

## Persistent Hematologic and Immunologic Disturbances in 8-Year-Old Dutch Children Associated with Perinatal Dioxin Exposure

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Perinatal exposure to Dutch "background" dioxin levels in 1990 was high, but comparable with that of other industrialized Western European countries. Exposure during the sensitive perinatal period may cause permanent disturbances. Therefore, we assessed the health status and various hematologic and immunologic parameters among our longitudinal cohort. A medical history was taken and venipuncture performed in a longitudinal cohort of 27 healthy 8-year-old children who had documented perinatal dioxin exposure. Linear regression revealed a decrease in allergy in relation to prenatal ( $p = 0.02$ ) and postnatal ( $p = 0.03$ ) dioxin exposure. Increases in CD4<sup>+</sup> T-helper cells ( $p = 0.006$ ) and in CD45RA<sup>+</sup> cells ( $p = 0.02$ ) were seen in relation to postnatal exposure. A persistently decreased platelet count ( $p = 0.04$ ) and increased thrombopoietin concentration ( $p = 0.03$ ) were seen in relation to postnatal exposure. This follow-up has shown a decrease in allergy, persistently decreased thrombocytes, increased thrombopoietin, and increased CD4<sup>+</sup> T-helper and increased CD45RA<sup>+</sup> cell counts. This study provides indications of effects at the stem cell level of perinatal dioxin exposure, persisting until minimally 8 years after birth. **Key words:** allergy, dioxin, hematology, human, immunology, pediatrics, perinatal, T cells. *Environ Health Perspect* 111:1519–1523 (2003). doi:10.1289/ehp.5715 available via <http://dx.doi.org/> [Online 14 May 2003]

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (henceforth jointly referred to as dioxins) belong to the group of most toxic substances known and have been associated with malignancy, congenital malformations, immunosuppression, and hematologic disturbances (Neubert et al. 1993; Pluim et al. 1994; ten Tusscher 2002; ten Tusscher et al. 1999, 2000; Vos et al. 1998; Ziegler 1997). Dioxins are formed as waste products of combustion processes, and municipal incinerators are among the primary sources of these compounds in the Netherlands. Heating of polychlorinated biphenyls (PCBs) is a notable source of polychlorinated dibenzofurans. Dioxins, which are poorly degradable in nature, persist in the environment and accumulate in the food chain via fish oils and animal fats (Theelen et al. 1993). These polychlorinated aromatic compounds are highly lipophilic and in mammals are stored primarily in adipose tissues. Their lipophilicity allows them to readily pass the placenta, whereupon they are stored in fetal adipose tissues (Krowke et al. 1990; van Wijnen et al. 1990).

In 1979, leakage of PCBs and polychlorinated dibenzofurans into rice oil caused a large-scale human poisoning in Taiwan, called Yu-Cheng disease. The children born to the poisoned mothers, and thus exposed in the womb to a high dose of PCBs and furans, developed severe respiratory problems during the first 6 months of life, and 25% of these

babies died before 4 years of age because of lung diseases. Those who survived had respiratory sequelae, such as pneumonia, which endured until 10 years of age, after which time a clinical improvement was seen. Additionally, the incidence of otitis media was increased and became a chronic problem in these children, many of whom developed cholesteatomas before 10 years of age (Guo 1999).

In 1986, high dioxin concentrations in the breast milk of Dutch mothers was reported, followed by similar findings in other industrialized countries (van den Berg et al. 1986; WHO 1989, 1996). This information indicated that fetuses and breast-fed children are exposed to relatively high "background" dioxin levels.

Pluim et al. (1994), studying the same group of children as the present cohort, found thrombocyte counts to be significantly decreased during the perinatal period, in relation to increasing postnatal dioxin exposure. The thrombocyte count was not performed at the 30-month follow-up (Ilsen et al. 1996). Pluim et al. (1994) also found a reduced number of granulocytes in relation to prenatal dioxin exposure. This was no longer evident at the 30-month follow-up performed by Ilse et al. (1996). Similarly, Weisglas-Kuperus et al. (1995, 2000) found reduced numbers of granulocytes during the perinatal period and an increase in infectious diseases, such as otitis media and chicken pox. At follow-up after

approximately 2 years, both the aforementioned Dutch studies (Ilsen et al. 1996; Weisglas-Kuperus et al. 2000) revealed that the previous lowering of these white blood cells was no longer evident. In Japanese breast-fed infants, the CD4<sup>+</sup> and CD8<sup>+</sup> counts and the ratio between the two lymphocyte subsets were shown to be influenced by dioxins (Nagayama et al. 1998).

