

NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT,
BILTHOVEN, THE NETHERLANDS

Report no. 529102007

**Developments in infant exposure to dioxins, furans
and PCBs in breast milk and potential health
consequences, in the Netherlands**

C.E.J. Cuijpers, M.J. Zeilmaker, G.W. van der Molen,
W. Slob, E. Lebret.

November 1997

This project has been performed in order and for the account of the Ministry of Public Health ,
Welfare and Sports, Health Inspectorate within the framework of the project 529102.

National Institute of Public Health and the Environment, the Department of Chronic Disease
and Environmental Epidemiology and the Department of Health Effect Research.
P.O. Box 1, 3720 BA Bilthoven, The Netherlands
phone:-31-30 - 274 3804, fax: -31-30 - 274 4407

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CONTENTS	P
MAILING LIST	2
LIST OF ABBREVIATIONS	6
SUMMARY	7
SAMENVATTING	8
1. INTRODUCTION	9
2. PBPK MODELING OF PCDDs AND PCDFs IN BREAST MILK	
2.1 Model Structure	11
2.2 Model Input	11
2.3 Individual PCDD and PCDF congeners in breast milk: Model simulations versus the empirical values of the 1988/1993 campaign	12
2.4 Model simulations of the I-TEQ levels in breast milk for 1988, 1993 and 1998 (predicted level)	13
2.5 Calculation of the postnatal (cumulative) exposure index of infants	13
2.6 Conclusions of the PBPK model	15
3. REVIEW OF THE EPIDEMIOLOGICAL STUDIES	
3.1.1 The Michigan study; Design	16
3.1.2 The Michigan study; Results	17
3.2.1 The Rotterdam/Groningen study; Design	17
3.2.2 The Rotterdam/Groningen study; Results	18
3.3.1 The Amsterdam study; Design	19
3.3.2 The Amsterdam study; Results	19
3.4.1 The North Carolina study; Design	19
3.4.2 The North Carolina study; Results	20
3.5.1 Discussion: Methodological aspects	20
3.5.2 Causality of the reported associations	21
3.5.2.1 Consistency	21
3.5.2.2 Strength of the found associations	22
3.5.2.3 Biological plausibility	22
3.5.2.4 Exposure response relationship	22
3.5.2.5 Exposure preceding effect (temporality)	22
3.6 Conclusions of the epidemiological review	23
FIGURE 1: Historic correction function	24

FIGURE 2: Simulations of the levels in human milk ('body burden') for 16 of the 17 dioxin/furan congeners	25
FIGURE 3: Simulations of the I-TEQ level in human milk ('body burden') for 1993 and 1988	27
FIGURE 4: Simulations of the I-TEQ level in human milk ('body burden') for 1993 and 1998; different birth cohorts	27
FIGURE 5: Dose-effect curves as reported by Jacobson et al.	28
FIGURE 6: Dose-effect curves as reported by Koopman-Esseboom et al.	29
APPENDIX I Overview of the PBPK model	30
APPENDIX II Table with the control variables included in the 4-year follow-up of the Michigan fish eater cohort, reported by Jacobson et al.	34
REFERENCES	35

LIST OF ABBREVIATIONS

OCP's	Organochlorine pesticides
PCB's	Polychloorbiphenyls
PCDD's	Polychloordibenzo- <i>p</i> -dioxins
PCDF's	Polychloordibenzofurans
TEF used	Toxicity equivalence factors in respect to 2,3,7,8-TCDD, in this report we the international TEF's (i-TEF's according to NATO/CCMS, 1988 and Van Zorge et al 1989) for dioxins and furans and the Interim WHO TEF's (Ahlborg 1994) for the planar and the other PCB's
TEQ	2,3,7,8-TCDD toxicity equivalent; the TEQ's are calculated by multiplying the levels of PCDD's, PCDF's and planar PCB's with its TEF value.
I-TEQ	TEQ value calculated by summarizing the values of the seventeen 2,3,7,8-substituted PCDD's en PCDF's expressed in TEQs by using the international TEF's (see TEF)
PCB-TEQ	TEQ value calculated by summarizing the values of the planar PCB's expressed in TEQs by using the Interim WHO-TEF's (see TEF)
TEQPCB- PCB's	TEQ value calculated by summarizing the values of the other dioxin like expressed in TEQs by using the Interim WHO-TEF's (see TEF)
PBPK	Physiologically based Pharmacokinetic
TDI	Tolerable Daily Intake
tTT4	Total thyroxin
tTT3	Total triiodothyroxin
FT4	Free thyroxin
TSH	Thyroid stimulating hormone
NBAS	Neonatal Behavioral Assessment Scale
nM	nanomolar
mU/l	milliunits per ml

SUMMARY

This report evaluates the time trend and the associated health effects of the pre- and postnatal exposure of infants to dioxins, furans and PCBs.

The time trends of the amounts of dioxins and furans in mother's milk in the 1988 and the 1993 campaign of the surveillance program "Man, Nutrition and the Environment" were analyzed by means of Physiologically Based Pharmacokinetic (PBPK) modeling. Based on this analysis the PBPK model predicted, in comparison with the outcome of the 1993 campaign, a decline of about 20%, in dioxin and furan levels in mother's milk which will be collected in the 1998 campaign of this program. The result has to be interpreted within the context of assumptions underlying the model. It appeared that the model underestimated the rate at which the amount of dioxins and furans declined in the period between 1988 and 1993. The most plausible explanation for this is that the historical correction function used by the model, to adjust for historical food intake, did not predict time trends in intake accurately enough. This is supported by data on time trends in foods which became recently available. Hence, the amounts of dioxins and furans in mother's milk which will be collected in the 1998 campaign are expected to be even lower than simulated by the model.

To determine the health risk for infants, which might be associated with the present (and future) exposure levels to dioxins, furans and PCBs, four epidemiological studies were evaluated in respect to their usefulness for a quantitative risk assessment. In these studies no adverse effects of the *postnatal* exposure (via breast milk) to dioxin, furans and PCBs on the neurological and the psychomotor development of children were convincingly demonstrated. The results with *prenatal* exposure are considered inconclusive. Due to methodological limitations of the studies in respect to exposure assessment and data analysis, the question whether (and to what extent) background exposure of infants to dioxins, furans and PCBs results in adverse developmental effects remains unresolved. Since the reported results did not show clear exposure-response relationships they are of limited use for quantitative risk assessment purposes.

SAMENVATTING

Dit rapport beschrijft een evaluatie van de tijdtrends en geassocieerde gezondheidseffecten, van pre- en postnatale blootstelling van zuigelingen aan dioxinen, furanen en PCBs.

De niveaus van dioxinen en furanen in moedermelk zoals gemeten in 1988 en 1993, in het kader van het Bewakingsprogramma Mens, Voeding en Milieu, zijn geanalyseerd met behulp van 'Physiologically Based Pharmacokinetic' (PBPK) modellering. Op basis van deze analyse kan (gemiddeld) een daling van de dioxine-niveaus in moedermelk van ca. 20% worden voorspeld voor de ronde 1998 ten opzichte van de niveaus in 1993. De resultaten dienen te worden geïnterpreteerd in de context van de aannames die ten grondslag liggen aan het model. Simulaties van de niveaus in 1988 en 1993 tonen aan dat het model de afname over deze 5-jaars periode onderschat. De meest plausibele verklaring hiervoor is dat de correctie voor de historische inname via voeding, zoals opgenomen in het model, niet accuraat is ingeschat, hetgeen mede wordt ondersteund door zeer recentelijk beschikbaar gekomen resultaten over historische gehalten in voeding. Tengevolge hiervan wordt verwacht dat de niveaus in moedermelk welke zullen worden bepaald in de 1998-ronde van het monitoringsprogramma, lager zijn dan de huidige modelvoorspellingen.

Om het gezondheidsrisico voor zuigelingen, welk geassocieerd is met de huidige (en toekomstige) blootstellingsniveaus van dioxinen, furanen en PCBs te bepalen, zijn vier epidemiologische studies geëvalueerd, met het oog op bruikbaarheid in een kwantitatieve risicoschatting. In deze studies werden geen nadelige effecten van *postnatale* blootstelling (via borstvoeding) op de neurologische en psychomotorische ontwikkeling van de zuigelingen overtuigend aangetoond. De onderzoeksresultaten van de *prenatale* blootstelling zijn niet doorslaggevend. Door methodologische tekortkomingen van de studies ten aanzien van de blootstellingskarakterisering en de data-analyse, kan de vraag of (en in welke mate) achtergrondblootstelling van zuigelingen aan dioxinen, furanen en PCBs resulteert in ontwikkelingsstoornissen, op dit moment nog niet worden beantwoord. De gerapporteerde resultaten bevatten geen duidelijke blootstellings-respons relaties hetgeen de bruikbaarheid van deze onderzoeksresultaten voor een kwantitatieve risicoschatting beperkt.

1. INTRODUCTION

In the Netherlands information on the time-trends of the amounts of individual dibenzo-*p*-dioxins (PCDDs), dibenzo-*p*-furans (PCDFs) and polychlorinated biphenyl's (PCBs) in breast milk is collected as part of the surveillance program 'Man, Nutrition and the Environment'. In this program human milk surveys are performed at five years intervals. Because of their persistent and lipophilic nature these components accumulate in the lipid fraction of the different body compartments. In lactating women, breast milk is an important route of PCDD, PCDF and PCB excretion. Since it is assumed that the concentration (amount per gram lipid) is equal for all body compartments, the levels of these compounds in breast milk can be taken as a biological index for the human "body burden". During pregnancy the mother's "body burden" determines the exposure level of the growing fetus to PCDDs, PCDFs and PCBs. Furthermore, just after birth, it also determines the exposure of infants who receive these compounds via breast feeding.

The health risks which are associated with these exposures is still subject of discussion. In the Netherlands the exposure of nursed infants to PCDDs, PCDFs and PCBs via mother's milk approximately amounts to 200 pg TEQ/kg/day, for the period of breast feeding. This exposure (of short duration) is substantially higher than the current Tolerable Daily Intake (TDI) for these compounds (10 pg TEQ/kg/day, for life-time exposure). Besides, recently the Health Council of the Netherlands reviewed the current knowledge on toxicity of PCDDs, PCDFs and PCBs in experimental studies in animals. Based on this analysis a reduction of the TDI for dioxin-like compounds from 10 to 1 pg TEQ/kg/day was proposed (Health Council, 1996).

