/g. The placenta appears to be at least a partial barrier for Cadmium. Cadmium transport includes a broad variety of mechanisms. Once in circulation, it mainly interferes with Ca and Zn transportation. On the other hand, placenta appears to be a weaker barrier for Pb than for Cd. In the case of Hg, predominantly the organic form is absorbed and readily crosses the placenta. In fetal blood, the organic mercury content is equal or even greater than in maternal blood, raising questions on normal fetal development. Placenta as a biomarker could be taken as an alternative to repeated maternal blood sampling for assessing lead exposure in utero. Placenta samples are usually obtained at the time of parturition, a one-time event. Hence, each pregnancy has to be looked upon as an RTM (real time monitoring) process since the affected species is exposed to the placental source of pollutants only during the course of that particular pregnancy. © 2001 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Several factors, such as lifestyle, diet, drug abuse, therapeutic excess and exposure to bacterial and virus type of contamination and environmental chemicals are linked with fetal abnormalities, in addition to maternal infection (compounded by under or malnutrition), hormones and a variety of chemical agents.

The placenta has a dual transport function in that it facilitates the passage of some bio-substances to the fetus while acting as a barrier to other materials. Besides nutrient substances crossing the placenta barrier, some harmful substances can also pass through placenta that can damage the embryo. For example, placenta is highly permeable to alcohol leading to fetal alcohol syndromes in the event of excess ingestion of alcohol. Similarly, some drugs also pass through the placental barrier leading to varying degrees of birth defects. The addiction of women during pregnancy to cocaine and heroin and the resulting ill effects that affect the fetus are well established. Similarly, a wide array of infectious agents (e.g. rubella and coxackie viruses) also

cross the placental barrier and infect the fetus with severe health consequences. The toxic effects of lithium (heart anomalies) resulting from its therapeutic use as an antidepressant agent, the role of methyl mercury inducing mental retardation, cerebral atrophy and blindness, the action of lead in impairing the central nervous system (Goyer, 1997), and several other metals and their compounds (Prasad, 1988) are good examples of the impact of chemicals. Similarly, the class of organic compounds consisting of organotin compounds (Bierkamper and Buxton, 1990), chlorinated hydrocarbons, polychlorobiphenyls and dioxins amongst others, represent another potent source of dangerous exposure to chemicals (Iyengar et al., 1998, Chapter 8; Jensen et al., 1991).

## 2. Toxic trace elements in human placenta

Among the recognized toxic trace elements, Cd, Pb and Hg (organic and inorganic) have been investigated extensively, unlike Al, As and Ni. Several sampling techniques have already been

Table 1  
Elemental composition of placenta: pre 1975 results<sup>a</sup>

Element	<i>n</i>	Method	Basis	Conc. as reported (µg/g)	Average conc. <sup>b</sup> based on wet weight (µg/g)
Al	60	AAS	Wet	1.1	1.1
As	6	NAA	Dry	0.02	0.0115
	?	NAA	Dry	0.13	
Cd	135	AAS	Dry	0.102	0.019
	45	AAS	Wet	0.021	
Hg	1061	AAS	Dry	0.12	0.0015–0.07 (range)
	6	NAA	Dry	0.06	
	9	MISC	Wet	0.072	
Pb	234	AAS	Dry	1.83	0.325
	55	AAS	Wet	0.35	

<sup>a</sup>Source: Iyengar et al. (1978); Snyder et al. (1975) — (ICRP-23).

<sup>b</sup>Wet to dry ratio of 6 was used for conversion (ICRP-23; Snyder et al., 1975).

Table 2  
Concentration of toxic trace elements in human placenta between 1976 and 2000