Presently, very little is known about the effects of perinatal dioxin exposure on the long-term health of children. Perinatal exposure to dioxins and related compounds may have consequences spanning many years, possibly because of their long half-life and their influence on homeostatic set points during the various developmental windows (Hurst et al. 2000; Weisglas-Kuperus et al. 2000). Consequently, we hypothesized that perinatal exposure to background levels of dioxins in the Netherlands would cause a persistent decrease in circulating thrombocyte counts. Similarly, the white blood cell counts could also be expected to be influenced in this manner.

In our ongoing study of the development of children with documented perinatal dioxin exposure, we determined various hematologic and immunologic parameters and possible clinical effects of the perinatal exposure, such as infections and allergy.

The medical ethics committees of De Heel Zaans Medical Centre (Zaandam, The Netherlands) and of the Academic Medical

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Centre (Amsterdam) approved the study. Informed consent was obtained from the children and their parents/guardians.

## Materials and Methods

**Subjects.** Eight years after Pluim et al.'s (1994) study, the study participants were again contacted. The perinatal dioxin exposure is documented for all these study participants. The children were born to healthy, well-nourished Caucasian women, between 23 and 38 years old (mean = 29.2 years), who intended to breast-feed for a minimum of 2 months. Their obstetrician or midwife recruited the mothers. Of the original 35 mother–infant pairs, one child was untraceable, four withdrew from the study, and three declined venipuncture, leaving 27 children in this follow-up study. The nonparticipants introduced no population bias, because their prenatal and postnatal exposures were spread across the group range (dioxin toxic equivalents: 9.05, 13.75, 19.95, 21.62, 30.52, 38.25, 47.16, 62.65). Table 1 summarizes the characteristics of the cohort.

The children underwent blood withdrawal on one of two Saturday afternoons in the spring of 1998 at De Heel Zaans Medical Centre. The dioxin toxic equivalency and cumulative toxic equivalency values as determined by Pluim et al. (1994) in the breast milk of their mothers were used as such within this follow-up study. Briefly, the concentration of dioxins, using the International Toxic Equivalency Factor (I-TEQ) method, in the mother's breast milk shortly after having given birth was taken as the prenatal dioxin exposure level of the child. The postnatal cumulative dioxin exposure was calculated as breast milk dioxin concentration multiplied by the amount of breast milk ingested during the breast-feeding period of the child. We assumed a 2.5% fat concentration in the milk, 700 g of daily milk intake during the exclusively breast-feeding period, and 350 g daily intake during the transition period to formula feeding (Pluim et al. 1994). Dioxin-like PCBs are not included in this calculation. To approximate the total dioxin concentration (i.e., dioxins plus dioxin-like PCBs), the dioxin concentrations, as presented in this study, need to be doubled, according to measurements done by Liem and Theelen (1997). Formula feeding, which replaces animal fats with plant fats, has undetectable levels of dioxins (Weisglas-Kuperus et al. 2000).

The mothers kept a diary during the breast-feeding period for data validation. The average length of breast-feeding was nearly 3 months (88 days), with the longest period being 6 months and a range of 1 week to 6 months. For the 27 participants, the prenatal dioxin exposure ranged from 8.74 to 46.6 (mean = 27.8) ng TEQ/kg fat (dioxin TEQ per kilogram fat, measured in breast milk).

The postnatal exposure ranged from 4.34 to 123.72 ng (mean = 43.4 ng) TEQ dioxin. These are exclusively dioxins and furans. The exposure is equivalent to the background exposures in The Netherlands in general during that period (Koopman-Esseboom et al. 1994; van den Berg et al. 1986; WHO 1989, 1996). Later (childhood) dioxin exposures are about 25 times less than during the fetal and breast-feeding period, and it can safely be assumed that all the subjects had similar childhood exposures because they all had similar diets (Patandin et al. 1999).