The most recent breast milk surveillance campaign in the Netherlands took place in June 1993. The results of this campaign were recently reported by Cuijpers *et al.* (1997). Based on the information about current levels the Health Inspectorate asked the following questions:

1. Can we describe the time trends of PCDDs, PCDFs and PCBs levels in breast milk?
2. Can we quantify the expected health risks, for the growing fetus and the nursed infant, associated with the 1993 exposure levels of PCDDs, PCDFs and PCBs?

Answering the first question will give insight in (developments) in PCDDs, PCDFs and PCBs *exposure levels*, whereas answering the second question will give insight in the exposure related *health effects*.

To address the first question we used a physiologically based pharmaco-kinetic (PBPK) modeling approach, which will be described in chapter 2. In this approach the following three steps can be distinguished:

- calibration of the existing PBPK model for dioxins and furans, by use of the empirical data of the 1993 mother milk survey,
- using the PBPK model to calculate the (cumulative) exposure of infants which potentially can, combined with exposure-response relationships, be used for a quantitative risk-assessment,
- prediction of (developments in) future levels; which can on the one hand be helpful in planning the numbers of the next mother milk survey (1998) and on the other hand give

insight in the rate at which the PCDD/PCDF and PCB associated health risk will change with further decreasing levels.

To answer the second question we reviewed a number of recently published epidemiological studies which addressed the effects of the background exposure to PCDDs, PCDFs and PCBs on the thyroid hormone status of young children and their cognitive and psychomotor development. The study results were evaluated in respect to their usefulness for a quantitative risk assessment of the pre- and postnatal exposure to PCDDs, PCDFs and PCBs of the fetus and the nursed infant, respectively. The review of the epidemiological studies will be discussed in chapter 3.

2. PBPK MODELING OF PCDDs AND PCDFs IN BREAST MILK

In this chapter the time trends of PCDDs, PCDFs and PCBs in mother's milk of the Dutch female population will be described, using a PBPK model. It will start with an overview of the PBPK model followed by the results of the model simulations and the calculated (postnatal) exposure index of the infants.

2.1 Model structure

The principle of Physiologically Based Pharmacokinetic (PBPK) modeling lies in a description of the absorption, distribution, metabolism and excretion of a chemical within a physiological context of the mammalian organism, i.e. organs which exchange the chemical and its metabolites via the blood. The kinetics of TCDD in (the average) male and (the average) female has been described by a PBPK model (Van der Molen *et al.* 1996). This model is equally applicable for dioxins or furans other than TCDD. Basically the model assumes that dioxins and furans in the body distribute between the blood, the liver, the bone, the muscle, the adipose tissue, the remaining organs and the breast milk. These compartments were chosen because of their different lipid composition (in the model highly lipophilic compounds are assumed to reside entirely in the lipid fraction of the organs and breast milk). Organ volumes were modeled such that they vary as a function of age. An overview of the model is given in the Appendix I (for further details see Van der Molen *et al.*, 1996). The organ lipid content (percentage) was assumed to be constant with age. In lactating women two mechanisms were held responsible for the elimination of dioxins and furans from the body: elimination via the liver and extra elimination by breast milk (the model represents primagravidae). The elimination via the liver was assumed to follow first-order kinetics. Due to the absence of clear insight in the elimination mechanism of dioxins and furans in humans no attempt was made to describe this elimination in more detail. The elimination of dioxins and furans via mother's milk was modeled by assuming that, during lactation (90 days), 30 g of milk lipid is removed daily from the body via breast milk. To model the extra elimination by breast feeding it was assumed that the concentration of the dioxins and furans in the lipid fraction of the milk is equal to their concentration in all other lipid fractions in the body. In describing the above mentioned uptake, distribution and elimination mechanisms quite a few model parameters are needed. With the exception of the rate constant for the elimination of the congener via the liver, all these parameters were obtained from the literature (see Appendix I and Van der Molen *et al.* 1996 for a more detailed overview of the parameters of the PBPK model). Given a known intake of various dioxins and furans (see below) the elimination rate constant (k) for the elimination via the liver is the only unknown model parameter. This parameter was obtained by fitting the model to the concentrations of Dutch milk data of 1993. It should be noted that, as with its other parameters, the model contains only one parameter value (point estimate) for the elimination of a particular dioxin or furan congener via the liver. However in the human population the value of this parameter is expected to vary substantially. In its current version the PBPK model does not take the inter-individual variation of this and all of its other parameter into account. The results of simulations performed with the PBPK model therefore have to be considered as a best estimate for the average Dutch female.

2.2 Model Input

Because 95% or even more of the human exposure to dioxins/furans is through food, model input is determined by the consumption of dioxin/furan contaminated foods. Age-dependent intakes of the dioxin and furan congeners were calculated with the aid of a statistical

exposure model (STEM) (Slob, 1993) using the data from the Dutch National Food Consumption Survey 1987 (Hulshof en van Staveren, 1991) in combination with concentration measurements in food around 1991 (Liem *et al.*, 1991). It should be kept in mind that the concentrations of congeners in food have changed in the past. This of course also holds for differences in food consumption habits. Hence, in comparison with the exposure as assessed in 1993 the exposure to dioxins and furans will have differed in the past. To account for these differences a historical correction function was calculated for the past exposure to dioxins and furans (see Figure 1). This function, which was obtained by fitting the PBPK model to the observed concentration of TCDD in human blood in the German population (assumed average elimination rates as estimated from internal concentrations of TCDD in Vietnam veterans, see Van der Molen *et al.*, submitted), represents the intake of TCDD at any time in the past relative to the intake of 1993. For example, in 1960 the exposure to dioxins and furans from food was assumed to be about three times the exposure to these compounds in 1993. The function depicted in Fig. 1 was assumed to represent the historic exposure of all dioxin and furan congeners. It was further assumed that from 1993 on the historical correction function remained unchanged. In the model the intake due to breast feeding was not accounted for, and the 'body burden' at birth was assumed to be zero.

2.3 *Individual PCDD and PCDF congeners in breast milk: Model simulations versus the empirical values of the 1988/1993 campaign*

The PBPK model simulates the breast milk levels (as an index for 'the body burden') of the individual dioxins and furans or their I-TEQ value in a cross section of the Dutch female population (individuals of 0-70 years of age). The simulations have been performed for 16 of the 17 dioxins and furans congeners which have also been monitored in the 1988 and the 1993 campaign of the surveillance program 'Man, Nutrition and the Environment'¹. To adjust model weights and body composition, to the subjects of 1993, the function for adipose tissue weight in the model (see Appendix I) was multiplied with the average ratio between measured adipose weight and model adipose tissue weight (at the subjects age) The same procedure was followed for the non-adipose tissue weight.

Figure 2 shows the results of the model simulations and the measured values for 1988 (model simulation: dashed curve; measurements: circles; "pooled samples") and 1993 (model simulation: solid curve; measurements: crosses; individual samples). This figure shows that the measured as well as the simulated amounts of dioxins and furans in breast milk in the 1988 campaign are significantly higher than in the 1993 campaign. In 1988 considerable discrepancy is observed between the measured and simulated levels of several congeners. In most cases the model systematically underestimated the measured values of the 1988 campaign (see for example the dioxin OctaCDD). This finding indicates that the PBPK model underestimated the decrease in the amounts of dioxins and furans in mother's milk in the period between 1988 and 1993.

The most likely explanation for this discrepancy is a rather inaccurate way in which the PBPK model estimates the historical exposure (intake).

From Figure 2 it can further be seen that relatively high amounts of dioxins and furans are present in the body's lipid fraction at young ages. This is mainly due to the relatively small amount of body fat at early age. Following the increase in body fat a small decrease in this concentration is seen around puberty (12 years).

¹for 1,2,3,7,8,9 HxCDF the value in all samples was below the level of detection in 1993, therefore this congener was not used in further model simulations.

The scatter of measured values around the simulated curve reflects the substantial variation due to inter-individual differences in body weight and composition (especially body fat), in elimination rates (almost no information on this is available), and (historical) intakes. As mentioned in the previous section the PBPK model does not take these variations into account.

2.4 *Model simulations of the I-TEQ levels in breast milk for 1988, 1993 and 1998 (predicted level).*

Figure 3 presents the simulated (and measured) I-TEQ values for the 1988 and the 1993 milk samples. As with the individual congeners the measured as well as the simulated I-TEQ values of breast milk declined in the period from 1988 to 1993. As with the individual congeners the PBPK model underestimates this decrease.

Figure 4 and table 1a summarize the results of the model simulations for the amounts of dioxins and furans which are expected in mother's milk in the 1998 campaign. As a reference the 1993 simulations were included in this figure too. To illustrate the difference between a simulation of the cross section of the entire female population and simulations of different birth cohorts, four curves of women aged 20, 25, 30 and 35 years are presented in this figure. From the latter (in combination with the data from table 1a) it appears that the body burdens for the females who are 20, 25, 30 and 35 years old in 1998 are expected to be 20-23% lower than females of the same age in 1993. Note that in the model this decrease is entirely caused by differences in the historical exposures of these women. As the model underestimates the decrease of the amounts of dioxins and furans in the period between 1988 and 1993 (see above) this may also be the case in the period between 1993 and 1998. Hence, the real decrease in the period between 1993 and 1998 could be significantly larger than is simulated. Since the real reason for the discrepancy between model and measured data are not sufficiently understood, these assumptions need to be verified against data from the next round of mother milk, in 1998.

Table 1a. Simulated I-TEQ values of breast milk for women aged 20,25, 30 and 35 years in 1993 respectively 1998.

age in years	I-TEQ, 1993	I-TEQ 1998	ratio I-TEQ'98/'93
20	15.60 pg/g fat	11.97 pg/g fat	0.77
25	19.39 pg/g fat	15.08 pg/g fat	0.78
30	22.73 pg/g fat	18.02 pg/g fat	0.79
35	25.64 pg/g fat	20.75 pg/g fat	0.81

2.5 *Calculation of the postnatal (cumulative) exposure index of infants.*

The expected cumulative exposure to dioxins and furans for the child receiving breast feeding was calculated using the model derived I-TEQ values as presented in Table 1a. These I-TEQ values were multiplied by (variable) duration(s) of breast feeding (3 and 6 months respectively 1 year). The results of this calculation are presented in table 1b. In interpreting the results shown in Table 1b it should be kept in mind that in the Netherlands 67% of the mothers begin to breast feed their baby. After three months the number of mothers who breast feed their child is reduced to 44% and after six months to 27% (CBS 1993).