Element	Reference	n, Method	Basis, unit	Mean $\pm$ S.D.	Average (range) <sup>b,c</sup> wet weight (ng/g)
Al	Ward et al., 1987 <sup>c</sup>	100, NAA	dry, ng/g	(700–5400)	250
As	Ward et al., 1987 <sup>c</sup>	100, NAA	Dry (ng/g)	69.0 $\pm$ 71.0	6 (3–12)
	Karp et al., 1977	19, MISC	Wet (ng/g)	3.0 $\pm$ – 1.0	
	Horvat et al., 1988 <sup>c,g</sup>	34, NAA	Wet (ng/g)	6.5 $\pm$ 5.8	
	Diaz-Barriga et al., 1995	18, AAS-HG	Wet (ng/g)	2.6 $\pm$ 0.4	
Cd	Schramel et al., 1988a	33, GFAAS	Dry (ng/g)	31.0 $\pm$ 15.0	4 (1–6)
	Centeno et al., 1996 <sup>a</sup>	25, AAS	Dry (ng/g)	26.2 $\pm$ 17.9	
	Fiala et al., 1998	688, AAS	Dry (ng/g)	18.02	
	Kantola et al., 2000 <sup>d,e</sup>	106, GFAAS	Dry (ng/g)	22.2 $\pm$ 11.4	
	Diaz-Barriga et al., 1995	18, GFAAS	Wet (ng/g)	1.3 $\pm$ 0.5	
	Truska et al., 1989	50, GFAAS	Wet (ng/g)	4.0 $\pm$ 0.37	
	Hubermont et al., 1978	70, GFAAS	Wet (ng/g)	(0.3–3.75)	
	Osman et al., 2000 <sup>e,f</sup>	106, ICP-MS	Wet (ng/g)	5.6	
Hg (total)	Capelli et al., 1986	22, AAS	Dry (ng/g)	76.0 $\pm$ 54.0	8 (2–13)
	Ward et al., 1987 <sup>c</sup>	100, NAA	Dry (ng/g)	(20.0–100.0)	
	Schramel et al., 1988a	26, CVAAS	Dry (ng/g)	26.0 $\pm$ 12.0	
	Yang et al., 1997	9, CVAAS	Dry (ng/g)	68.0 $\pm$ 18.0	
	Karp et al., 1977	19, MISC.	Wet (ng/g)	8.0 $\pm$ 1.0	
	Kuhnert et al., 1981	22, GLC	Wet (ng/g)	6.7 $\pm$ 4.7	
	Horvat et al., 1988 <sup>c,g</sup>	34, NAA	Wet (ng/g)	12.9 $\pm$ 7.7	
	Truska et al., 1989	50, CVAAS	Wet (ng/g)	2.2 $\pm$ 1.0	
Hg (methyl)	Capelli et al., 1986	18, AAS	Dry (ng/g)	50 $\pm$ 40	7 (1–14)
	Yang et al., 1997	9, CVAAS	Dry (ng/g)	22 $\pm$ 12	
	Kuhnert et al., 1981	22, GLC	Wet (ng/g)	1.4 $\pm$ 1.1	
	Tsuchiya et al., 1984	226,	Wet (ng/g)	14 $\pm$ 8.0	
	Horvat et al., 1988 <sup>c,g</sup>	Extraction	Wet (ng/g)	7.0 $\pm$ 5.1	
Ni	Thieme et al., 1986	30, ASV	Dry (ng/g)	51.0 $\pm$ 22.0	36 (9–62)
	Ward et al., 1987 <sup>c</sup>	100, NAA	Dry (ng/g)	373.0 $\pm$ 89.0	
	Centeno et al., 1996 <sup>a</sup>	25, AAS	Dry (ng/g)	330.0 $\pm$ 270.0	
	Karp et al., 1977	19, MISC.	Wet (ng/g)	18.0 $\pm$ 18.0	
Pb	Schramel et al., 1988a	33, ASV	Dry (ng/g)	116.0 $\pm$ 43.0	34 (5–60)
	Centeno et al., 1996 <sup>a</sup>	25, AAS	Dry (ng/g)	30.9 $\pm$ 37.8	
	Tsuchiya et al., 1984	110, AAS	Wet (ng/g)	45.0 $\pm$ 34.0	
	Truska et al., 1989	50, GFAAS	Wet (ng/g)	43.0 $\pm$ 30.0	
	Romero et al., 1990	15, GFAAS	Wet (ng/g)	(15.0–92.0)	
	Radomanski and Sikorski, 1992	127, AAS	Wet (ng/g)	60.0	
	Osman et al., 2000 <sup>e,f</sup>	89, ICP-MS	Wet (ng/g)	8.7	

<sup>a</sup>Content in parenchyma tissue of placenta.

<sup>b</sup>Average ratio used for conversion dry/weight = 6.

<sup>c</sup>These data are results from the cited references in this table.

<sup>d</sup>The original value was given in  $\mu\text{g/g}$ , it is obvious that the units were mixed up.

<sup>e</sup>Authors also assessed reference material.

<sup>f</sup>Data converted from moles into weight units.

<sup>g</sup>Content in amnion of placenta.

reviewed and discussed in Part 1. Here we deal only with concentration data for toxic elements.

### 2.1. Pre-1975 data

As with essential trace elements, most of these results belong to the 1950s and 1960s when only a few possibilities existed to carry out trace element determinations (Table 1). Similarly, the use of certified reference materials (CRM) to validate methodology was rarely in practice during earlier years. However, it should be recognized that the pre-1975 data provided an opportunity to highlight the usefulness of elemental composition data in biological systems. Hence, the pre-1975 contributions found in the literature for placenta have proved to be useful.

### 2.2. Post-1975 data

These are presented in Table 2. Improved analytical techniques (as discussed in Part 2), in particular nuclear activation techniques in various versions and atomic absorption spectrometry among others, have been pivotal in establishing reliable results and for a subsequent re-evaluation of the toxic elemental composition of placental tissue. The reassessment of the values suggests that for many toxic trace elements, the concentrations have been reduced. For example, concentration for Al decreased 4-fold, for Cd at least 3-fold, and most conspicuously for Pb by a factor of almost 10 (Table 3). These findings indicate either considerable improvement of analytical methods or the reduced exposure to those pollutants. Conceivably, it must be a combination of both since contamination control procedures and the rest of the analytical methodology have vastly improved with time, and several environmental regulatory procedures are in effect to minimize emissions from contaminant sources. Establishment of reliable reference values for Pb, in this case leading to a significant reduction from earlier values (for whatever reason), is of great importance because the placenta does not seem to be an efficient barrier for Pb which penetrates readily (Baranowska, 1995; Nashashibi et al., 1999). It has been reported that the fetus is highly

Table 3

Impact of improved analytical techniques on the elemental composition in placenta

Element	Average/(range) conc. (ng/g) wet weight	
	Pre-1975	After-1975
Al	1100	250
As	11.5	6 (3–12)
Cd	19	4 (1–6)
Hg	(0.0015–0.07)	8 (total) (2–13) 7 (methyl) (1–14)
Ni	–	36 (9–62)
Pb	0.325	34 (5–60)

susceptible to Pb, resulting in retardation of growth and mental development (Richter et al., 1999). A provisional estimate for the placental Ni content has been proposed. For this metal, no evaluations were available in the pre-1975 recommendations. The data are tabulated in Table 3.

## 3. Discussion

The discussion will be restricted to post-1975 results in the following sections, and refers, therefore, to data listed in Table 2. Transport of toxic metals has been the subject of a number of investigators (Hinkle et al., 1987; Goyer, 1990; Diaz-Barriga et al., 1995; Vallee and Ulmer, 1972; Kajiwara et al., 1996; Lin et al., 1997; Reichrtova et al., 1998; Kantola et al., 2000; Osman et al., 2000) and the reader may refer to these sources for details. In the following section, comments are offered on the concentration levels found in the literature.

### 3.1. Aluminum

Only one investigation reported results for Al (Ward et al., 1987) in 100 placental samples and the average concentration appears to be 250 ng/g (Table 2). Because Al has an affinity for many of

the same biological ligands as the essential mineral cations Ca, Mg, Zn, Fe and Mn, it is expected to show a pattern of developmental concentrations that is similar to these elements in the brain (Golub et al., 1996).