Urinary mercury and blood lead levels were determined. The mercury levels were all < 1 µg/L, with the exception of one child who had a level of 5 µg/L, and the lead levels ranged from 16 to 24 µg/L. All mercury and lead values were low and within the Dutch acceptable levels. No relation was seen between lead and mercury levels and the outcomes tested, so it seems unlikely that lead and mercury toxicity would confound the results.

**Medical history.** A complete medical history was taken for each child, and a brief history was taken of the families of the children. Among other complaints, such as coughing spells and dyspnea, histories of asthma, bronchitis, pneumonia, otitis, chicken pox, measles, and allergies were noted. Allergies to milk and chicken products were the only food allergies we noted. Hay fever and allergy to animals and to dust mites were noted as allergy. A physician, mainly the family general practitioner, reportedly diagnosed all the allergies.

**Hematology and immunology.** The following parameters were determined in venous blood, collected in EDTA-coated tubes, using routine procedures: hemoglobin (Hb), hematocrit, erythrocytes (red blood cells, RBCs), RBC mean volume (MCV), mean Hb mass of RBCs, mean Hb concentration of RBCs, leukocytes and differential blood counts, and platelets. Additionally, centrifuged plasma was saved and frozen. This was later used for measuring the thrombopoietin concentrations, which was performed using a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay, as described previously (Folman et al. 1997). Thrombopoietin, a regulatory protein that is continually synthesized at a constant level by the liver, is necessary for regulating platelet numbers in the blood. Megakaryocytes bind the protein to produce thrombocytes in the bone marrow, and circulating platelets bind and remove thrombopoietin from the circulation, resulting in a feedback mechanism (Nathan and Orkin 1998).

**Phenotyping white blood cells by flow cytometry.** The following monoclonal antibodies were used: monoclonal antibodies including the Simultest IMK-lymphocyte kit (No. 340182; Becton Dickinson and Co.,

Alphen a/d Rijn, The Netherlands) [CD45-FITC/CD14-PE, isotype control, CD3-FITC/CD19-PE, CD3-FITC/CD4-PE, CD3-FITC/CD8-PE, CD3-FITC/CD16-PE and/or CD3-FITC/CD56-PE (PE, phycoerythrin; FITC, fluorescein isothiocyanate)]. In addition, CD45RO-PE, CD45RA-PE, and CD29-PE were used in combination with CD4-FITC. All materials were obtained from Becton Dickinson (San Jose, CA, USA). The staining method was performed in accordance with the manufacturer's factory manual (Becton Dickinson), with some modifications. In brief, all incubations were carried out in V-bottom microtiter plates at 4°C. Microtiter wells were filled with 45 µL of EDTA-treated blood and 10 µL of monoclonal antibodies (combined) and incubated for 30 min at room temperature. Red cells were lysed with 160 µL lysing fluid for 10 min and centrifuged at 300 × g for 5 min. Cells were washed twice in 150 µL 0.1% NaN<sub>3</sub> in phosphate-buffered saline and centrifuged at 300 × g for 5 min. Finally, the stained cell suspension was fixed in 100 µL 1% paraformaldehyde. Cells were stored in the dark, at 4°C. Immunofluorescence analysis was performed using a single-laser FACScan (Becton Dickinson Immunocytometry, Mountain View, CA, USA).

**Statistics.** Except for the dichotomous outcomes, linear regression (dose–response) analysis, a two-sided test, was performed using SPSS 10.0 (SPSS, Chicago, IL, USA). The dichotomous outcomes (medical history) were analyzed by binary logistic regression using SPSS 10.0. *p*-Values were calculated with standard *p* = 0.05 as criterion for statistical significance. A best-fitted line was applied to the scatter diagrams. None of the children smoked. Correction for mothers who smoked during pregnancy and/or at the time of testing did not influence the statistical outcomes presented here.