Table 1b. Simulated cumulative exposure of infants to dioxins and furans in mother's milk. Age of the mothers: 20-35 years. Periods of breast feeding : 3 months to 1 year. Exposure data were taken from Fig. 4.

period of breast feeding	year	age range (in years)	exposure (pg TEQ/g milk fat times weeks)
3 months	1993	20 - 35	187.2 - 307.7
	1998		143.6 - 249.0
6 months	1993	20 - 35	374.2 - 615.4
	1998		287.3 - 498.0
1 year	1993	20 - 35	811.2 - 1333.3
	1998		622.4 - 1079.0

2.6 *Conclusions of the PBPK model*

In the present study a physiologically based modeling approach to describe time trends of dioxin and furan “body burdens” in Dutch females was presented. In this approach the amounts of dioxins and furans in mother’s milk were used as a measure for the female “body burden”. In turn, the latter entities can be used as an exposure index for the fetus as well as the nursed infant.

The model simulations predict a reduction of the “body burden” (and so the fetus and infant’s exposure level) of dioxins and furans, expressed in the I-TEQ level of breast milk, in the order of magnitude of 20% for 1998 compared to the levels of 1993. This result has to be interpreted within the context of the assumptions underlying the PBPK model. For instance, in simulating the amounts of dioxins and furans in mother’s milk, a historical correction function was used to simulate the exposure of the Dutch population to PCDDs and PCDFs in the past. This correction function predicts that the exposure to these compounds is declining from 1960 onwards. Notwithstanding this correction the model significantly underestimated (compared to the measured values) the decrease in the amounts of dioxins and furans in breast milk in the period between 1988 and 1993. The historical correction function of the PBPK model apparently did not predict the time-trend of the exposure to these compounds accurately enough. This conclusion is supported by data on the time-trend of the intake of dioxins and furans via the food in the Netherlands. These data show that the real decrease of the exposure to dioxins and furans in the Netherlands via the food in the period between 1974 and 1994 is greater than simulated by the model (Liem et al. 1997). Consequently, for some dioxins and furans an even larger decrease than simulated by the model may be expected, in the milk samples which will be collected in the 1998 campaign. Adaptation of the historical correction function to the new available information on time trends in food, will certainly improve future model simulations of time trends in mother’s milk.

In predicting the time-trend of the amounts of PCDDs, PCDFs and PCBs in breast milk the PBPK model cannot, yet, replace the empirical monitoring of these compounds in mother’s milk, but it will profit from the new data by further validation.

The simulations were based on a model containing average values for (historical) daily intake, the female body composition (although calibrated to the 1993 women) and the elimination rates of the congeners from the body. However, as demonstrated in Fig. 2, substantial inter-individual differences in these parameters seem to exist. Due to these inter-individual differences, the distributions of fetus and infants exposure can not yet be accurately assessed based on the current version of the PBPK model. Future model simulations can be made more realistic by using distributions in stead of point estimates for these parameters. Such stochastic approaches may provide more useful data on the infant exposure distributions.

3. REVIEW OF EPIDEMIOLOGICAL STUDIES

In several epidemiological studies the relationship between background exposure of infants to dioxins and/or PCBs and potential health effects has been investigated. The aim of the present chapter is to evaluate whether or not these studies provide useful data for a quantitative risk assessment, *i.e.* whether they provide exposure-response relationships.

The studies reviewed in this chapter included the studies of Jacobson *et al.* (1984, 1985, 1990, 1996) (further referred to as the Michigan study), Koopman-Esseboom (1995) and Huisman (1996) (both studies further referred to as the Rotterdam/Groningen study), Pluim *et al.* (1993) (further referred to as the Amsterdam study), Rogan and Gladen (1986; 1991) and Gladen *et al.* (1988; 1991) (further referred to as the North-Carolina study).

3.1.1 *The Michigan study; Design*

The Michigan study comprised a prospective study on the cognitive and intellectual development of children which were prenatal exposed to PCBs (Fein *et al.*, 1984; Jacobson *et al.*, 1985; 1990; 1996). The study population consisted of 313 children, 242 of whom were born to mothers who had reported a (mean) annual consumption of 7 kg PCB contaminated fish for a (mean) period of 16 years before pregnancy. The level of PCB was determined as total PCB. The total amount of PCBs in cord blood and in mother's milk were taken as measures for prenatal PCB exposure. A sample of cord blood was collected shortly after delivery. Postnatal exposure to PCBs was assessed by the product of the amount of PCBs in mother's milk and the duration of breast feeding. A sample of mother's milk was collected 0.2 to 4.5 months post partum (median: 1 month). Information on the duration of breast feeding was collected at 2, 4, 5 and 7 months after delivery. The children were evaluated at birth and at the age of 5 and 7 months, 4 and 11 years. Weight, body length, head circumference and gestational age were determined at birth. The behavioral aspects of the newborn were determined by means of the Neonatal Behavioral Assessment Scale (NBAS score), a semi-quantitative measure for neonatal muscle tone and reflexes. At the age of 5 months the cognitive development was determined by means of the Bayley Scales of Infant Development. At the age of 7 months the visual recognition memory was tested by means of the Fagan test. The Fagan test examines a baby's ability to divide its attention over old and novel visual stimuli. The outcome of the Fagan test represents the way in which memory and abstraction processes operate in children. These processes are considered the similar to the ones used by older children and adults in solving intelligence tests. At 4 years of age the children were tested on the McCarthy Scales of Children's Abilities and on tests which focus on assessing reaction time, short-term memory processing, visual discrimination and sustained attention. The McCarthy Scales of Children's Abilities provide testing of the children on verbal, perceptual performance, quantitative, memory and motor capacities. At the age of 11 years children were tested with the Wechsler Intelligence Scales for Children IQ test, the spelling and arithmetic subtests of the Wide Range Achievement Test and the word and passage-comprehension subtests of the Woodcock Reading Mastery tests.

In the Michigan studies extensive information on potential confounding variables was obtained (see Appendix II). In their analyses the investigators included only "potential confounders", defined as variables related with the exposure to PCBs at $p < 0.10$. The correlation's between PCB exposure measures and confounding variables assessed in the 4 years of follow-up phase of the Michigan study are presented in table II-1 (Appendix II). The investigators reported that with one exception, (maternal PBB levels), the correlations did not exceed 0.19, and concluded that prenatal PCB exposure is only weakly related to part of the control variables (mother milk 8/28 = 29%, cord blood 5/28 = 18%).

In different reports on the study population different sets of confounders were used. The choice which confounders to use and which not was however not discussed.

3.1.2 *The Michigan study; Results*

In the Michigan study infants born to mothers who had reported the consumption of PCB contaminated fish weighted 160-190 gram less at birth. The cord serum PCB levels predicted a significantly lower birth weight (birth weights at cord serum levels of ≤ 3 ng/ml (N=166) and > 3 ng/ml (N=75) were 3.57 kg and 3.41 kg, respectively) a smaller head circumference and a shorter gestation (Fein *et al.*, 1984). The concentration of PCBs in cord blood remained correlated with smaller size until the age of 5 months. This finding however has to be interpreted with caution. The authors state that the effect of the consumption of PCB contaminated fish on birth weight is of a comparable size as the effect of smoking during pregnancy on birth weight. From the original paper it is however unclear whether maternal smoking during pregnancy was incorporated as a confounder in the analysis of the association between birth weight and maternal fish consumption. At birth the consumption of contaminated fish showed a negative correlation with NBAS scores. For the concentration of PCBs in cord blood such a relation was not found. Neither maternal fish consumption nor cord levels of PCBs were related to the outcome of the Bayley scales at 5 months of age. As shown in Fig. 5a the concentration of PCBs in cord blood was reported to be negatively associated with the outcome of the Fagan test at 7 months. At 4 years of age cord serum and milk PCB levels were negatively associated with the outcome of the McCarthy Scales of Children's Abilities (see Figs. 5b and 5c). At this age children were also subjected to tests of cognitive processing. Inconsistent correlations of the outcome of these tests with prenatal PCB exposure were reported (for example cord blood PCB levels were found to be negatively correlated to the outcome of these tests but PCB levels in mother's milk not). At the age of 11 years negative effects of prenatal PCB exposure on the Wechsler Intelligence IQ score were most prominent in the highest exposed children (see Fig. 5d). Similar results were reported for several other test items of the Wechsler test.

In none of the above mentioned development tests a negative association was found between the postnatal exposure to PCBs via mother's milk and the performance of the children in these tests.

3.2.1 *The Rotterdam/Groningen study; Design*

The Rotterdam/Groningen study investigated, in addition to PCBs, the effects of dioxins and furans on the postnatal development of children (Koopman-Esseboom, 1995; Huisman, 1996). The study population consisted of 418 mother-child pairs. Half of the infants were breast fed (only mothers who breast-fed their children for at least 6 weeks were included in this group) and half received formula feeding which contained negligible amounts of dioxins, furans and PCBs. The children were born to mothers who had been exposed to background levels of dioxins, furans and PCBs via the food. Prenatal exposure was assessed for PCBs by measuring the concentration of the non-planar PCBs 118, 138, 153 and 180 in cord blood and in maternal blood. Maternal blood was collected in the last month of pregnancy. Mother's milk was collected in the second and the sixth week after delivery. Over a 24 hr period samples of each breast feeding were collected and pooled into one representative 24 hr milk sample. In this sample 17 dioxins and 26 PCBs were determined. Next to mother's milk information on the duration of breast-feeding was collected. The duration of breast feeding was categorized into a short exposure of 6-16 weeks and a long exposure of 17-30 weeks.

Total thyroxin (tTT₄), total triiodothyroxin (tTT₃), free thyroxin (FT₄), and thyroid stimulating hormone (TSH) were measured in the plasma of infants 9 to 14 days after delivery and at 3 months of age.