### 3.2. Arsenic

The average concentration appears to be 6 ng/g, spread over a range of 3–12 ng/g. Significant differences in As content of human placenta under exposed (5.9 ng/g) and normal (2.6 ng/g) environmental conditions have been demonstrated (Diaz-Barriga et al., 1995). It has been shown that placenta partially blocks the passage of Arsenic. Maternal and fetal blood As levels were not available from this study; hence, a firm conclusion awaits further information.

### 3.3. Cadmium

The average concentration appears to be 4 ng/g, spread over a range of 1–6 ng/g based on wet weight placenta under non-exposed environment. However, other investigators (Goyer, 1993; Tsuchiya et al., 1984) have reported up to 32 and 30 ng/g based on wet weight, respectively. Although no specific reference to sources contributing to elevated levels of Cd in the environment was seen in the discussions, it would seem that the exposure levels for Cd were higher than generally anticipated under normal exposure conditions (e.g. subjects living away from industrial sources of emissions).

The principal sources of Cd include industrial pollution, food and cigarette smoking (Philp, 1995). A heavy smoker can easily be exposed to 1.5–60 µg of Cd everyday, based on 120 ng (Norman, 1977) up to 2 µg (Elinder et al., 1983) of Cd per cigarette. Fifty percent of inhaled Cd and 6% of ingested Cd can be absorbed (Philp, 1995), most of which is bound to a cysteine-rich protein, metallothionein (MT) in the liver and kidney. MT is an important protective mechanism that prevents xenobiotics to permeate through placenta, but its binding is reported to be unstable and, therefore, it does not definitely indicate that Cd cannot reach the fetus through placenta (Snah

and Miller, 1985). Metallothionein binds both the essential and toxic metals and, therefore, it plays an important role in fetal nutrition and protection. It has been shown that Cd perturbs Ca adsorption or transport in kidney, intestine and placenta (Ando and Matsui, 1987).

Cd induces metallothionein and replaces Zn from the enzyme-binding site (Vallee and Ulmer, 1972). Placental Cd increase and Zn decrease are associated with the age of the pregnant women, the number of pregnancies (multiparae) and the increase of smoking (Fiala et al., 1998). In a Finnish study conducted by Kantola et al. (2000), birth weights correlated inversely with the length of time the mothers smoked. The group found that Cd starts to accumulate in the placenta during the first trimester ( $4.5 \pm 7.1$  ng/g, wet weight) and peaked at term ( $22.2 \pm 11.4$  ng/g). The data also demonstrate an inverse accumulation of Zn and Cd throughout the pregnancy in the placenta and maternal blood samples. Elevated Cd concentrations in placenta from smoking mothers were also found by Bush et al. (2000). Animal experiments have demonstrated that the protective capacity of the placenta against exposure of the fetus to cadmium is strongest during the third trimester of pregnancy (Ahokas and Dilts, 1979). In a Swedish study conducted by Osman et al. (2000), smokers had significantly higher cadmium concentrations in blood and placenta. Cord blood cadmium was only approximately 10% of that in maternal blood. The results showed that cadmium passage through placental barrier was restricted.

### 3.4. Lead

The average concentration appears to be 34 ng/g, spread over a range of 5–60 ng/g. In an extensive study conducted in former Yugoslavia, placenta samples collected under exposure conditions ranging from low, mid and high in Pb, its levels in placenta (compared with no known additional sources of exposure) were found to be 75, 150 and 450% higher, respectively (Loiacono et al., 1992). The effects of chronic exposure to Pb and the impact on its concentration in the umbilical cord and placental tissue (both body and membrane) have been studied (Baghurst et al.,

1991). The results indicated modest increases in Pb concentrations in the placental body. Significantly, the Pb concentrations in membranes from late fetal deaths (2.73 mg/kg, dry) were very high when compared with those obtained from normal births (0.78 mg/kg, dry). In a Zambian study the mean blood levels were 412 and 370 ng/ml for mothers and infants, respectively (Clark, 1977). However, there is no indication of analytical quality control (AQC) and, therefore, it cannot be ruled out that part of the discrepancy may stem from analytical sources of error. Interestingly, as commented by the authors, the infants did not show any noticeable ill effects from this high Pb levels suggesting that the actual exposure level could have been lower than what was measured. High intake of Pb through drinking water (Hubermont et al., 1978), and by exposure to elevated Pb in the urban environment (Tsuchiya et al., 1984) have also been shown to moderately elevate Pb burdens in maternal blood and placenta as well as the blood Pb of the newborn (or cord blood), confirming efficient transfer of Pb through placenta. Other documented exposures to Pb, increase in placental Pb and concomitant stillbirth occurrences have been seen in cases studied around the Stoke-on-Trent area in the UK (Khera et al., 1980). The occupationally exposed women were employed in pottery, lithography and painting jobs. Other examples are documented in the literature (Baglan et al., 1974; Hubermont et al., 1978; Takaes von et al., 1984a,b; Truska et al., 1989). Therefore, pregnant women must be prevented from exposure to Pb in drinking water as well as other emission sources. In a Swedish survey placental concentration of lead ranged between 0 and 130 ng/g. Lead levels in cord blood were the same as in maternal blood (11 ng/ml). Cord blood Pb was a negative predictor of child's birth weight, length and head circumference. The results showed that Pb easily crossed the placental barrier (Osman et al., 2000).