## Results

**Clinical findings.** The medical histories of the subjects revealed a significant decrease in allergy in relation to prenatal dioxin exposure [*p* = 0.023; slope = −0.141; 95% confidence interval (CI), 0.769–0.981], as well as in relation to postnatal exposure (*p* = 0.030; slope = −0.060; 95% CI, 0.892–0.994; Table 2). It must be borne in mind that the definition of

**Table 1.** Characteristics of the cohort (*n* = 27).

Characteristics	Occurrence
Sex [ <i>n</i> male (%)]	12 (44)
Breast-feeding period (days)	87.6 (7–180) <sup>a</sup>
Mother's smoking ( <i>n</i> )	6
Prenatal dioxin exposure (ng/kg fat)	27.8 (8.74–46.62) <sup>a</sup>
Postnatal cumulative dioxin exposure (ng)	43.4 (4.34–123.72) <sup>a</sup>

<sup>a</sup>Values shown are mean (range).

allergy in this study is broad. All those reporting allergies were female. Linear regression of only the female subjects showed a more significant negative relation with prenatal ( $p = 0.008$ ; slope =  $-0.035$ ; 95% CI,  $-0.058$  to  $-0.011$ ) and postnatal ( $p = 0.006$ ; slope =  $-0.017$ ; 95% CI,  $-0.028$  to  $-0.006$ ) exposure. The immunoglobulin E status was not determined. No relation was seen between the eosinophil granulocytes and perinatal dioxin exposure.

We found no relation between perinatal dioxin exposure and diseases such as otitis, pneumonia, and chicken pox.

**Hematology.** The platelet counts ranged from  $248$  to  $449 \times 10^9/L$  (mean =  $334 \times 10^9/L$ , SD =  $52.6 \times 10^9/L$ ). The normal range is from  $150$  to  $350 \times 10^9/L$  for adults, which is generally also used for children, although normal values for children have yet to be thoroughly determined. None of the subjects exhibited thrombocytopenia, whereas nine children had more platelets than the adult standard. This is commonly seen in children and is considered to be without clinical relevance. Linear regression of thrombocytes versus prenatal dioxin exposure revealed an insignificant decrease ( $p = 0.45$ ; slope =  $-0.79$ ; 95% CI,  $-2.91$  to  $1.33$ ) in relation to increasing exposure. Linear regression of platelets versus cumulative postnatal exposure revealed a statistically significant decrease ( $p = 0.04$ ; slope =  $-0.67$ ; 95% CI,  $-1.32$  to  $-0.02$ ) in relation to increasing exposure (Figure 1). This is similar to what Pluim et al. (1994) found in the same children at 11 weeks of age. Based on these results, the concentration of the platelet synthesis-regulating protein thrombopoietin in the frozen plasma was evaluated.

One subject was excluded from the thrombopoietin analysis because of a suspected cross-reaction with the mouse immunoglobulin antibodies of the thrombopoietin test. The thrombopoietin concentrations, measured in the remaining 26 serum samples, ranged from 8 to 42 (mean = 17) aU/mL (arbitrary units per milliliter). The adult normal values for thrombopoietin are between 5 and 40 aU/mL. Thrombocytes versus thrombopoietin revealed a statistically borderline significant inverse correlation ( $p = 0.07$ , with a slope of  $-2.37$ ; 95% CI,  $-4.95$  to  $0.21$ ). This suggests that interference in the production of thrombocytes is more likely than an enhanced destruction as explanation for the thrombocyte decrease.

**Table 2.** Medical history results.

	No. (%) (n = 27)	Prenatal exposure			Postnatal exposure		
		Slope	95% CI	p-Value	Slope	95% CI	p-Value
Allergy (yes)	8 (30)	-0.141	0.769–0.981	0.023*	-0.06	0.892–0.994	0.03*
Eczema (yes)	8 (30)	-0.11	0.785–1.023	0.105	-0.035	0.919–1.015	0.17
Asthma (yes)	6 (22)	-0.038	0.876–1.059	0.436	0.008	0.979–1.038	0.581
Otitis (yes)	9 (33)	0.011	0.923–1.108	0.811	-0.003	0.967–1.029	0.866

\*Statistically significant.