Developmental tests were performed at the age of two weeks and 3, 7 and 18 months. At the age of two weeks the neurological development of the children was examined with the so-called Prechtl test. In this test a number of items which characterize a child's ability to maintain its posture and to perform neurological reflexes are scored and combined to a Neurological Optimality Score. The neurological condition of the children was also investigated at the age of 18 months. Test items at this age included a child's motor functions such as grasping, sitting, crawling, standing and walking as well as the fluency with which these functions are performed.

At the age of 3 and 7 months children were tested with the Fagan test. The mental and psychomotor development of the children was assessed at the age of 3, 7 and 18 months with the aid of the Bayley Scales of Infant Development.

In the study information on several confounding factors was obtained by questionnaire; education (father, mother), duration breast-feeding, parity, gender, obstetrical optimality score, birth weight, maternal weight, smoking and alcohol use. However, a major part of the statistical analysis of the study was performed by means of univariate techniques. When multivariate analyses were performed different sets of confounders were used in the analysis. The selection of the confounders however was not discussed by the authors. Furthermore, they did not give insight in the correlations between individual confounders and exposure parameters.

3.2.2 *The Rotterdam/Groningen study; Results*

In investigating the relation between the infant's dioxin, furan en PCB exposure via mother's milk and their thyroid hormone status the children were classified as being exposed to high or low amounts of dioxins, furans and PCBs. Low and high exposure were defined as exposure to mother's milk which contained a higher or lower TEQ content than the median TEQ level of the sampled milk (30.8 pg TEQ/g milk fat). At two weeks of age the TT₄ concentration in the highest exposed group was found significantly lower than in the lowest exposed group (160 vs. 178 nM). For TSH the reverse relationship was found (2.6 vs. 1.9 µIU/ml). At 3 months of age only the TSH levels was found to be significantly increased in the highest exposed group. At this age no effects of mother's milk on the children's serum TT₄ levels were found anymore. It should be noted that the statistical analysis of the thyroid hormone was not corrected for confounding or modifying effects.

With respect to the effects on postnatal development inconsistent results were reported. Only part of the TEQ parameters assessed in mother's milk, was found to be negatively associated with the neurological status of the children at two weeks of age. When prenatal exposure was assessed by the concentration of PCBs in cord blood this association was found at 18 months but not at two weeks of age.

A negative association was found between prenatal exposure, as assessed by the concentration of PCBs in maternal blood, and the psychomotor development at the age of 3 months. However, when the concentration of PCBs in cord blood was taken as a measure of prenatal PCB exposure this association was not found.

Prenatal exposure to PCBs was not correlated with the outcome of the Fagan test at 3 and 7 months, the psychomotor development at 7 and 18 months and the mental development at 3, 7 and 18 months.

As shown in Fig. 6 positive effects of breast feeding were found. At the age of 7 months the results of the Fagan test were positively correlated to the dioxin, furan and PCB exposure via

mother's milk. The authors ascribed this effect to the presence of (beneficial) lipophilic compounds other than dioxins, furans and PCBs in mother's milk (Koopman-Esseboom, 1995). A similar result was found for the psychomotor development at 7 months. At this age breast-fed children in the lowest exposure (expressed in TEQ) category scored significantly higher in the psychomotor test than formula fed children. However, though this positive effect was not observed for children in the higher exposure groups, the psychomotor scores in these groups did not differ from the control group, i.e. "formula fed" children. The latter scores therefore were not considered to represent an adverse effect of breast feeding on the psychomotor development of the children.

3.3.1 *The Amsterdam study; Design*

The Amsterdam study focused on the relationship between the concentration of dioxins and furans, but not PCBs, in mother's milk and the amounts of thyroid hormones in the blood of newborns. The study population consisted of 38 infants who were born to mothers who had been exposed to background levels of dioxins and furans via the food. Cord blood was collected immediately after birth. Blood samples were taken from the infants at 1 and at 11 weeks after birth. In the plasma total thyroxin (tTT₄), free thyroxin (fTT₄), total triiodothyroxin (tTT₃), thyroxin-binding globulin (TBG) and thyroid stimulating hormone (TSH) were determined. Milk samples were collected three weeks after delivery. Exposure was assessed by measuring 17 dioxin and furan congeners in mothers milk. The median TEQ content of mother's milk was taken as a reference point for the division of the population into a low and a high exposure groups (low exposure: sample content below the median, group mean 18.6 ng TEQ/kg milk fat, range 8.7-28.0; high exposure: sample content above the median, group mean 37.5 TEQ ng/kg milk fat, range 29.2-62.7). Although information on several characteristic of the mother e.g. smoking, was obtained, univariate analysis was performed.

3.3.2 *The Amsterdam study; Results*

In the Amsterdam study thyroid hormone levels were compared in newborns who were exposed to relatively low and to relatively high levels of dioxins and furans in mother's milk (see above). Birth weight was similar in both groups. In cord blood none of the measured thyroid levels differed between the low and the high exposed group. At 1 week of age the total TT₄ level was significantly higher in the high exposure group (low exposure group: 155 nM²; high exposure group: 179 nM). This effect was also found at 11 weeks of age (low exposure group: 111 nM; high exposure group: 122 nM). Similarly TSH levels were increased in the high exposure group at 11 weeks of age (low exposure group: 1.8 mU/l; high exposure group: 2.5 mU/l). It should be noted that the statistical analysis of the thyroid hormone levels was not corrected for confounding variables.

3.4.1 *The North-Carolina study; Design*

The North-Carolina study originally consisted of the offspring of 880 women. The development of more than 700 of these children was followed during a 3 to 5 year follow-up period after birth. The children were born to mothers who had been exposed to background levels of PCBs via food. The exposure of the children was determined by the sum of the concentrations of PCBs 125 and 146 in cord blood and mother's milk collected at birth (colostrum) and at 6 weeks, 3, 6 and 12 months after birth.

² nM: nanomolar; mU/l: milliunits per ml

The development of the children was assessed at birth, at 6-month intervals until 2 years and thereafter yearly until 5 years of age. The behavioral aspects of the newborn were determined by means of the Neonatal Behavioral Assessment Scale (NBAS score). Infant cognitive and motor development was tested with the Bayley Scales of Infant Development at 6, 12, 18 and 24 months of age. At the age of 3, 4 and 5 years the children were tested with the McCarthy Scales of Children's Abilities.

The investigators included a comprehensive list of potential confounders in their statistical analysis; e.g. race, maternal age, prior breast-feeding, smoking alcohol use, occupation, education, maternal weight and others. Significant associations with PCB levels were reported for the following confounders: race, maternal age, prior breast-feeding, smoking, alcohol use, and occupation.

3.4.2 The North-Carolina study; Results

At birth no correlation between birth weight and the concentration of PCBs in mother's milk was found. However, a negative correlation between the PCB content of mother's milk and the NBAS score was found (less muscle tone, lower activity levels, hyporeflexivity). A similar outcome was reported at 6, 12, 18 and 24 months of age for the outcome of the Bayley Scales of Infant Development. At the age of 3, 4 and 5 years this relationship was not found anymore for the outcome of the McCarthy Scales of Children's Abilities.

In none of the above mentioned development tests a negative association was found between the postnatal exposure to PCBs via mother's milk and the performance of the children in these tests.

3.5.1 Discussion: Methodological aspects

The epidemiological studies give inconsistent results about the association between the prenatal (intra-uterine) and postnatal (via breast milk) exposure to dioxins, furans and PCBs and the postnatal development of children. No clear exposure-response relationship was reported, by any of the studies. From a risk assessment perspective these studies can be regarded as elements in hazard identification. For a quantitative risk assessment additional criteria with respect to analytical and methodological issues must be fulfilled.

In the Michigan study different indicators for the prenatal exposure of children to PCBs were used in reporting exposure-effect relationships: prenatal exposure was either assessed by the consumption of PCB contaminated fish by the mother's, the PCB concentration in cord blood or the PCB concentration in mother's milk (see Figs 5b and 5c). Nowhere in this study do the investigators motivate their choice for one measure of exposure over the other. One of the consequences of using different measures of exposure is that the results as reported in the different sub-analyses are difficult to compare. When the concentration of PCBs in cord blood was taken as a measure of prenatal exposure the reported relationships are difficult to interpret, since two of the four exposure classes are below the reported detection limit of 3 ng/ml (Fig. 5). Moreover, we have some concern about the validity of the total PCB measurements as used in the (early) U.S. studies. However, the U.S. investigators do not discuss the restrictions of using the total-PCB measurement in respect to their exposure characterization. This undermines the reported exposure response relationships.

Comparison of the results between studies is complicated by the absence of a consistent definition of pre- and postnatal exposure. In fact, if the assumption that the dioxin and PCB levels in breast milk reflect the mother's body burden is correct, one could only speak of postnatal exposure if these levels, often measured shortly after delivery, are multiplied by duration of breast feeding. Thus, exposure levels in maternal blood, cord blood and breast

milk (shortly after delivery) would, based on this assumption, be expected to result in similar effects. However, this was not found in the reviewed studies.

Unfortunately the data analyses in the Rotterdam/Groningen study are mainly univariate. In a number of statistical analyses subjects were categorized and groups wise compared on effects of pre- and postnatal exposure to dioxins, furans and PCBs. Using the same information in a (multivariate) regression analyses would have made a more appropriate analysis of the data. As mentioned the results shown in Figure 6 suggest a positive effect of breast feeding on the development of children. If true, such a relationship would have appeared in a much more straightforward way when the available information (postnatal exposure vs. developmental score of individual children) was analyzed with a multiple regression (analysis of variance) model. Further analyses of the data of the Rotterdam/Groningen study may allow more definitive conclusions on the negative and /or positive effects on the development of children attributed to the intra-uterine PCDD, PCDF and PCB exposure or exposure via breast feeding. These kind of analyses will also provide insight in the 'exposure-response relationship' if one exists.

In none of the reviewed studies the controlling for confounding factors, if done, was consistently reported. Often criteria for the selection of specific confounding factors are not mentioned. This hampers a systematic comparison of the results of these studies.

In conclusion, the reported findings do not allow the derivation of a quantitative exposure-response relationship, nor can meta-analysis be performed.

3.5.2 Causality of the reported associations

Hill (1965) has proposed a number of criteria which are helpful in interpreting observed associations in epidemiological studies as causal ones (Rothman, 1986).

