Perinatal dioxin exposure in the Netherlands: the Amsterdam-Zaandam cohort, and a 15 year follow-up.

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Introduction:
In 1987 in the Amsterdam – Zaandam region in the Netherlands a study was started to the effects of Poly-Chlorinated- Dibenzo- Dioxin (PCDD's) and Poly-Chlorinated- Dibenzo-Furans (PCDF's) exposure in the perinatal period and lactation.
In 1990 a grant became available making it possible to extend the study to 120 mother-baby pairs. Out of these 120, 44 mother-baby pairs were included in the study, because the babies must be breastfed at least 11 weeks exclusively.
Levels of dioxins and furans using ITEQ were in this group between 8-63 ng/kg milkfat in their breastmilk 3-4 weeks after birth.
In the period 1987-1990 14 mother-baby pairs were studied with levels between 29-93 ng/kg milkfat. In this group no laboratory tests were done.
The levels measured in breast milk were used as prenatal exposure. Postnatal exposure through breast milk was calculated as the concentration measured in breast milk multiplied by the amount of breastmilk the baby consumed during the breastfeeding period.
In the following you find a summary of our findings in this birth cohort until puberty.

In the Perinatal Period:
Main findings in the 44 mother-baby pairs were [(1-6)]:
Effects:
A significant effect on thyroid metabolism was found in relation to prenatal exposure in the baby. There was a tendency to an increased T4 level in cord blood that became significant at the time-points 7 days and 11 weeks after birth. At 11 weeks also the thyroid stimulating hormone (TSH) level was significantly increased.
A significant lowering in poly nuclear white blood cells and monocytes were found in the first weeks after birth.
After 11 weeks a significant lowering of blood platelets was found in relation to the cumulative exposure through breast milk .
An increase in the liver enzymes Aspartate-Aminotransferase (AST) and Alanine-Aminotransferase (ALT) were found in relation to postnatal exposure.
A significant negative relation was found between the congener 2,3,7,8- TCDF and the vitamin K1 level at the age of 11 weeks.
A diet used by the mother during a week either carbohydrate-rich or fat- rich and both dioxin low did not influence the dioxin levels in breast milk.
No effect was seen on growth or development of the baby at the age of 6 months.

The effect on thyroid hormone metabolism was interpreted as an interference of dioxins with the thyroid regulatory system. We hypothesize that dioxins ultimately decrease the nuclear T3 receptor occupancy of the neuron by either an effect on the enzyme 5'-deiodinase (D2), which results in a decreased conversion of T4→T3 or because of an effect on the transporter protein Monocarboxylate transporter 8 (MCT 8) that brings T3 from the folliculo-stellate cell, a sort of glia-cell into the neuron.

We hypothesize that the negative effect on the number of blood platelets might be explained by accumulation of delta-Amino-Laevulinic –Acid (ALA) in bone marrow due to interference of dioxins with the Heme synthesis

pathway. An increase in ALA stimulates the differentiation of the stem cell into erythroblasts and erythrocytes at the cost of the differentiation into megakaryocytes and blood platelets.
At the age of 2 years and 7 months:

The Amsterdam- Zaandam group was studied at the age of two years and seven months. Signs of enhanced neuromotor maturation were found and it was hypothesised that this may be due to the thyroxine-agonistic action of dioxins [7]. Enhanced neuromotor maturation is not necessarily a favourable effect. No longer effects were found on thyroid-hormone- regulatory system or on the numbers of white cells or on the liver-enzyme levels. Blood platelets were not controlled.

At the age of 7-12 years:
A follow-up study was done of the original 14 mother-baby pairs, that entered the study between 1987-1990 and the 44 mother-baby pairs from the original group born in 190-1991. We were able to control 42 children. Not all participated in every study.
The children (41) were controlled for brain function with help of visual evoked responses and Electro-Encephalo-Graphy (EEG) and Magneto-Encephalo-Graphy (MEG). Psychological and neuromotor tests were done. In the blood cytochrome P-450 activity was tested with help of a paraxanthine/caffeine molar ration in venous blood after a caffeine loading test. Furtheron TSH, FT4, ALT and AST were measured.
Haematological and immunological parameters were controlled and related to the health status (allergy) and infections.
Lung function was measured.
Dental examination was preformed.