### 3.5. Mercury

Total Hg in human placenta under no known sources of environmental burden appears to vary from 2 to 13 ng/g, with an average value of 8

ng/g based on wet weight. Similar concentrations are found for methyl-Hg. Mercury is present in the earth's crust and is methylated by bacteria in aquatic environments to methylmercury (organic). It is then concentrated by the food chain. Hence, predatory fish and sea mammals have the highest levels. Following an outbreak of MeHg poisoning in Iraq, a dose-response relationship was determined, indicating that an exposure in the range of 10–20 ppm might adversely affect the fetus (Cox et al., 1989). Dietary MeHg in the human is almost totally absorbed in the gastrointestinal tract and rapidly enters the bloodstream (WHO, 1990). Approximately 95% is taken up by red blood cells, and then distributed through the body over the next 3–4 days. The brain is the primary target organ. Kajiwara et al. (1996) investigated MeHg placental transport in pregnant rats by administering MeHg intravenously.

In pregnant women, MeHg readily crosses the placenta (Myers and Davidson, 1998) and has a high affinity for fetal hemoglobin. Levels in fetal blood are approximately 25% higher than in the mother (Amin-Zaki et al., 1976); similar findings were reported by Klopov (1998). In the fetus, exposure to Hg disrupts normal developmental processes such as neuronal migration and organization of gray matter (Choi et al., 1978). In a study carried out by Nishima et al. (1977) on pregnant women from the city of Tokyo, both total Hg and MeHg found in cord blood ( $25 \pm 10$  and  $13 \pm 6$  ng/g, respectively) were higher than those found in the maternal blood ( $14 \pm 6$  and  $6 \pm 3$  ng/g). Vimy et al. (1990) found that mercury from dental amalgam appeared in maternal and fetal blood and amniotic fluid within 2 days after placement of the amalgam tooth restorations in sheep. Mansour et al. (1973) showed that the organic mercury had a stronger ability of transfer through placenta. The reported results suggest that Hg (both total and methylated forms) readily crosses the placental barrier. High concentrations of inorganic mercury in umbilical cord blood and placental tissue, may be due to the reason that in the body, methylmercury can undergo biotransformation to inorganic mercury by demethylation (Dock et al., 1994; Berlin et al., 1975).

Hubermont et al. (1978) who investigated the placental transfer of Hg in women living in a rural area of Belgium, have demonstrated that the Hg levels of blood samples from newborns (15.9 ng/ml) was higher than that found in maternal (14.5 ng/ml), and placental (9.7 ng/g) samples. In contrast, in a Czechoslovakian study involving 50 subjects (two groups belonging to an industrial environment matched with controls) from Truska et al. (1989), found practically no difference in the total Hg concentrations in maternal blood erythrocytes and plasma, cord blood erythrocytes and plasma and placenta. In an extensive study, Tsuchiya et al. (1984) determined total as well as methyl Hg in over 200 placental and related samples from Japanese women from the Nagoya city area, a typical urban environment characterized by high-density traffic and large population. These results showed that total Hg content in placenta ( $185 \pm 452$  ng/g) was significantly higher than in maternal ( $19 \pm 36$  ng/g) and cord ( $30 \pm 62$  ng/g) blood. Furthermore, the umbilical cord content of both total and MeHg showed the same tendency as the placenta. Although these findings may indicate that placenta probably acts as a barrier, it may be applicable only to high-level exposure situations coupled with chemical forms of Hg in the exposed environment (Tsuchiya et al., 1984).

### 3.6. Mercury level in maternal blood, umbilical cord and placenta

Yang et al. (1997) found out that in maternal

blood, placental tissue and umbilical cord blood, the concentrations of inorganic, organic and total mercury was significantly higher in the exposed group than in the non-exposed group, shown in Table 4 (Yang et al., 1997).

The concentration of organic, inorganic and total mercury in the umbilical cord blood and placental tissue were significantly higher than those in maternal blood, strongly indicating the presence of the transplacental pathway of mercury. The organic mercury was highly related to that of umbilical cord blood, showing the organic mercury had a stronger ability of transfer through placenta.

### 3.7. Nickel

Not many investigations are seen for Ni in Placenta. From available sources, the concentration of Ni in placenta appears to be in the range of 9–62 ng/g wet weight, with an average value of 36 ng/g.

Ni is released into the environment from many sources, e.g. during mining, smelting, and refining operations and considered to be an occupational hazard. Klopov (1998) revealed that nickel concentrations in cord blood were higher than in maternal blood, thus confirming the ability of nickel to pass through the placental barrier. It was reported that spontaneous and threatened abortions were increased in pregnancies in nickel-exposed workers (Chashschin et al., 1994). However, it is unclear at what exact exposure level this occurred. Results from Reichrtova et al.

Table 4  
Mercury level in maternal blood, umbilical cord and placenta ( $\mu\text{g/l}$ )<sup>a</sup>

Mercury	Maternal blood		Umbilical cord blood		Placenta	
	Exposed (mean $\pm$ S.D.)	Control (mean $\pm$ S.D.)	Exposed (mean $\pm$ S.D.)	Control (mean $\pm$ S.D.)	Exposed (mean $\pm$ S.D.)	Control (mean $\pm$ S.D.)
Organic	6.98 $\pm$ 3.16 <sup>c</sup>	2.50 $\pm$ 1.60	9.73 $\pm$ 7.44 <sup>b</sup>	4.18 $\pm$ 1.67	36.24 $\pm$ 18.66 <sup>b</sup>	21.52 $\pm$ 11.69
Inorganic	10.84 $\pm$ 4.22 <sup>c</sup>	4.27 $\pm$ 1.88	16.78 $\pm$ 7.23 <sup>c</sup>	6.22 $\pm$ 2.76	91.07 $\pm$ 23.49 <sup>d</sup>	46.56 $\pm$ 25.12
Total	17.85 $\pm$ 4.51 <sup>c</sup>	6.77 $\pm$ 2.12	26.51 $\pm$ 8.91 <sup>d</sup>	10.40 $\pm$ 3.25	127.31 $\pm$ 23.91 <sup>d</sup>	68.08 $\pm$ 18.88

<sup>a</sup>Data pooled from Yang et al. (1997).

<sup>b</sup> $P < 0.05$ .

<sup>c</sup> $P < 0.01$ .