3.5.2.1 Consistency

The criterion of *consistency* refers to the repeated observation of an association in different populations under different circumstances.

As far as comparisons can be made the studies discussed do not demonstrate strong consistency. For example, the relationship between the prenatal exposure to PCBs and a reduced birth weight which was found in the Michigan study was not confirmed in the North-Carolina study.

Furthermore, in the Rotterdam/Groningen study the prenatal exposure to dioxins and furans was found to be negatively associated with thyroid hormone levels (TT₄) in the blood of newborns whereas in the Amsterdam study the levels of this hormone were significantly increased in children receiving the highest prenatal dioxin/furan exposure. In both studies TSH levels increased up to 3 months after birth. The data analyses in both studies were univariate correlations and comparison of high and low exposed infants. Preliminary results of thyroid hormone levels in the RIVM-infant population demonstrate that the found correlation structure is far more complex and that analyses between prenatal exposure to dioxins and furans and the postnatal thyroid status of infants should be performed in a multivariate fashion to control for potential confounding and effect modification (Fiolet *et al.*, in preparation).

The negative effects of the prenatal exposure to PCBs on the Fagan score as reported in the Michigan study were not confirmed in the Rotterdam/ Groningen study. On the contrary, in the latter study a positive association between the dioxin/furan and PCB exposure via breast milk and the Fagan score was found.

Also, at birth the prenatal exposure to PCBs was found to be negatively associated with the NBAS score in the North-Carolina study whereas this association was not found in the Michigan study.

Finally, even within one study inconsistent results were reported. At the age of two weeks a significant negative association between the child's NOS score and the dioxin/furan and PCB exposure via breast milk, but not with prenatal exposure (cord- nor maternal plasma) was found in the Rotterdam/Groningen study. In contrast at 18 months of age a negative association between the NOS score and the prenatal (cord-, maternal plasma), but not the postnatal (breast milk), exposure was found.

In conclusion, the results of the various studies are considered inconsistent. Multivariate and pooled analyses could potentially shed some light on these apparent inconsistencies.

3.5.2.2 Strength of found associations

Strong associations are more likely to be causal than weak associations. For, weak associations are more likely to be explained by undetected biases. In the studies discussed here the found associations in general are weak. Furthermore, insight in potential confounding and bias is to a large extent lacking in these studies.

3.5.2.3 Biological plausibility

Biological plausibility relates to the question whether reported epidemiological associations are, from a mechanistic point of view, realistic. Regarding this criterion the associations which have been reported in the epidemiological studies (effects on the thyroid hormone status and postnatal development) are all induced by the prenatal exposure of experimental animals to dioxins, furans and PCBs (Morse, 1996; Levin *et al.*, 1988; Tilson *et al.*, 1990; Schantz *et al.*, 1995; 1996).

3.5.2.4 Exposure-response relationship

A criterion which supports causality is the existence of an *exposure response relationship*. Neither the Michigan, the North-Carolina nor the Rotterdam/Groningen study provide clear evidence for an exposure-response relationship. On the basis of animal experiments such a relationship is however to be expected. (Morse, 1996; Levin *et al.*, 1988; Tilson *et al.*, 1990; Schantz *et al.*, 1995; 1996).

3.5.2.5 Exposure preceding effect (temporality)

In a causal relationship the *exposure needs to precede the effect(s)*. In the associations under study this would indeed be the case; exposure during pregnancy and early infancy, effects occurring in the period between birth and the onset of puberty. However, the limitations of the reviewed studies as discussed above do not allow us to draw firm conclusions in regard to this criterion.

3.6 *Conclusions of the epidemiological review*

The quoted studies do not indicate an adverse effect of the *postnatal* exposure (via mother's milk) to dioxins, furans and PCBs on the postnatal development of children.

The investigators of the reviewed studies conclude that neurotoxic effects in children exposed to relatively high *prenatal* levels of dioxins, furans and PCBs cannot be excluded. However, in the light of the rather ill defined exposure assessment in these studies, the limited data analysis and the inconsistencies of some of the reported effects we do not support this conclusion. In our opinion the question whether (and to what extent) background exposure of infants to dioxins, furans and PCBs unequivocally leads to developmental problems in the exposed children, remains to be demonstrated.

The results of the quoted epidemiological studies merely serve a function in the hazard identification of the toxic effects of the *prenatal* exposure of children to dioxins, furans and PCBs. At present these results cannot be used in the quantitative risk assessment of such exposure. To obtain data suitable for a quantitative risk assessment, multivariate (re)analyses with rigorous model specification for inclusion of confounders and effect modifiers of these studies are necessary.

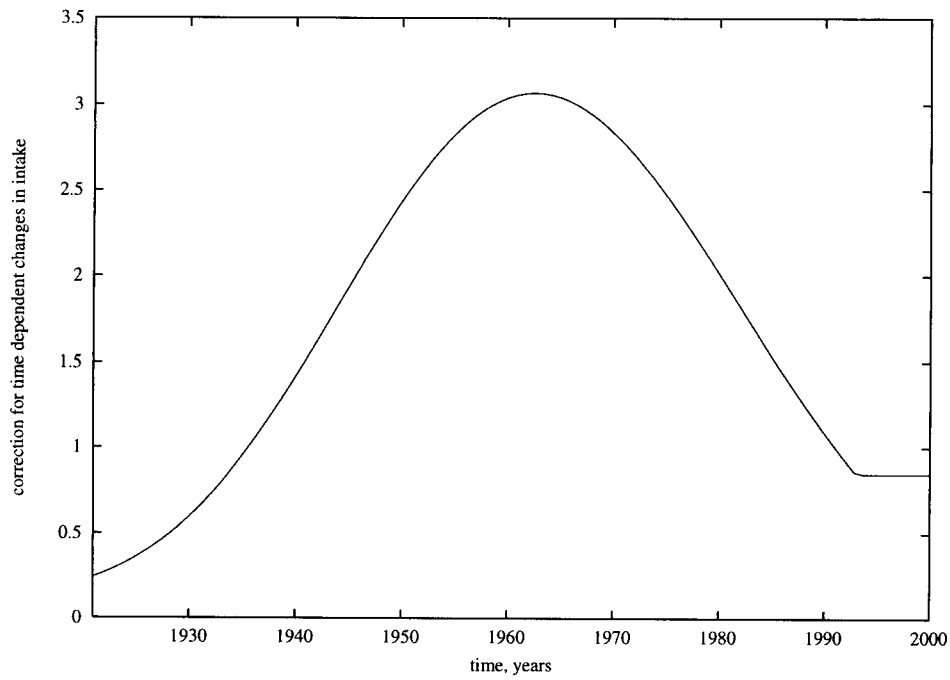


Fig. 1. Historic correction function for time dependent changes in dioxin intake. Dioxin intake is assumed to be unchanged from 1993 onward.

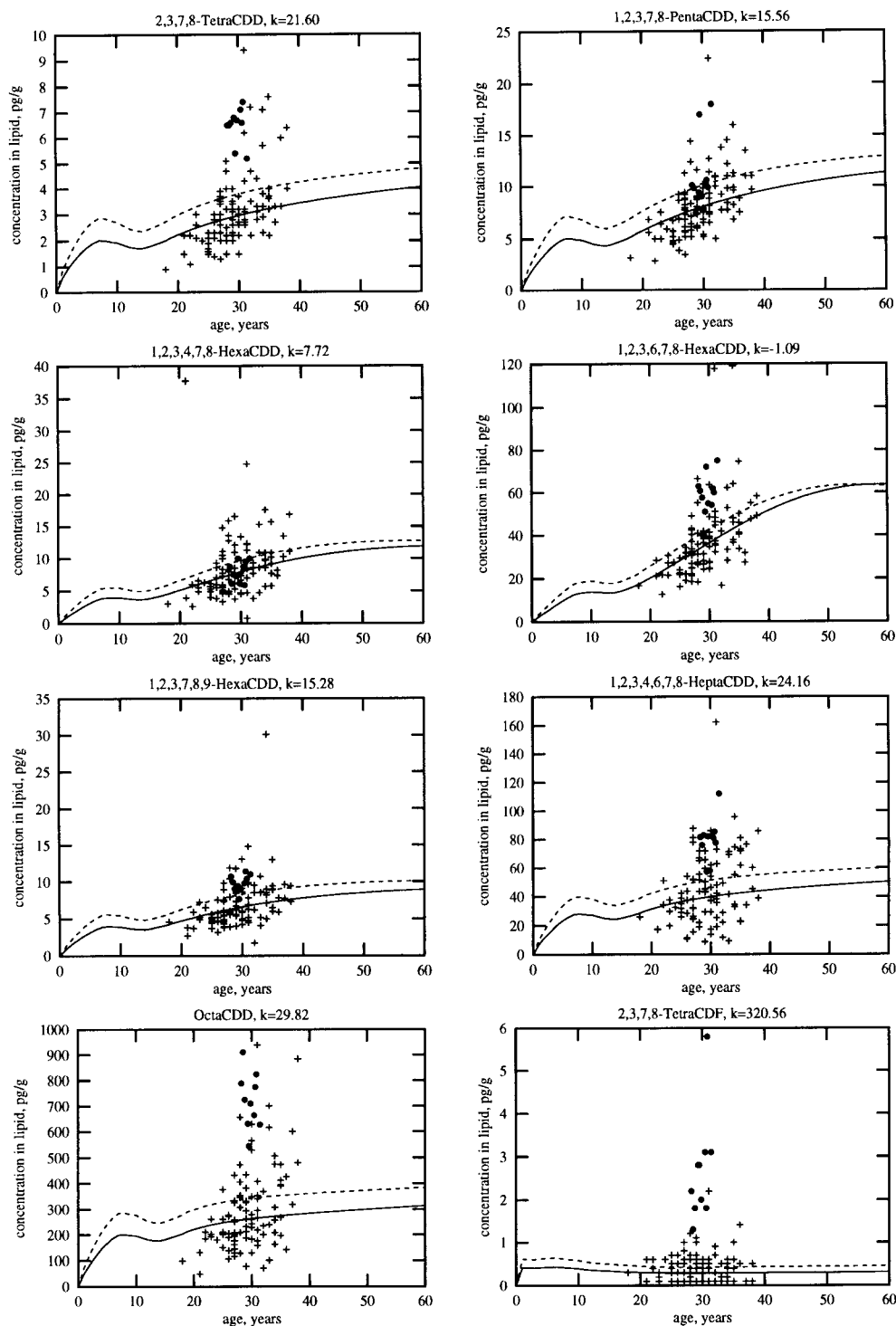


Fig. 2. Simulations of the levels in human milk (as an index of the ‘body burden’) in 1993 (solid curve) and 1988 (dashed curve). The crosses represent the measured levels in human milk, in 1993 (individual samples), the circles represent the measured levels in human milk in 1988 (pooled samples). (k = the elimination rate constant for the elimination via the liver of the specific congener, obtained by fitting the model to the concentration of Dutch milk data of 1993). In the 1993 data set (OCV levels in human milk) the non-detects are assigned half their level of detection (Cuijpers et al. 1997).

