

## Residues of PCDDs and PCDFs in human milk samples in Ahmedabad, India

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### Introduction:

Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated dibenzo furans (PCDFs) represent a class of organic environmental pollutants. They are unwanted byproduct of incineration, uncontrolled burning and certain industrial processes. They are persistent in nature and bioaccumulates through food chain. These are hazardous to human health and environment<sup>1-3</sup>. The residues of these toxicants have been detected in human adipose tissue, blood and milk<sup>4-7</sup>.

Their health effects include skin disorders, induce stillbirths, cancer and also disrupting endocrine functional properties through aryl hydrocarbon receptor (AhR) mediation<sup>8-10</sup>. The affinity for AhR has been used to build a toxic equivalency factor (TEF) list; which is used in risk assessment of these toxic halogenated aromatics<sup>11, 12</sup>. Dioxins were first identified in human milk in 1980<sup>13</sup>. The concern about the presence of these compounds in breast milk and their effects on breast-fed infants health is raising worldwide<sup>14</sup>.

In Fukuoka, epidemiological studies showed that 13 women in Yusho families had given 11 live births and two stillbirths. Ten babies had shown the characteristic grayish dark brown-pigmented skin at birth and majority of the babies were small for dates<sup>15</sup>. Later it was confirmed that PCDD/Fs were transferred through females to their fetus via placenta and breast milk<sup>16-17</sup>. It has been also reported that after 7 years of exposure the 13 children were seen to be apathetic and dull with low IQs<sup>18</sup>. A low male to female sex ratio at birth was reported after the Seveso accident<sup>19</sup>.

WHO has coordinated two rounds of follow up studies on levels of PCDD/Fs and PCBs in human milk and the data shows a decreasing trend during the last 30 years. However, in India there is no data available on the exposure and residues of these contaminants. This study presents first time the levels of dioxin and furans in human milk samples collected from the Ahmedabad city in India.

### Methods and Materials:

Breast milk samples were obtained from 23 primiparas, who were in good health and hospitalized at Ahmedabad, India. Samples were collected from the subjects whose infants were less than 2 weeks of age. Subjects were selected randomly and written consent was obtained from all the subjects after explaining the detailed purpose and importance of the study. The detailed exposure assessment questionnaires, including health status, food frequency pattern, smoking status, medication use were obtained. The work history and residential history of mother and father was also taken in to consideration.

Breast milk samples minimum (20 ml) were collected and frozen immediately and stored at  $-80^{\circ}\text{C}$  until analysis were performed. The thawed milk samples were homogenized and representative samples were spiked with standard to check the efficiency of the procedure. The extraction of the samples were carried out according to the EPA approved conventional procedure and the clean up of the samples were performed on silica gel, alumina and finally on carbon column. All the reagent and chemicals were analyzed to check the presence of background contamination. The lipid was extracted by acetone/hexane partitioning.

The quantification and confirmation of the residues were carried out on HP 6890 GC equipped with micro ECD and Perkins Elmer Auto XL gas chromatograph with Turbo mass (Mass spectrometer). The capillary columns, HP-5 and PE-5 MS were used. The dimensions of the columns were 30m X 0.25mm X 0.25 $\mu\text{m}$  and 30m X 0.25mm X 0.5 $\mu\text{m}$  respectively. The instrument conditions were as follows- MS were operated in selected Ion monitoring mode. The conditions were Injector temperature –  $250^{\circ}\text{C}$ , EI Source -  $280^{\circ}\text{C}$ , Transfer line -  $280^{\circ}\text{C}$  Mass scan – 50m/z to 500m/z. Oven temperature programming were as  $90^{\circ}\text{C}$  for 2min, Rate  $20^{\circ}\text{C}/\text{min}$  to  $200^{\circ}\text{C}$ , hold for 2 min, Rate  $3^{\circ}\text{C}/\text{min}$  to  $300^{\circ}\text{C}$ , hold for 1 min, Carrier gas helium, Flow rate 1ml/min. For GC the conditions were - Injection mode-Split/Split less, Injector temperature- $220^{\circ}\text{C}$ , Detector temperature- $275^{\circ}\text{C}$ , Oven temperature programming: Initial temperature- $150^{\circ}\text{C}$  for 2 min then increase up to  $270^{\circ}\text{C}$  at the rate of  $5^{\circ}\text{C}$  per min, Total run time-46 min, Carrier gas-Nitrogen gas, Purge Flow-60 ml/min.