<sup>d</sup> $P < 0.001$ .

(1998) have revealed the occurrence of nickel and lead in all three placental zones — basal plate, chorionic villous tree and chorionic plate. The lead and nickel appearance in the maternal, intermediate and periumbilical zones of full-term

human placenta demonstrated a continuous transport of metals from mother's side during the fetal/embryonic period. Simultaneously, a toxic effect of heavy metals on various placental structures may be chronic, resulting in the structural

Table 5  
As, Cd, Hg and Pb in placenta in exposure conditions (ng/g)

Country	As	Cd	Hg	Ni	Pb	Remarks (ref)
Australia					93	Port Pirie, lead smelting plant, lead bearing dust (Baghurst et al., 1991)
Belgium					133	Lubramont, rural; drinking water = 247 ng/ml (Hubermont et al., 1978)
India	62	10				New Delhi, lower class hospital (Sriramachari et al., 1996)
Japan		30	185 + 452 (2–3166)			Nagoza city: general industrial exhaust; heavy traffic. (Tsuchiya et al., 1984)
Mexico	5.9	10.9			122.5	San Luis Potosci city; smelting and metallurgy activities (Diaz-Barriga et al., 1995)
Poland		110			500	Upper Silesia; coal and metal mining and smelting (Baranowska, 1995)
Poland		127			510	Upper Silesia; coal and metal mining and smelting (Baranowska, 1995)
Russia				154	102	Norlisk, largest metallurgic production in the world (Klopov, 1998)
United Kingdom					350 (290–450)	Stoke-on-Trent; pottery, workers, painters, etc. (Khera et al., 1980)
Yugoslavia		15?				T. Mitrovica area (Loiacono et al., 1992)
Many countries		11–19				Smoking and Cd (Clark, 1977)

and functional impairments, respectively (Reichertova et al., 1998).

### 3.8. Intrauterine growth retardation (IUGR)

IUGR presents an increased risk of morbidity and mortality to the newborn. It may occur in 20% of children in some populations of developing countries, and in approximately 7% in industrial countries. IUGR is most probably multifactorial and may include maternal infection, malnutrition, placental dysfunction, hypertension, preeclampsia, smoking professional and environmental exposure (Kalinka et al., 1996). Nutrition may significantly influence the course of pregnancy, especially in groups with high exposure to toxic metals, particularly Pb and Cd (Bencko et al., 1995). Richter et al. (1999) studied the relationship between the concentrations of Pb and Zn in placenta of women with Intrauterine fetal growth retardation. They detected in the control group significantly lower Pb levels ( $11.31 \pm 5.79$  ng/g) and significantly higher Zn levels ( $20.52 \pm 11.92$   $\mu$ g/g) in placental tissue. The IUGR group had rather low levels of Zn ( $14.3 \pm 4.97$   $\mu$ g/g) and a higher level of Pb (15.24 ng/g). Higher age is associated with higher Pb levels in placental tissue, whereas Zn levels decrease. Richter et al. (1999) advised supplementation with Zn and substances that participate in defoliation mechanisms (ascorbic acid, folic acid) in a risk population.

### 3.9. Variations of toxic trace elements in placenta

Several environmental factors induce changes in metal concentrations in blood and placenta. Industrial activities related to mining, metallurgy, smelting, emissions, and even tobacco smoke are all known to affect the concentration levels of heavy metals. Literature data available in this regard are summarized in Table 5. Mostly Cd and Pb are studied in some detail while a number of other elements namely Al, As, and Ni need additional investigations. Data from different countries, especially for elements such as Pb show great differences, but a definite conclusion whether or not that these differences are true geographic indications, must wait until the quality

of the analysis is beyond doubt, since not all studies have validated their methods by analyzing certified reference materials. The use of analytical techniques that require minimum sample manipulation (e.g. solid sampling) are being applied to placental analysis and the results are encouraging. Thorough homogenization is the key and can be easily achieved by the use of brittle fracture technique (Iyengar and Kasparek, 1977). Furthermore, with the exception of methyl-Hg, systematic information about speciated analytes for several elements are not available. This is extremely important to have a clear understanding of the toxic effects and risk assessment. All these concerns should be addressed in the future.

## 4. Biomonitoring for assessment of environmental exposure

The use of biomarkers to monitor exposure to environmental chemicals is now frequently used in epidemiological investigations (*Science of the Total Environment*, 1993). General exposure-related biomarkers that are routinely collected in human subjects are blood, urine and breath. In specific cases, scalp hair is also used, but it is not free of objections due to the fact that it can be extraneously contaminated. On the other hand, studies using placenta are rather limited for a variety of reasons. A major reason is that problems of sample collection and handling (as discussed in part 1 under 'Sampling of placenta for analytical analysis') and ascertaining representativeness. In addition, placenta appears to be a less known analytical specimen among biological trace element researchers. All this has contributed to the fact that only a handful of epidemiological studies have been carried out, to elucidate the ability of placenta to act as a barrier to toxic metals such as As, Cd, Hg and Pb.

The potential of placenta or any other specimen as a biomarker depends upon the possibility to associate the presence of a chemical (in the form of metals, non-metals or any other pollutant sources) to a specific, and preferably a measurable biological parameter (i.e. a metabolite as functional indicators or some kind of measurable

biological effect). Having identified such a link the next task is to use it as a monitoring tool. Such procedures are carried out as either real-time monitoring (RTM) or as long-term monitoring (LTM) operations.

#### 4.1. RTM vs. LTM

The RTM is a means of frequently checking short-term changes of pollutant profiles using routine clinical specimens such as blood and urine (and occasionally also breast milk and saliva). The samples collected for RTM are analyzed as soon as access to the laboratory is available. Usually, these samples are not stored for a long time unless the project planning includes extended storage. On the other hand, samples for LTM should be reliable indicators of the long-term body burden of the chemicals identified in them.