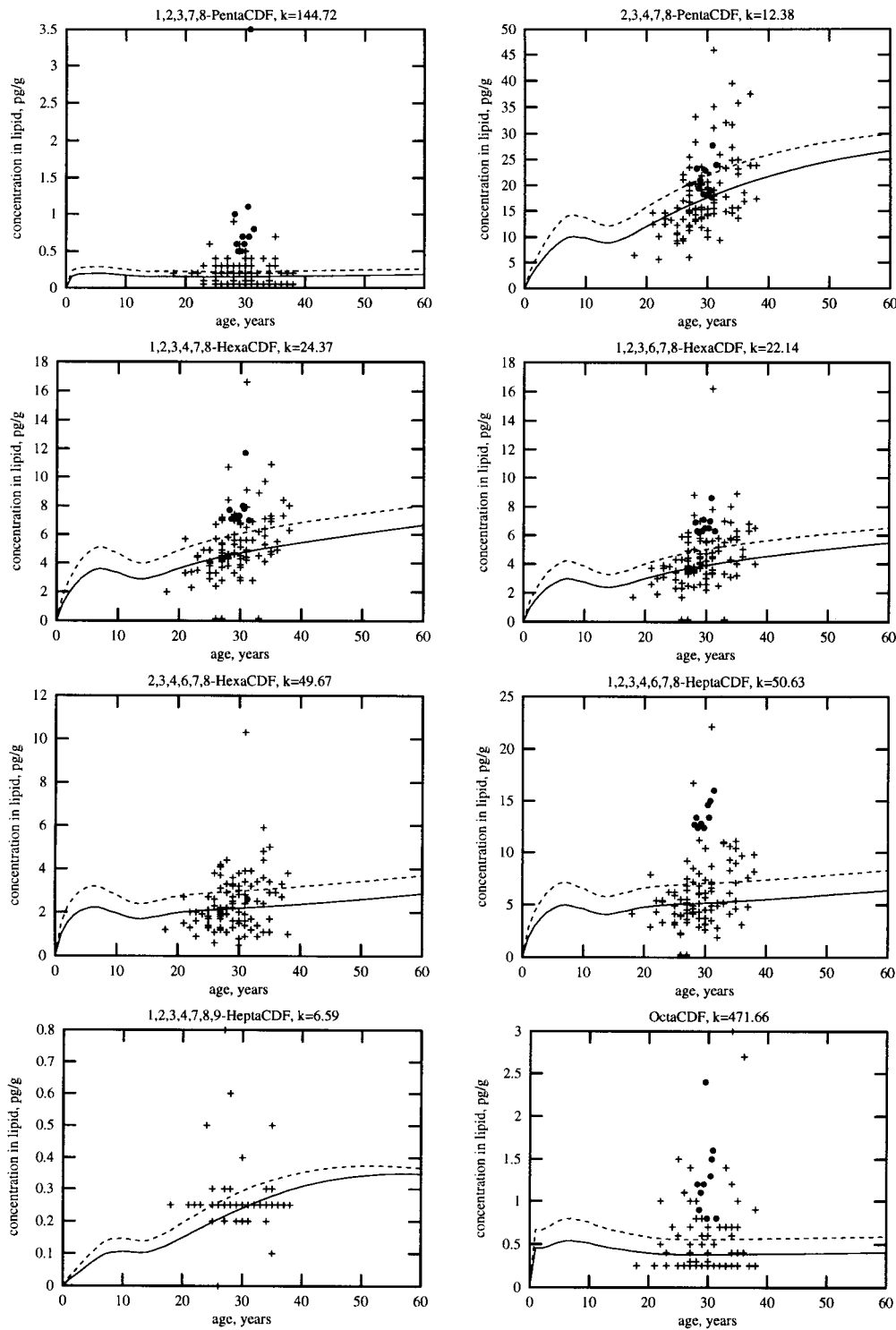


Fig. 2. (continued): Simulations of the levels in human milk (as an index of the ‘body burden’) in 1993 (solid curve) and 1988 (dashed curve). The crosses represent the measured levels in human milk, in 1993 (individual samples), the circles represent the measured levels in human milk in 1988 (pooled samples). (k = the elimination rate constant for the elimination via the liver of the specific congener, obtained by fitting the model to the concentration of Dutch milk data of 1993). In the 1993 data set (OCV levels in human milk) the non-detects are assigned half their level of detection (Cuijpers et al. 1997).

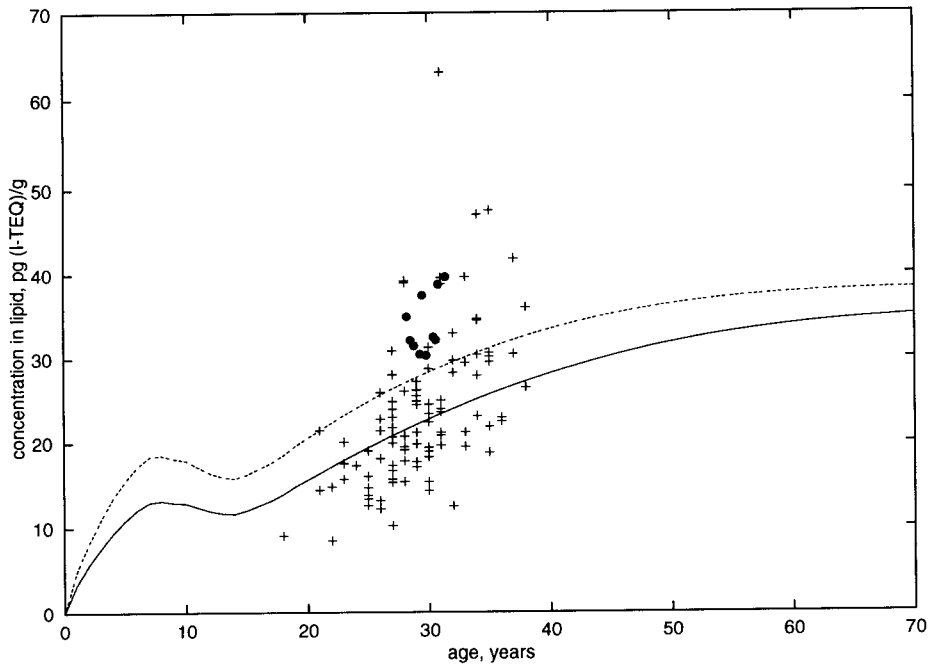


Fig. 3. Simulations of the I-TEQ levels in human milk (as an index of the 'body burden') in 1993 (solid curve) and 1988 (dashed curve). The crosses represent the measured I-TEE levels in human milk, in 1993 (individual samples), the circles represent the measured -I-TEQ levels in human milk in 1988 (pooled samples).

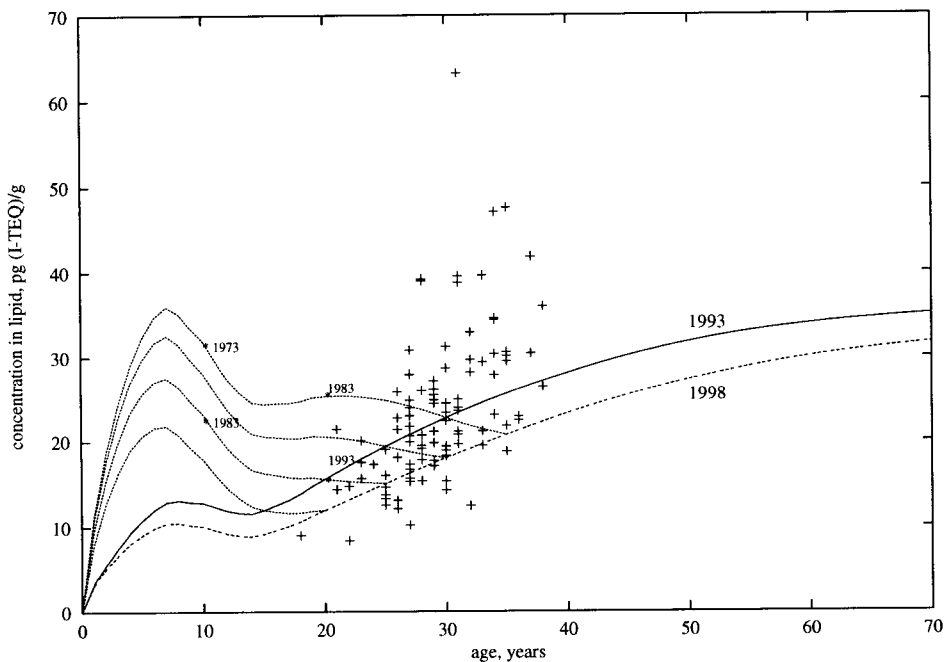
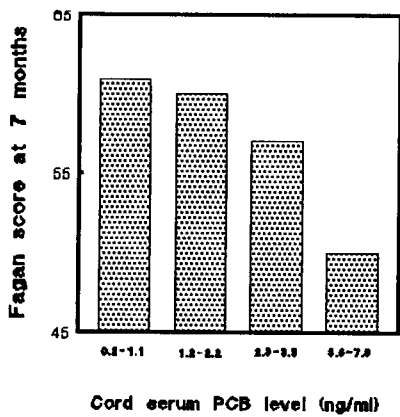


Fig. 4. I-TEQ levels in milk (as an index of the 'body burden') by age for a measured cross section in the female population in 1993 (crosses), and a simulated cross section in the female population in 1993 (solid curve), a simulated cross section in 1998 (dashed curve), and 4 simulated birth cohorts of women who will be 20, 25, 30 en 35 years old in 1998.