Linear regression of thrombopoietin versus prenatal dioxin exposure revealed an insignificant increase ( $p = 0.31$ ; slope =  $0.15$ ; 95% CI,  $-0.15$  to  $0.45$ ). Linear regression of thrombopoietin versus cumulative postnatal dioxin exposure revealed a significant increase ( $p = 0.03$ ; slope =  $9.98E^{02}$ ; 95% CI,  $0.008$ – $0.191$ ) in relation to increasing exposure.

To our knowledge, no studies have shown a relation between dioxin exposure and RBC counts. In the present study, such parameters as RBC counts and MCV showed no relation with either prenatal or postnatal dioxin exposure.

**Immunology.** We found a significant increase ( $p = 0.006$ ; slope =  $0.083$ ; 95% CI,  $0.026$ – $0.140$ ) in CD4<sup>+</sup> (T-helper) cells (mean = 39.2, range = 25.27–49.05) in relation to increasing postnatal dioxin exposure. The T-cell/B-cell ratio (mean = 3.89, range = 1.5–6.2) was borderline significantly raised ( $p = 0.086$ ; slope =  $0.042$ ; 95% CI,  $-0.006$  to  $0.090$ ) in relation to the prenatal dioxin exposure. We found a significant increase in the CD45RA<sup>+</sup> cell count (mean = 27.3, range = 16.1–41.3) in relation to the postnatal dioxin exposure ( $p = 0.019$ , slope =  $0.078$ , 95% CI,  $0.014$ – $0.141$ ). Other immunologic parameters revealed no relation with either the prenatal or postnatal dioxin exposure.

## Discussion

Although the somewhat limited size of the cohort obviously cautions interpretation of the data, we have found indications of persistent effects of perinatal dioxin exposure in later childhood. Below we separately discuss our findings of a decrease in allergy, relative thrombocytopenia, and immunologic disturbances.

### Allergy

The observed decrease in allergy was not altogether unexpected. In another group of Dutch children, born in the same period as our subjects, a decrease in allergy in relation to current levels of PCBs was also detected (Weisglas-Kuperus et al. 2000).

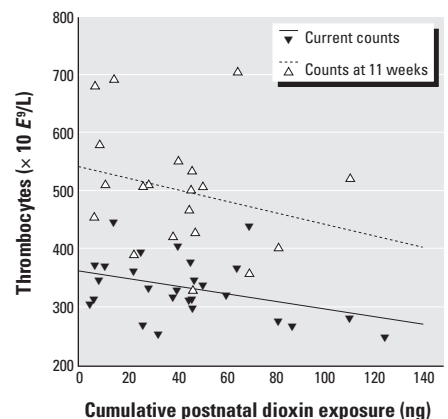
A decrease in allergy sounds beneficial, but this may be deceptive. The decrease could have its pathogenesis in, for example, a relatively deficient immune memory system, an altered antigen receptor, or an insufficient mobilization of immune cells. Yet it is also possible that perinatal dioxin exposure “protects” against allergies by means of interference

in the Th1:Th2 ratio. However, considering the toxicity of dioxins, any relation should be viewed with suspicion.

We are inclined to explain the decrease in allergy as a long-term effect of perinatal dioxin exposure because of the relation seen with the perinatal exposure. However, the current levels of dioxins and/or PCBs playing a role cannot be ruled out, because these levels might be related to the pre- and postnatal exposures. Among 4-year-olds, the levels of PCBs were four times higher in breast-fed infants than in bottle-fed infants (Patandin et al. 1997). The current levels of dioxins and PCBs in our study children were not measured. However, with use of a model, breast-feeding has an effect of 5% higher levels of PCBs and dioxins, at 8 years of age (Patandin et al. 1999).

## Laboratory Findings

**Platelets in the present study.** This study has shown a significant decrease in thrombocytes and borderline increase in thrombopoietin concentration, both in relation to perinatal dioxin exposure. The platelet count and thrombopoietin concentration show an inverse relation. This indicates the cause of the relative thrombocyte decrease to be a suppressed production of platelets, rather than increased destruction (Nathan and Orkin 1998). The strong inverse correlation between thrombopoietin and platelets, as well as the fact that the scatter diagrams (not shown) reveal clear gradients, lends credence to the dioxin influence at the megakaryocyte-to-thrombocyte differentiation stage or earlier in the thrombopoiesis. The significant decrease in the number of granulocytes, as detected by Pluim et al. (1994) and Weisglas-Kuperus et al. (1995) in the perinatal period, may support our hypothesis of a production problem at stem cell level, showing disruption of two cell lines. In this study, dioxin effects on red blood cells (third cell line) could not be demonstrated.