The following effects at this age were found in relation to the perinatal exposure to dioxins:
Brain function studies revealed a significant negative effect on the latency times and amplitude in relation to prenatal and postnatal dioxin exposure. Spontaneous alpha frequency and alpha amplitudes were not affected. IQ-test and neurologic examination were not related to dioxin exposure. However in the behavioural field there were more social problems, and more aggressive behaviour as reported by the teachers. And an increase in anxious-depressed feelings as reported by the parents. [8]
The Cytochrome P450-activity (Cyp 1A2) was not related to either the pre- or postnatal exposure as measured with help of a caffeine loading test. Neither were the thyroid hormone levels, TSH-levels and liver enzyme levels ALT and AST. [9]
The follow-up findings in haematology and immunology and health status revealed a decrease in allergy, persistently decreased thrombocytes, increased thrombopoietin, increased CD4+ T-helper and increased CD45RA counts. So indications of persistent effects at stem cell level until minimally 8 years after birth.
Lung function (FEV1/FVC) was negatively related with prenatal and postnatal dioxin exposure. An increase in bronchial obstruction and a reduction in lung function in relation to both a prenatal and postnatal dioxin exposure. [10]
No abnormalities were found in dental enamel in our cohort.

The persistent effect on brain function is of great concern. Together with behavioural problems this might be a basis for an increase in criminality. The same effect on behaviour was also described in the Yucheng-cohort. [11]
Dioxins might cause a defective myelination, have a direct effect on neurons or works as an endocrine disruptor by inhibiting important enzymes like the 5'- deiodinase (D2) necessary to convert T4 → T3 in glia-cells. Myelin is formed by glia-cells especially in the period of the brain spurt from 30 weeks gestational age with a peak around birth and then slowing down until adolescence. Most neurons are formed in the early trimester of pregnancy. Interestingly the folliculo-stellate cells involved in the thyroid hormone metabolism in the pituitary gland and hypothalamus are a sort of glia-cells some with HLA- markers like the white blood cells. In these cells T4 is deiodinated to T3 by the enzyme 5'deiodinase (D2). Knock-out mices for this D2 enzyme demonstrates a thyroid hormone resistance. In humans the genetic disease of thyroid hormone resistance is wellknown to cause the Attention Deficit Hyperactivity Disorder (ADHD). [12] Halogenated dioxins and biphenyl derivatives have a structural specific interaction with the iodothyronine-5'deiodinase in rat liver, resulting in a decrease in the conversion of T4 → T3. [13] This makes the inhibition of this enzyme by dioxins a candidate for causing the hypothyroid state intra-cellularly in the neuron as we found around birth.
The finding, that the Cyp 1A2 activity is not influenced by dioxins in the perinatal period might be related to the fact that activity in vivo is present not earlier than 6 months after birth. [14] An abnormal imprinting of this activity under the influence of dioxins in the perinatal period could not be demonstrated.
The finding of a persistent lower number of blood platelets and the abnormalities in the immune cells were found in relation to the perinatal exposure, as is the decreased allergy and the chest congestion. However we cannot

exclude that current levels might play a role. Current levels were not measured. Effects on immune cells are interesting. Vos discussed already long ago the possible relation of dioxins with auto-immune diseases based on immuno-suppression. [15] Diseases like anorexia nervosa, more seen in the offspring of DES-mothers are probably based on auto-immune processes against the hypothalamus. There is a proven rise in anorexia in general and in other auto-immune diseases like type 1 diabetes, asthma and Crohn's disease in the last decades that must be related to environmental causes. Interestingly in Crohn's disease quite recently a defect in innate immunity is demonstrated and it is hypothesized that this abnormality is the cause of the disease. In this respect is the modulation of serum complement levels (belonging to innate immunity) following exposure to PCDD's in mice noteworthy. [16]

The findings in lung function are new. We hypothesize an effect on growth of the lung as is also seen in the offspring of smoking mothers. [17]
An important protein for both the lung and innate immunity is the Surfactant protein A produced by the Clara-cells in the lung. Clara-cells are susceptible to the toxic effects of dioxins and hyperproliferation is described. This protein is also important for the prevention of Otitis Media, as it is expressed in the Eustachian Tube. [18] In the Rotterdam studies an increase in Otitis Media is described in relation with current PCB-levels. [19]

At the age of 13-18 years; puberty
In 2005 the cohort was studied again. We were able to trace and include in this study 35 children out of the original 60. Pubertal development and growth are assessed. Current dioxin- and PCB-levels are measured. Lung function, haematology, lipid spectrum, and glucose metabolism will be studied.
A relation was found between the initiation of breast development and prenatal dioxin exposure. [20] This finding confirms the finding by Fenton c.s. in rat studies. [21] Retardation in breast development was also found in a cohort study in Belgium in relation with current dioxin exposure. [22] Abnormalities in growth of a tissue or organ prenatally is always tricky, because it makes the tissue or organ vulnerable for later development of malignancies.

In conclusion:
In many organs subtle abnormalities are found at follow-up of a birth cohort in relation to the background levels of dioxins in the eighties and nineties of last century in the Netherlands. An effect on reproduction must wait for later research.

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