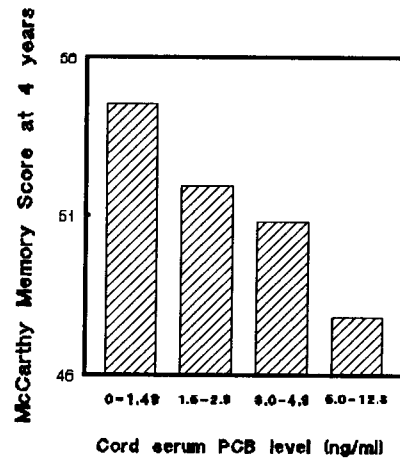
Fig. 5 Dose-effect relationship between prenatal PCB exposure and the performance of children in developmental tests, as reported by Jacobson et al. (1990; 1996). Prenatal exposure to PCBs was assessed by measuring their concentration in cord blood (5A and 5B) or in mother's milk (5C and 5D).

- A. Fagan score at 7 months of age. Group N's were 20, 21, 20 and 20.
- B & C McCarthy Memory Score at 4 years of age. Group N's were 48,43,32 and 10.
- D. IQ scores at eleven years of age. Group N's were 21,45,46,36 and 30. IQ scores in the range of 70-80 are indicative for mild mental retardation.

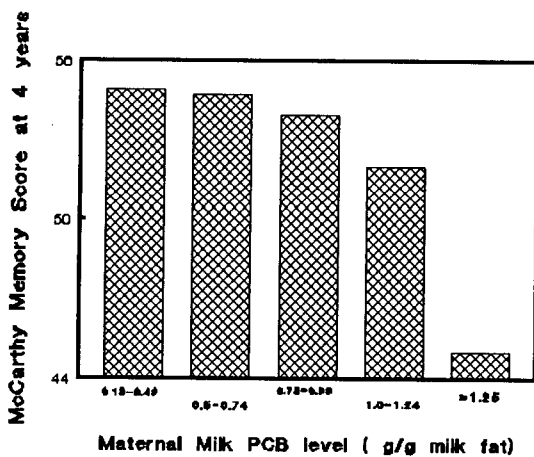
A



B



C



D

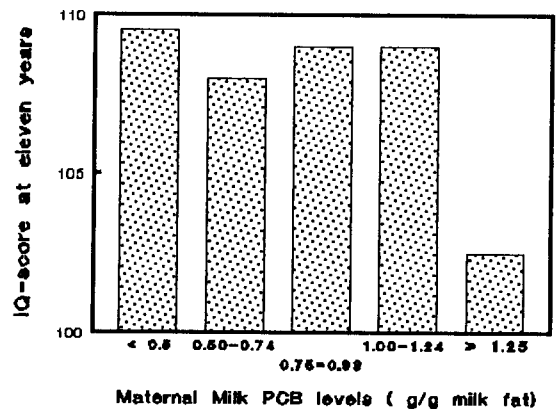
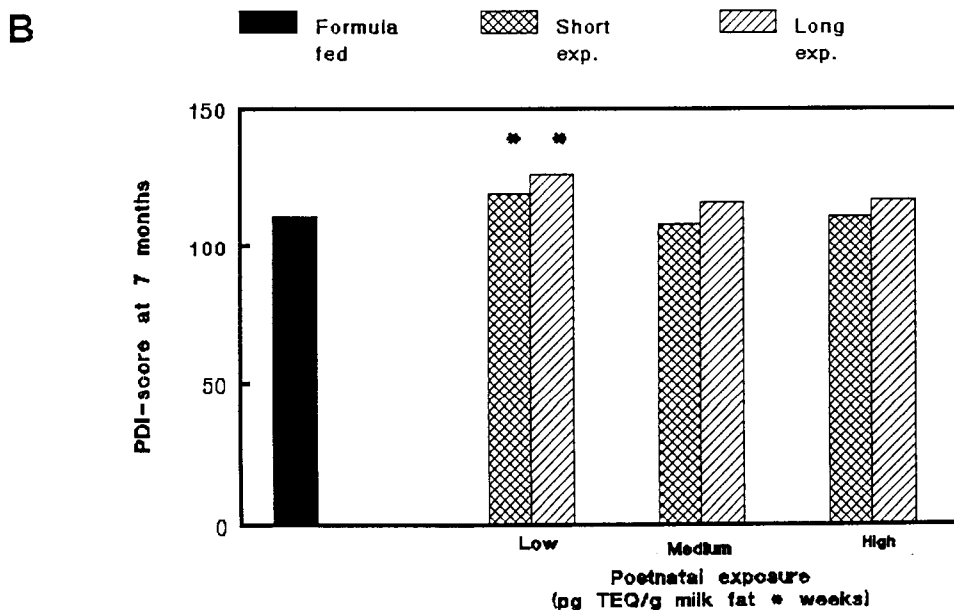
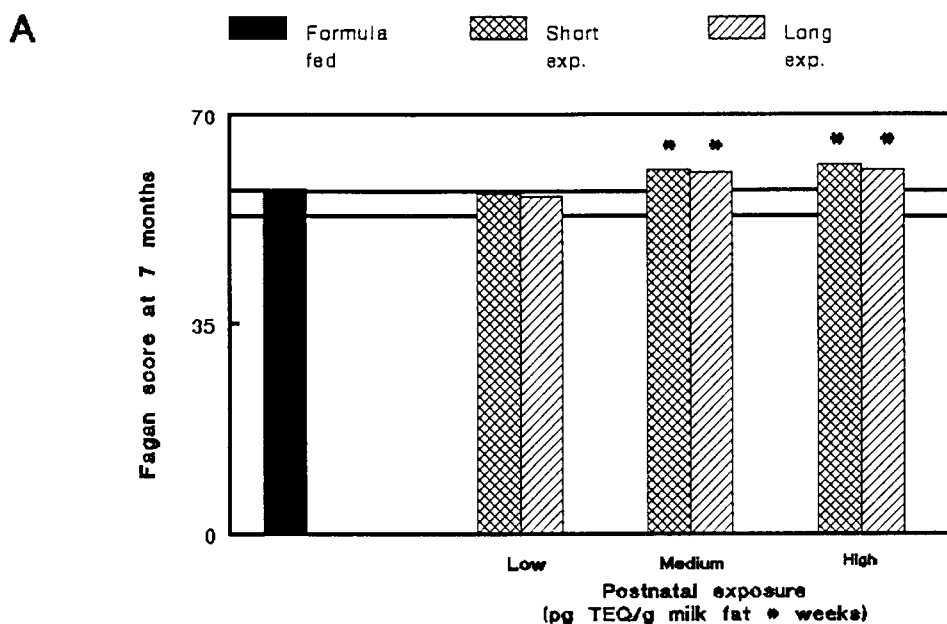


Fig. 6 The effects of postnatal exposure to dioxins, furans and PCBs in mother’s milk and the performance of children in developmental tests as reported by Koopman-Esseboom (1995).

- A. Fagan score at the age of 7 months. Upper line: Fagan score considered as normal. Lower line: Fagan score considered indicative for a delay in mental development.
- B. Psychomotor development (PDI-score) at 7 months.

Postnatal exposure to dioxins, furans and PCBs was quantified by multiplication of the duration of breast feeding with the TEQ content of these compounds in mother’s milk at two weeks after birth. The resulting exposure measure was divided into three categories: low exposure (168-769 pg dioxin/furan/PCB TEQ/g milk fat times week, N=27), medium exposure (770-1289 pg dioxin/furan/PCB TEQ/g milk fat times week, N=26), and high exposure (1290-4340 pg dioxin/furan/PCB TEQ/g milk fat times week, N=27). Asterisk: differing significantly from controls (formula fed infants). Short: duration of breast feeding 6-16 weeks. Long: duration of breast feeding 17-30 weeks. The effects of postnatal exposure to dioxins, furans and PCBs on the psychomotor development (PDI-score) at the age of 7 months (after Koopman-Esseboom, 1995)



Appendix I

Overview of the model

(by G.W. van der Molen)

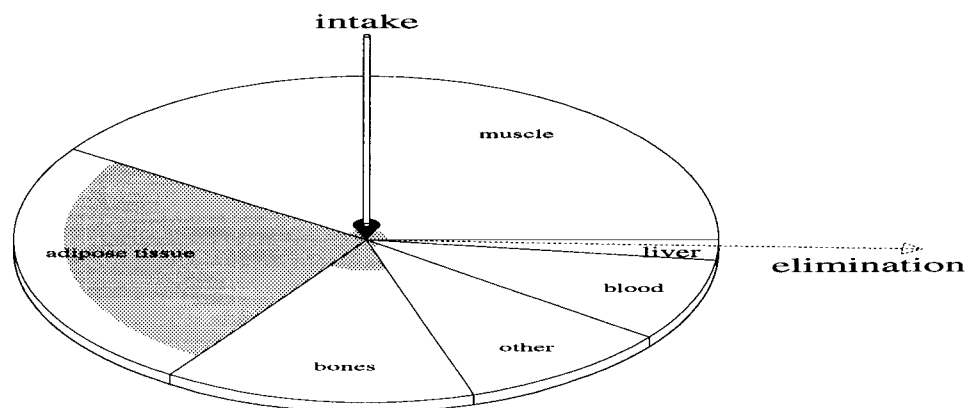


Figure I-1. Schematic representation of the toxicokinetic model for dioxins and furans in the human body. The pie-parts represent physiological body compartments, each consisting of a lipid part, represented by the gray areas, and a non-lipid part, represented by the white areas.

Basic structure:

Intake: Model input is determined by the consumption of dioxin/furan contaminated foods.

Distribution: Upon entering the body, dioxins and furans are rapidly distributed among the lipid fractions of the body compartments (represented by the gray areas). Amounts of dioxins and furans are assumed to be negligible in the non-lipid fractions (represented by the white areas). The exchange between the lipid fractions is rapid compared to the elimination from the body, so that the concentrations in the lipid fractions are considered to be in a semi-steady state, and thus are equal in each compartment.

Elimination: Elimination takes place as a first order process from the liver, so elimination is proportional to liver weight and the concentration in the liver. This process can either take place through metabolism in the liver, or through excretion of dioxins and furans into the feces via bile.