Examples are hair (with some limitations), adipose tissue (for organic pollutants), and liver for both organic and inorganic pollutants. Also, samples obtained for LTM are an integral part of a long-term preservation scheme. Such a storage facility, when technically improved, transforms itself into what is now known as a specimen bank, which facilitates the preservation of specimens for decades if necessary, and enables deferred chemical characterization.

Concerning biomonitoring, it is crucial to define clearly in what context the information on the composition of a given biological specimen has been acquired. This is because, the criteria governing the acceptability of samples for the clinical diagnosis of deficiency or toxicity differ markedly depending upon the purpose of the investigation. For example, biological monitoring of pollutants, environmental pollution (i.e. expo-

Table 6  
Suitability of human clinical specimens for biological monitoring programs<sup>a,b</sup>

Metal	Specimen	Exposure	Risk assessment
Antimony	B <sup>c</sup>	+ <sup>g</sup>	? <sup>j</sup>
Aluminium	U <sup>d</sup>	++ <sup>h</sup>	?
Aluminium	B	+++ <sup>i</sup>	+++
Arsenic (inorganic)	U, P <sup>f</sup> ? (see text)	++	+
Arsenic (organic)	U	++	?
Cadmium	B, U, P	+++	+++
Chromium	U	++	+
Cobalt	U	+	?
Lead (inorganic)	B, P? (see text)	+++	+++
Lead (organic)	U, P? (see text)	+	+
Manganese (inorganic)	B, U	?	?
Manganese (organic)	U	++	?
Mercury (inorganic)	B, U, P? (see text)	++	++
Mercury (organic)	B, H <sup>e</sup> , P? (see text)	+++	+++
Nickel	B, U	+	?
Selenium	B, U	++	++
Tin	B, U	?	?
Vanadium	B, U	+	?

<sup>a</sup>Source: WHO, 1996.

<sup>b</sup>Pooled information from reference 1 (See Iyengar, 1989, Chapter 4).

<sup>c</sup>Whole blood, plasma or serum.

<sup>d</sup>Urine.

<sup>e</sup>Hair.

<sup>f</sup>Placenta.

<sup>g</sup>Weak.

<sup>h</sup>Moderate.

<sup>i</sup>Well established association.

<sup>j</sup>Not known.

sure), for nutritional surveillance and monitoring, and forensic investigations (Iyengar, 1989).

#### 4.2. Common biomarkers in practice

An indication of the suitability of various human tissues including placenta, and body fluids for use in the biomonitoring of a number of trace elements and in assessing exposure to toxic elements, is presented in Tables 6 and 7. The feasibility of the selective monitoring of essential and other trace elements through analysis of whole blood and its components or of hair, urine, feces or milk is discussed elsewhere (WHO, 1996). By combining this information with what is known about placental physiology (e.g. as a barrier for selected trace element transport) as discussed in an earlier section of this report, several useful approaches for monitoring can be developed for practical use.

As in many cases, making decisions based on trace analytical results whether it is to assess health effects of the presence of a pollutant, or for judging the suitability of a biological specimen as a biomarker that involves some kind of analytical measurements to be performed on it, depends on how well the biological meaning of the specimen is understood and how consistent the measurements are. The placenta is no exception to this situation and, if any, it presents a situation that is complicated by its physiology, susceptibility

to great variations during sampling and, finally, the analysis itself, all of which can be truly challenging in some cases. Trace metal determinations are particularly prone to all of the above parameters.

It is an essential requirement that the maternal and fetal compartments collected for investigation be accurately defined to facilitate meaningful interpretation of analytical data on such specimens. Furthermore, metals and non-metals (and among metals each metal) may be expected to exert their own metabolic characteristics, may mutually influence concentration levels and distribution within the placental tissue, as well as their movement across the placenta. Considering that during the term of pregnancy women undergo extensive changes in body composition resulting in the redistribution of chemical constituents, any additional burdens from environmental and other sources are superimposed on the changes already taking place. This combination of events becomes an important parameter in data interpretation and presents a difficult situation.

### 5. The placental role as a biomarker

Slikker and Miller (1994) concluded that the human placenta can represent an environmental record of exposure for its 9 months of existence.

Table 7  
Human clinical specimen suitable for assessing exposure to toxic elements<sup>a</sup>

Tissues	Arsenic	Cadmium	Lead	Inorganic mercury	Methyl mercury
Blood	X	X	X	X	X
Bone			X		
Brain				X	X
Faeces		X	X		
Hair	X		X <sup>b</sup>		X
Kidney		X	X	X	
Liver		X	X		X
Placenta	See text	X	X	See text	See text
Teeth			X		
Urine	X	X	X	X	X

<sup>a</sup> Pooled information from reference 1 (See Iyengar, 1989, Chapter 4).

<sup>b</sup> Sampling and preparatory critical steps.

Several heavy metals such as Cd, may be preferentially bound by the placenta retarding passage to the fetus and, therefore, reducing damage to the fetus. On the other hand, it is certainly possible that the placenta can be selectively damaged resulting in malformations, growth retardation or fetal death. Placenta as a biomarker has been already suggested by Baglan et al. (1974) as an alternative to repeated maternal blood sampling for assessing lead exposure in utero. To understand the role of placenta as a biomarker, it is useful to compare it with amniotic fluid, which is a recognized specimen in determining congenital abnormalities. This process is repeated if needed at different intervals, or if reconfirmation of the diagnosis is needed. Thus, amniotic fluid is used as an established diagnostic specimen. In the case of placenta, samples are usually obtained at time of parturition, a one time event. Hence, each pregnancy has to be looked upon as an RTM process since the affected species is exposed to the placental source of pollutants, only during the course of that particular pregnancy.