All the standards were procured from Accu standard USA and Wellington Canada. Other chemicals used were of high purity grade from Sigma and Merck.

### Result and Discussion:

We have measured the five congeners of PCDD/Fs in human milk samples collected from 23 primiparas. Table –1 shows the age, height, weight and dietary habits of the subjects. It also shows the Total PCDD/Fs and percentage of lipid in milk samples collected from the subjects.

The concentration of dioxin is reported by a quantity, that is Toxic Equivalent Quotient (TEQ) of total Dioxins (PCDDs + PCDFs). The toxicity of dioxin differs for every congener and mainly depends on number and position of chlorination. The toxicity of 2, 3, 7, 8 tetra chlorination is the strongest, the other isomer is expressed by Toxic equivalent factor (TEF), as relative toxicity for 2, 3, 7, 8- tetra chlorination. Table 1 shows the Total PCDD/Fs in pg-TEQ/g. The value ranges from 2.49 to 14.24 with an average of 6.22. Reports from other countries

showed a wide variation in residues of dioxin in human milk samples (6 to 40 pg-TEQ/g)<sup>20</sup>. Average % of lipid in the milk samples was 2.92%. The percentage of lipid ranges from 1.62% to 5.00%. Fig 1 shows the relationship between the total Dioxin and the lipid content in breast milk. Figure showed a good correlation ( $r^2 = 0.7814$ ). We have selected primiparas as subjects because the residues of Dioxins are significantly at higher levels in primiparas than in multiparas<sup>21</sup>. The subjects were selected randomly. From the table, it was found that out of 23 infants born only 6 were male and 17 were females. Figure 2 shows the ratio of male and female infants. Survey of the literature showed a reduced proportion of male births in several countries. Results of the many studies showed that there is an increased probability of female births with increasing Dioxin levels of father's Serum<sup>22, 23</sup>. Exposure of males during their pre and puberty years may be especially relevant as fathers exposed where less than 19 years of age sired significantly more girls than boys. Mother's serum Dioxin levels were not a significantly predictor of the probability of a male birth. This indicates that the pre and pubertal years may be very sensitive period to dioxin action in human males<sup>24</sup>. Age of the subject's ranged from 18 to 30 years. The average height and weight of the mothers were 150.57 cm and 47.22 Kg respectively. From the table, it was observed that most of the subjects are vegetarian. Only two subjects included non-vegetarian food like meat and fish frequently in their diet. However, all the subjects ate the food supplied by the hospital during their hospitalization period. The higher residues in subject No 4 and 19 might have been because of their intake of non-vegetarian diet.

Breast milk is an ideal medium for assessing exposure to Dioxins with long half-life. These toxicants persist in breast milk as they do in the environment. Breast milk monitoring serves as an indicator of past human exposure or environmental conditions. The Infant level of TCDD at birth are 25% of the maternal levels<sup>25</sup>. Breast fed infants typically ingest TCDD at 50-100 fold higher level than adults, on a baby weight basis<sup>26,27</sup>. Infants absorb 90% of the ingested<sup>28</sup>. TCDD and may exceed the adult acceptable daily intake.

This is the first report to document the extent of PCDD/Fs contamination in human milk samples collected from India. This study shows the presence of PCDD/Fs in the Indian environment. This is a base line information because quite a few samples were analyzed from only one city of the country, therefore, a systematic nation wide monitoring is warranted to get the actual pattern of exposure.