Model parameters:

The model is suitable for a wide age range (from 0 to 70 years), because the model parameters which cannot be assumed to be constant during a life-time are incorporated as age-dependent functions.

Intake: For each congener, the intake is the product of the age dependent intake rate of the average Dutch female and the time dependent correction factor for historical changes in the

intake rate. The age dependent intake rates were calculated with the aid of a statistical exposure model (Slob, 1993) using the data from the Dutch National Food Consumption Survey 1987 (Hulshof en van Staveren, 1991) in combined with concentration measurements in food around 1991 (Liem *et al.*, 1991). The derivation of the correction function for historical changes in the intake rate, which we assume to be the same for each congener, is explained in the main text.

Body weight: Data collected during the Dutch National Food Consumption Survey were utilized to construct descriptive functions (of age) for the body weight of males and females. (Dutch Ministries of WVC and LNV, 1988; Hulshof and Van Staveren, 1991). Figure I-2 shows pie-chart representations of the human body at different ages. The surface of the charts correspond to body weight.

Body composition: Body composition is also age dependent. In figure I-2 these changes are illustrated by pie-chart representations of the human body. The following paragraph from the article by van der Molen *et al.* (1996) describes how these functions were obtained:

Adipose tissue weights as percentages of body weight given by Widdowson (1981) for ages 0-17.5 years, and by Deurenberg (1991) for ages >18 years were linearly interpolated. Deurenberg gives equations for adipose tissue weight (as percentage of body weight) as functions of age, sex and Body Mass Index (BMI) for adults and children. BMI was calculated as a function of age by using weights and lengths from the Dutch national food consumption survey (Dutch Ministries of WVC and LNV, 1988; Hulshof and Van Staveren, 1991).

Liver volume as a proportion of body weight declines after the age of 24 years according to Wynne *et al.* (1989). In their article they give values at the ages of 24 and 91 years, estimated by linear regression analysis. These values were multiplied by a specific weight of $1.06 \text{ (g cm}^{-3}\text{)}$ and by the body weight function discussed above, resulting in liver weight as a function of age. For children only liver-weight measurements of babies were found in the literature. The growth of liver weight between zero and 24 years was assumed to be similar to the body-weight curve.

For blood, bones, and 'remaining' organs percentages of body weight in adults (Sinclair, 1985) were used to calculate compartment weights at the age of 25 years. Weights of these compartments were taken to be constant from 25 years on. Thus, for adults only weight of the muscle compartment is still unknown. In the model it is set to body-weight minus all other compartment weights. In this way muscle weight decreases in aging people. For persons under 17.5 years of age male and female muscle weights as reported by Widdowson (1981) were used. For children no data on age dependency of blood, bone, and 'remaining' organ weights were found, and we used growth curves with a slope similar to that of the body weight curve to describe these compartment weights as functions of age.

Lipid fractions of compartments: No information about age dependency of the lipid fractions of compartments was found, so these were assumed to be constant. Parameter values and their source are presented in table I-1.

Table I-1 Lipid fractions of human tissues on wet-weight basis.

compartment	parameter	value	source
blood	f_b	0.0052	Päpke (1989)
liver	f_l	0.049	Lentner (1981)
bone	f_{bo}	0.186	Clarys (1985)
muscle	f_m	0.064	Ryan (1985b), Lentner (1981)
adipose tissue	f_a	0.859	Beck (1990), Duarte-Davidson (1993), Patterson (1986), Ryan (1986), Ryan (1985b), Schecter (1985)
remaining organs	f_o	0.049	Beck (1990), Lentner (1981), Ryan (1985b)

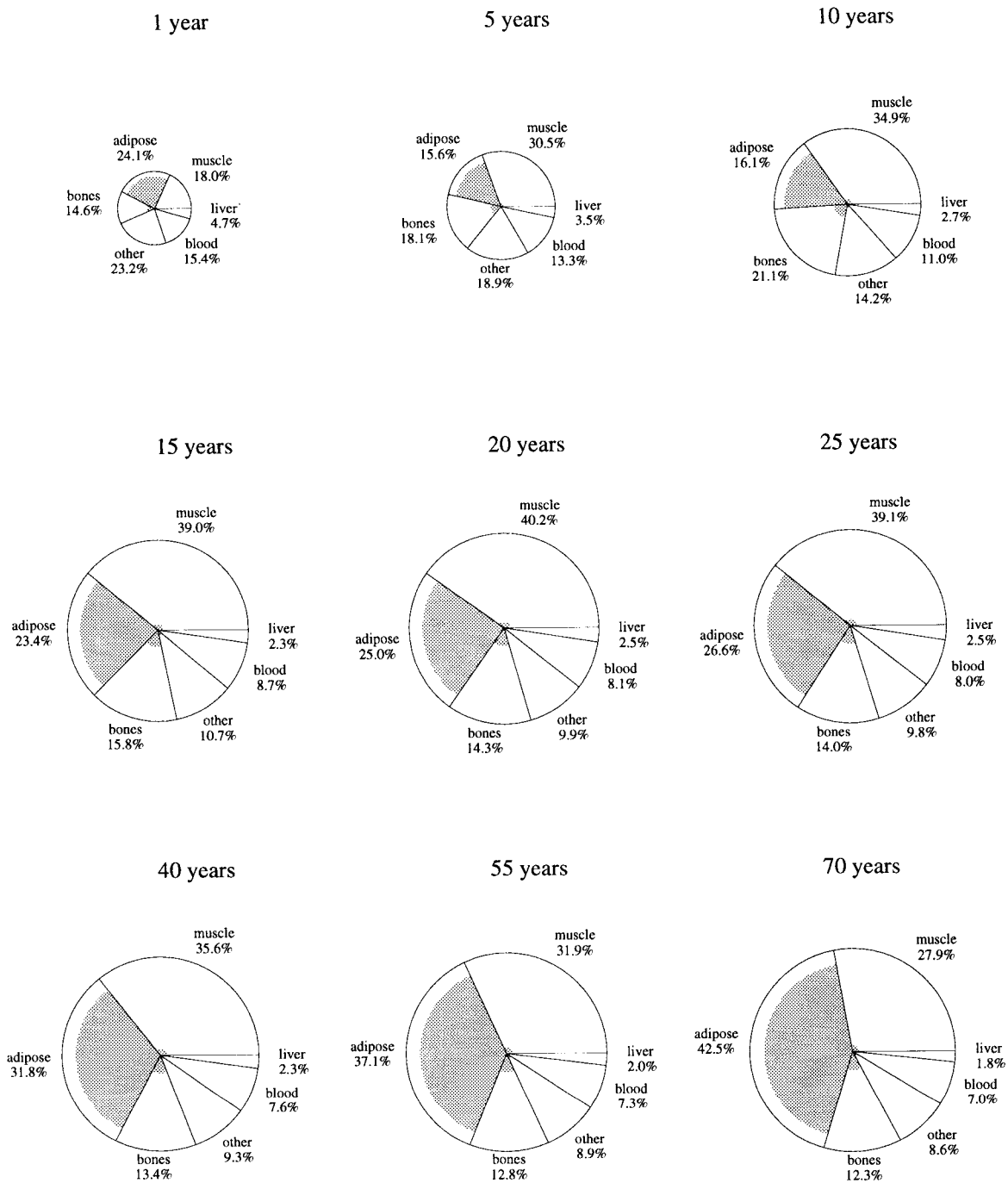


Figure I-2. Schematic representations of the average female human body. The pies represent the human body; the surface corresponds to body weight. The pie-parts represent physiological body compartments, each consisting of a lipid part, represented by the gray areas, and a non-lipid part, represented by the white areas. Lipid percentage's of all compartments (shown in table I-1) are assumed to be constant with age. Body weight and composition are functions of age (and dependent on gender).

Appendix II

Table II-1: Correlations of PCB exposure measures with control variables included in the 4-year follow-up of the Michigan fish eater cohort. (as reported by Jacobson J.L. and Jacobson S.W. 1996)

Variable	cord serum PCB level ^a (N=146)	maternal milk PCB level (N=120)
Socioeconomic status	0.04	-0.07
Home Inventory ^b	0.01	0.07
Maternal verbal competence ^c	0.10	-0.13 [†]
Maternal education (years)	0.00	-0.00
Maternal age	0.16 [§]	0.05
Maternal weight ^d	0.05	-0.05
Maternal height ^d	0.01	0.15 [†]
Paternal weight ^d	-0.04	0.04
Paternal height ^d	0.09	-0.04
Marital status ^e	-0.04	0.01
Maternal employment (h/wk)	0.05	0.19 [§]
Number of children	0.10	-0.11
Delivery complications ^f	0.02	0.10
Gravidity	0.17 [§]	-0.01
Parity	0.13 [†]	-0.09
Sex of child ^g	-0.09	-0.03
Familial stress ^h	0.06	0.15 [†]
Preschool experience ⁱ	0.02	-0.06
Alcohol prior to pregnancy ^a	0.01	0.12 [†]
Alcohol during pregnancy ^a	0.12 [†]	0.06
Smoking prior to pregnancy	0.02	0.02
Smoking during pregnancy	0.04	0.09
Polybrominated biphenyls ^{a,j}	0.06	0.27 [‡]
4-year serum DDT ^a	-0.03	0.17 [§]
4-year blood lead	0.02	-0.18 [§]
Age at 4 year testing	0.00	0.09
Medication prior to testing ^k	-0.02	-0.05
Examiner	4.41 [‡]	<1

^alog transformed

^bHome observation for measurement in the Environment -Preschool version

^cPeabody picture vocabulary test - Revised

^dUsed as control variable only in analyses of physical growth

^eUnmarried =0: married =1

^fNo =0: Yes =1: coded yes for any of the following: emergency cesarean section, labor longer than 20 h, placenta previa or abruptio, toxemia cyanosis, fetal distress, meconium staining, infected cord, or knot in cord.

^gMale =0: female =1

^hHighest level of stress reported on a 6-point scale in any of four domains: financial, health, marital or other.

ⁱNo =0: Yes =1

^jAs assessed in cordserum, milk, and 4 year serum, respectively

^kAntihistamines, cough syrup, or seizure medication within 24 h prior to testing

* P< 0.001 † P< 0.10 ‡ P< 0.01 § P< 0.05

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