**Figure 1.** Scatter diagram of thrombocytes versus postnatal dioxin exposure, including the thrombocyte counts at 11 weeks after birth, as determined by Pluim et al. (1994).



The subjects in this study group all have thrombocyte and thrombopoietin concentrations falling within the limits of "normal," as is to be expected in an "average, healthy" subject group. However, follow-up far into adulthood is certainly justified, to be vigilant for later clinical problems. We are tempted to hypothesize that the persistently reduced number of thrombocytes seen during childhood may lead to a clinically evident shortage in later life, with increased risk of coagulation problems. This cohort was selected on pregnancy and birth-weight optimality. Therefore, the elicited results may underestimate those in a general population cohort.

The persistently decreased thrombocyte count relative to the postnatal dioxin exposure is remarkable because Pluim et al. (1994), who studied the same group of children at 11 weeks of age, detected the same. Then, too, the reduced thrombocyte count was related to the postnatal dioxin exposure. This might be caused by a persisting effect of the exposure to dioxins in the perinatal period. This reduction can be induced by either a reduced production of thrombocytes (suggesting dioxin toxicity at the megakaryocyte level in the bone marrow) or an increased sequestration of ineffective platelets (suggesting toxicity at the gene level). An increased destruction is excluded by the finding of the inverse correlation between thrombopoietin and thrombocyte levels. We hypothesize that dioxins may negatively influence the production of platelets, thereby necessitating a compensatory increase in thrombopoietin production. It is not known whether the level of thrombocytes is genetically determined or if the set point for this feedback system remains constant throughout life under healthy circumstances. Should this be the case, our findings can be explained as a toxic effect on this system in the perinatal period, with effects persisting into later life. Another explanation for these outcomes is that we have detected a continuing effect of dioxin exposure, with current body levels still able to negatively influence thrombocytopoiesis. This was seen in Japanese workers after chronic exposure (Watanabe et al. 2001) and in two women after acute poisoning (Geusau et al. 2001). The two subjects of the latter study had current dioxin concentrations 1,000-fold higher than our cohort subjects, yet the subjects of the Watanabe et al. (2001) study had levels not much higher than those of the mothers of our cohort.

**Platelets in the animal studies.** Zinkl et al. (1973) described enduring thrombocytopenia in adult rats and guinea pigs treated with high-dosage 2,3,7,8-tetrachloro-*p*-dioxin (TCDD). Kociba et al. (1976) reported lower thrombocyte counts for adult rats given 1 µg TCDD/kg/day, although no difference was seen between lower dosage and control groups.

In a study of TCDD-treated rhesus monkeys, McConnell et al. (1978) described hypoplastic bone marrow after single high doses. Murante and Gasiewicz (2000) reported effects of TCDD exposure on marrow progenitor cells in mice. Their data indicate that proliferation and/or differentiation processes of hemopoietic stem cells are influenced by TCDD administered 5–6 weeks postpartum.

Bone marrow and megakaryocyte findings have been inconsistent (Gupta et al. 1973; Weissberg and Zinkl 1973; Zinkl et al. 1973). Fine et al. (1989) reported a reduction in lymphocyte stem cells in the offspring after a single dose of 10 µg/kg body-weight TCDD in maternal mice on gestational day 14.

Various dioxin studies have reported bleeding among laboratory animals exposed to dioxins, albeit at far higher doses compared with the perinatal exposure of this study group. Viluksela et al. (1997, 1998) noted bleeding to be the most common sign of lethality, apart from wasting syndrome. They noted significantly decreased platelet counts among the highest exposed groups in their subchronic/chronic dosage study, with platelet counts particularly low in rats moribund as a result of hemorrhage or anemia. They concluded that hemorrhage, as a cause of death, is a result of thrombocytopenia and prolonged prothrombin times. Shiverick and Muther (1983) showed that fetuses of pregnant rats treated with a daily oral dosage of 1 µg/kg TCDD had a 66% incidence of visceral lesions, characterized by intestinal hemorrhage. Olson et al. (1990) found gastrointestinal bleeding to be the most sensitive indicator of *in utero* exposure to TCDD, in the fetuses of maternal rats exposed to 1.5 µg/kg. The incidence of bleeding was  $7.7 \pm 5.5\%$ . Similarly, Gupta et al. (1973) reported bleeding among TCDD-treated rats and guinea pigs.