In the case of fetus, exposure to placental sources of pollutants is an in utero event. Hence, the usefulness of placenta as a biomarker comes into picture in an unusual way; an assessment of the presence of a pollutant in placenta, and subsequent means to reduce the pollutant burden of the maternal environment (while beneficial for future pregnancies) does not necessarily help the infant (from the present pregnancy). At this stage, it is the mammary barrier that is the deciding factor until the infant is weaned. In cases where the placenta acts even as a partial barrier (e.g. to Cadmium), it suggests a decrease of the harmful effects, if any. However, in the case of organometallic compounds such as MeHg, although the measured levels tend to be similar in maternal and fetal specimen due to a lack of barrier-effect, the information is still very useful as a forewarning since the neurological effects of MeHg are likely to show up later in adult life (Guzelian et al., 1992). Preventive measures to reduce post-natal exposure to MeHg is warranted under these conditions. A similar case can also be made for Pb.

### 5.1. Arsenic

The best evidence for placenta as a biomarker for As has come from studies of Diaz-Barriga et al. (1995). Placental samples collected from an industrial belt known for smelting and related metallurgical operations, 2.3 times greater As levels were measured in samples from industrial areas than those collected from rural districts, some 300 km away. Measurement of As was also undertaken in water, soil, air and household dust samples, and were shown to contain higher levels in the industrial environment than those from rural source, confirming a generally high exposure, and the link between exposure and elevated As levels. The As levels were also high in scalp, pubic hair and urine in the exposed subjects (Diaz-Barriga et al., 1995). Considering that hair is susceptible to external contamination (although a good indicator for As exposure), and that placental As correlates with that of urine (as a RTM) and placenta as an intermediate long-term indicator of As exposure, the combination of placenta and urine can be considered as a practical tool for monitoring pregnant women.

### 5.2. Cadmium

In a study from former Yugoslavia, placenta collected under different environmental conditions have shown that those samples coming from industrial areas (predominantly smelting operations), showed a 50% increase in placental Cd (Loiacono et al., 1992). In another study investigating Cd levels in blood in mother–newborn pairs from the surrounding of Cu smelters in Sweden (Lagerkvist et al., 1992), and a control area reported that the Cd levels of the newborns were approximately 70% of those in the mothers. Berlin et al. (1992) measured Cd in placenta of workers from a battery factory and found that the placental Cd levels were positively correlated with maternal blood Cd concentrations. Several studies have reported similar findings as summarized in various reports (Karp and Robertson, 1981; Goyer, 1995). Literature data for Cd in maternal and fetal blood and placenta are not consistent. This problem is compounded by analytical dif-

difficulties associated with measuring very low concentrations of Cd in blood and related matrices, and the difficulty in meaningfully classifying the so-called normal and other types of exposure of the investigated subjects to environmental Cd. With the exception of samples clearly separated due to occupational exposure, control populations from rural and urban areas need to be carefully qualified (e.g. food and living habits, age, etc.) to be reference groups. Notwithstanding any of these drawbacks, placental Cd concentrations have been found to be positively correlated with maternal blood Cd concentrations, and there are clear indications that fetal blood Cd levels are below that of maternal blood Cd levels, an indication that placenta at least acts as a partial barrier, thereby lessening the harmful effects to the fetus. As an accumulating organ during the 9-month period, the placenta can be used to monitor the Cd exposure of pregnant mothers (Lagerkvist et al., 1996).

Based on the well designed study of Hubermont et al. (1978), at term the Cd concentration of placenta reaches a 10-fold high average value in comparison with that found in maternal blood, providing the most convincing evidence of the barrier role played by placenta in preventing Cd from reaching the fetal tissues. Another compelling evidence is presented in a study by Baranowska (1995). In this investigation, samples of maternal and neonatal blood as well as placenta collected from the intensely industrial Upper Silesian region of Poland (98% of all the coal mined, 100% of the Zn and Pb are processed, 53% of steel production, 35% coke consumed and 29% of energy generated) were analyzed and the following results obtained. Cadmium in venous blood, cord blood and placenta equalled 4.9, 1.1 and 110 ng/g, respectively. Thus, high concentrations of Cd were found in placenta as a barrier in sequestering excess Cd from the blood and minimizing transfer to the fetus.

Furthermore, the review presented by Miller et al. (1988) indicates that there is sufficient evidence to support variation of Cd levels in placenta to reflect the geographic differences (8 ng/g in the Netherlands to 176 ng/g in the Ruhr Valley in Germany) and related environmental

sources. Also, a distinct correlation has been established between smokers and non-smokers (Lagerkvist et al., 1992), designating smoking as the most important environmental exposure in relation to Cd. Diaz-Barriga et al. (1995) measured 8.1 times greater Cd levels in samples from industrial areas than those collected from rural districts, some 300 km away. Measurement of Cd was also undertaken in water, soil, air and household dust samples, and were shown to contain higher levels in the industrial environment than those from the rural source confirming the link between excessive exposure and elevated Cd levels. Among other samples, hair appeared to be unsuitable while urine showed 8.8 times greater Cd levels when compared with samples from control areas. Thus, placenta has been shown to block Cd efficiently. Similar to the case of As, the combination of urine (as a RTM) and placenta as an intermediate-term indicator of Cd exposure, the combination of placenta and urine provides a practical tool to monitor pregnant women. Thieme et al. (1986) have used placenta to monitor for Cd in the urban areas of Munich. Ward et al. (1987) have reported highly significant negative relationships between placental Cd and Pb levels, and birth weight. Cadmium accumulation in the placenta is not unequivocal. Truska et al. (1989) have reported that in their study that included samples from industrial exposure areas, no Cd uptake was found. However, results from Mexico city found that maternal blood cadmium was significantly correlated with cord blood cadmium levels, while cord blood was correlated with newborn blood cadmium. Previous smoking habits of the mother increased maternal blood cadmium concentrations significantly, but did not modify cadmium concentrations of either the cord or the newborn. The latter suggests the existence of a placental barrier for cadmium. Birthweight was found to be inversely associated with cord blood cadmium levels (Galicia-Garcia et al., 1997).