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**TABLE-1 : Demographic characteristics of primiparas, lipid & TEQ of PCDD/Fs in Human milk samples**

S.NO	DIOXIN (pg- TEQ/g)	AGE (Years)	HEIGHT (Cm)	WEIGHT (Kg)	LIPID (% gm)	MALE	FEMALE	DIET
1	5.26	23	149.5	47	1.80		F	Vegetarian
2	6.68	22	148.5	40	3.80		F	Vegetarian
3	4.41	20	139	44.5	2.80	M		Vegetarian
4	6.53	25	144.50	38	3.00		F	Mixed
5	2.57	20	150	45	1.80		F	Vegetarian
6	3.62	23	160	55	1.82			Vegetarian
7	7.55	25	152	52	2.80		F	Vegetarian
8	2.49	20	150	46	1.62			Vegetarian
9	8.49	25	154	44	4.20	M		Vegetarian
10	6.91	20	158	59	2.50		F	Vegetarian
11	2.59	20	148	40	1.80		F	Vegetarian
12	7.37	25	156	46	3.60		F	Vegetarian
13	11.00	20	152.5	50	4.50		F	Vegetarian
14	8.10	21	143	50	3.50	M		Vegetarian
15	8.22	20	152	54	3.80		F	Vegetarian
16	3.38	20	145	44	3.00		F	Vegetarian
17	4.61	30	147.5	50	2.60		F	Vegetarian
18	3.49	20	150.2	40	2.00		F	Vegetarian
19	14.24	18	153.5	47	5.00	M		Mixed
20	3.06	21	159	52	1.80	M		Vegetarian
21	6.28	20	155	51.5	2.00		F	Vegetarian
22	9.01	25	143	47	4.20		F	Vegetarian
23	7.10	22	153	44	3.20		F	Vegetarian
<b>Mean</b>	<b>6.22</b>	<b>21.96</b>	<b>150.57</b>	<b>47.22</b>	<b>2.92</b>			
<b>Min</b>	<b>2.49</b>	<b>18</b>	<b>139</b>	<b>38</b>	<b>1.62</b>			
<b>Max</b>	<b>14.24</b>	<b>30</b>	<b>160</b>	<b>59</b>	<b>5.00</b>			
<b>SD</b>	<b>2.95</b>	<b>2.77</b>	<b>5.38</b>	<b>5.31</b>	<b>1.01</b>			

Figure 1

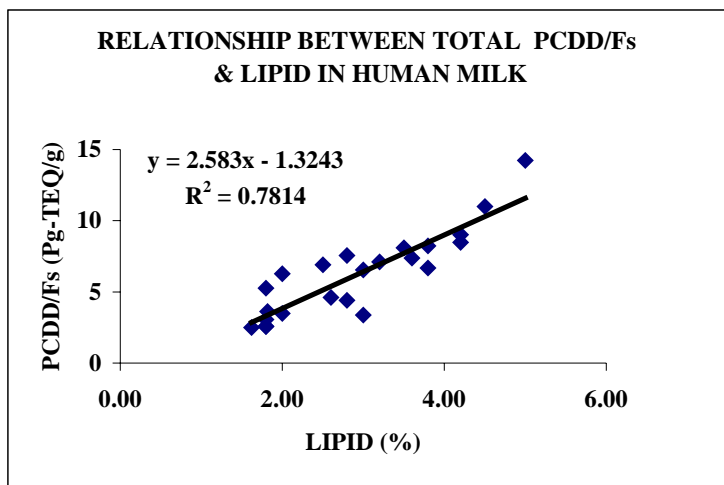
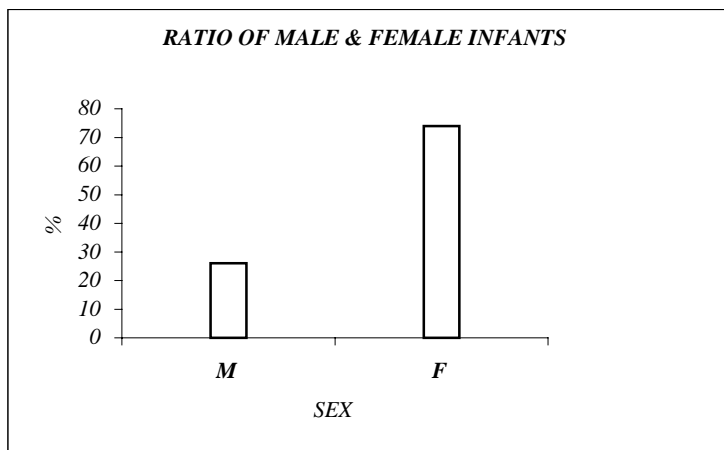


Figure 2



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