**Platelets in the human studies.** In a letter to the editor of *The Lancet*, Laporte (1977) focused attention on a study of Vietnamese exposed to the dioxin-contaminated defoliant Agent Orange, used extensively during the Vietnam War. The study reported gastrointestinal bleeding, caused by thrombocytopenia and prothrombin deficiency, related to the dioxin exposure. Prothrombin deficiency might be a result of vitamin K deficiency caused by PCBs or dioxins, as is hypothesized by Koppe et al. (1989) and has been found in the rat studies of Bouwman et al. (1990). Clotting times and prothrombin times were not measured because of limited amounts of blood permitted to be drawn by the institutional ethics committees.

**T cells.** It has long been known from animal and human studies that the thymus and T-helper cell (CD4<sup>+</sup>) are sensitive to dioxin exposure (Vos and Moore 1974; Vos et al. 1998; Weisglas-Kuperus et al. 2000; Zinkl et al. 1973). Most studies have revealed a decrease

in the CD4<sup>+</sup> population. In contrast, we have shown a significant tendency toward an increasing T-helper cell population with increasing postnatal dioxin exposure.

Studies in TCDD-sensitive mice revealed that TCDD might affect polymorphonuclear cells at the level of hematopoiesis, via a direct interaction with granulocyte precursor cells, or modulate polymorphonuclear cells at different stages of maturation. This suggests an effect on hematopoietic stem cells (Ackerman et al. 1989).

Total and dioxin TEQ levels in the breast milk correlated significantly with increased T-cell subpopulations in human infants of the Rotterdam study (Weisglas-Kuperus et al. 2000); additionally, significantly decreased B-cell markers were observed after postnatal exposure. In a later follow-up study at 42 months of age (Weisglas-Kuperus et al. 2000), prenatal PCB exposure was associated with an increased number of peripheral T cells, as well as with an increased number of CD8<sup>+</sup> (cytotoxic) and TcR $\alpha\beta$ <sup>+</sup> T cells. At 42 months of age, prenatal PCB-exposure was also significantly related with more CD3<sup>+</sup>HLA-DR<sup>+</sup> (activated) T cells as well as lower antibody levels to measles and a higher prevalence of chicken pox. Current PCB levels were associated with a higher prevalence of recurrent middle ear infections and a lower prevalence of allergic reactions, as already mentioned (Weisglas-Kuperus et al. 1995). The fact that the results seen are related to different exposures may indicate that current levels are still high enough to cause interference in immunology. Interestingly, our study has shown an increase in CD45RA<sup>+</sup> cells in relation to cumulative postnatal dioxin exposure. Oughton et al. (1995) also saw this in their study on long-term TCDD exposure in mice. We detected an increase in CD4<sup>+</sup> helper T cells in relation to perinatal dioxin exposure. In the Rotterdam study the number of CD45RA<sup>+</sup> cells correlated positively with the incidence of bronchitis at 18 months of age, but at 42 months this was no longer visible (Weisglas-Kuperus et al. 1995, 2000). Otitis and pneumonia were not related to dioxin exposure, in our study, but the limited size of our group may play a role here. However, our cohort showed a reduction in lung function and increase in bronchial obstruction, both relative to increasing perinatal dioxin exposure (ten Tusscher et al. 2001).

In conclusion, perinatal dioxin exposure, even at Dutch background exposure levels in 1990, produces a persisting negative effect on thrombocyte numbers, in conjunction with an increased thrombopoietin concentration, indicating a production problem at stem cell level. A decrease in allergy and increases in T-helper cells and CD45RA<sup>+</sup> cells were seen in relation to cumulative postnatal dioxin exposure. This persistent influence in average children with

background exposures is a new finding and warrants further research.

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