### 5.3. Lead

Evidence for placenta as a possible biomarker for Pb has also come from the studies of Diaz-Barriga et al. (1995). Placental samples collected

from an industrial belt showed 2.2 times greater Pb levels than those collected from rural districts. Measurement of Pb was also undertaken in water, soil, air and household dust samples, and were shown (except from water) to contain higher Pb levels in the industrial environment than those from rural source, providing a link between exposure and elevated Pb levels. The Pb levels were also high in samples of pubic hair (but not so in scalp hair), but not significantly different in urine specimens in either of the populations. In another investigation (Baranowska, 1995), samples of maternal and neonatal blood as well as placenta collected from the intensely industrial Upper Silesian region of Poland (98% of all the coal mined, 100% of the Zn and Pb are processed, 53% of steel production, 35% coke consumed and 29% of energy generated), were analyzed and the following results obtained. Pb in maternal blood, cord blood and placenta equalled 72.5, 38.3 and 500 ng/g, respectively. Thus, high concentration of Pb was found in placenta, but the levels observed in maternal blood sample is only twice that of cord blood samples indicating efficient transfer of Pb to the fetus. The accumulation of lead in maternal blood depends on the trimester of gestation (Schell et al., 2000).

Although high levels of Pb in the environment are also reflected in placenta, in terms of protecting the fetus the barrier factor appears to be insufficient. Based on the study of Hubermont et al. (1978) investigating the influence of low and high (11.8 and 247  $\mu\text{g/l}$ ) Pb in drinking water, a significant difference in the Pb levels in maternal and cord blood and in placenta was found in the two groups of subjects. Although the differences in placental and blood Pb levels were small, the maternal and fetal blood Pb levels were correlated with water Pb. The similarity of the influence of water Pb on both maternal and fetal blood levels indicates a rapid transfer of Pb from mother to fetus, and masks the influence of placenta as a barrier, if any. Therefore, for monitoring purposes during pregnancy, water analysis in combination with maternal blood levels provides a good tool and emphasizes the need to prevent undue Pb exposure during pregnancy (Hubermont et al., 1978). However, placental Pb

appears to have some value as an intermediate-to long-term indicator of Pb exposure, and appears to be a useful tool for monitoring pregnant women.

Thieme et al. (1986) have used placenta to monitor for Pb in the urban areas of Munich. Measurements in mother's venous blood, placenta, and cord blood showed (venous blood, Pb = 72.50 ng/ml, Cd = 4.90 ng/ml; in placenta, Pb = 0.50  $\mu\text{g/g}$ , Cd = 0.11  $\mu\text{g/g}$ ; in cord blood, Pb = 38.31 ng/ml, Cd = 1.13 ng/ml) that elevated concentrations of lead and cadmium were found in placenta and in maternal blood, whereas in neonatal blood there was an increased concentration of lead and only traces of cadmium. It is concluded that the placenta is a better barrier for cadmium than for lead (Baranowska, 1995). That placenta has only a small filtering effect was also shown by Nashashibi et al. (1999). Finally, Baghurst et al. (1991) have observed in Port Pirie, South Australia that there is considerable inter-individual variation in the efficiency of transfer of Pb from maternal blood to placental tissue. Port Pirie has a large lead smelting plant and a history of past contamination with lead-bearing dusts (Body et al., 1988).

#### 5.4. Mercury

Monitoring for Hg through placenta appears to be still evolving. The animal experiment demonstrating that the ratio of MeHg concentrations in the placenta to that in blood of the newborn being close to 1, indicates that the placental value can serve as an indicator (Mansour et al., 1973). Maternal and fetal blood are equally effective. Monitoring the maternal and fetal blood for Hg, especially in women consuming large quantities of fish has been shown to be effective. Although placenta was not studied in this case, when maternal blood Hg levels are elevated, then higher cord blood levels are found (Dennis and Fehr, 1975). Furthermore, fetal levels have been shown to rise more sharply than corresponding maternal levels. Similar results have been reported by Baglan et al. (1974). On the other hand, based on the study of Hubermont et al. (1978), placental barrier does not exist for Hg. In a Japanese study using amni-

otic fluid as a monitoring specimen, it was demonstrated that inorganic Hg was consistently higher than that of organic Hg by a factor of 3 (Suzuki et al., 1977). These investigations also reported that Hg levels changed according to gestational age for both forms of Hg, with the highest value being observed in the seventh month of gestation. The reasons for the increase of Hg during the seventh month of pregnancy are still unclear. Interestingly, it was observed that most of the fetal Hg was in the inorganic form, contrary to the supposition that transplacental route favors organic forms of Hg, and neither is there evidence of biotransformation of organic Hg in the fetus to inorganic Hg (Suzuki et al., 1977).

## 6. Concluding remarks

Reliable results for toxic trace elements and organic pollutants in placenta are still scarce and, therefore, well-designed epidemiological scale studies are needed to establish baseline values as well as changes arising from exposure to specific industrial and other environmental conditions. As a clinical specimen, the easy accessibility to placenta makes it very attractive to study the environmental impact of several elements considered harmful to health.

Consistently high concentrations of Cd found in placenta and in maternal blood (but not in the neonatal samples) clearly confirm the role of placenta as a barrier in sequestering excess Cd from the blood and, thereby, minimizing its transfer to the fetus. A distinct correlation has been established between smokers and non-smokers, thus identifying smoking as an important source of environmental exposure to Cd. Placental Pb has some value as an indicator of Pb exposure (long- and short-term), and appears to be a useful tool to assess maternal health during pregnancy. However, maternal blood appears to be a more reliable indicator that can also be used to predict the status of Pb in the fetus. In combination with urine (as a real time monitor), the placenta is a practical tool to monitor pregnant women for Arsenic exposure. Hair continues to be an effec-

tive and practical indicator specimen for Arsenic. For Hg, based on available information, the human hair is a better specimen especially for monitoring MeHg, while the use of placenta for this purpose appears to be still evolving.

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