BENEFIT AND RISK ASSESSMENT OF BREASTMILK FOR INFANT HEALTH IN NORWAY

- Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety

Vitenskapskomiteen for mattrygghet
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Gry Hay has participated as an observer from the Norwegian Directorate of Health.

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Extensive summary

Introduction

The present benefit and risk assessment of breastmilk and contaminants in breastmilk was initiated by the Norwegian Scientific Committee for Food Safety (VKM). The overall objective is to provide a balanced assessment of the benefits of breastmilk against the possible risks from exposure to contaminants in breastmilk with focus on Norwegian conditions. The aim is to contribute to a foundation for decision-makers when providing recommendations on the length of exclusive and partial breastfeeding.

The composition of breastmilk is tailored for the needs of the newborn. Provided that the nutritional needs of the mother are met during pregnancy and breastfeeding, breastmilk covers all the nutritional requirements of the infant the first months of life, with the exception of vitamin D. Breastmilk also contains a number of specialised components, including growth factors, factors with anti-microbial and anti-inflammatory properties and selected immunological components which boost the maturation of the infant's immune system. Infant formula fulfils the infant's established nutritional needs, but does not provide the specific protective factors which are present only in breastmilk.

However, studies over the last four decades have shown that polluting chemicals have accumulated in the environment, biomagnified in the food chain, are in our bodies, and consequently in breastmilk. The levels of lipid-soluble persistent contaminants in the foetus, the newborn child and in breastmilk largely reflect the amount of these in the mother's body.

Thus, breastmilk contains nutrients and protective immunological factors which have a positive effect on infant health, but may also contain contaminants. Particularly lipid-soluble and persistent contaminants accumulate in the infant during breastfeeding. This has contributed to a debate among experts agreeing that breastfeeding is beneficial, but discussing the advisable length of breastfeeding.

Breastfeeding in Norway

Breastfeeding prevalence is higher in Norway than in most European countries. 80% of the infants are breastfed at 6 months of age and 46% at 12 months. Mean breastfeeding duration is about 10 months.

Norwegian health authorities recommend that infants are exclusively breastfed for 6 months with a total duration of at least 12 months. However, only a minority of Norwegian mothers breastfeed exclusively for the recommended 6 months. The prevalence of exclusive breastfeeding declines rapidly from 3 months onwards with only 9% being exclusively breastfed at 6 months.

Mean breastmilk consumption in exclusively breastfed infants increases from approximately 700 ml/day at age 1 month to 850 ml/day at age 6 months. The amount of breastmilk provided to the child is not very different between the partially and exclusively breastfed infants during the first 4 months. From 7 months, breastmilk consumption in partially breastfed infants may be about 500 ml/day.

There are a few conditions where breastfeeding is contraindicated. Among these are some metabolic disorders, infections and use of certain pharmaceuticals.

Nutrients and immunological components in breastmilk

The positive health effects of breastmilk relates to nutritious as well as immunological properties.

An infant who is exclusively breastfed for the first 6 months of life has, provided adequate nutrition of the mother, all the nutritional needs covered with the exception of vitamin D. Therefore, worldwide, the recommended daily intake of nutrients for infants is derived from the nutrient concentrations in breastmilk multiplied with the average intake of breastmilk.

The composition of nutrients in breastmilk varies by stage of lactation, the time of day and during a given feeding. The concentration of some nutrients also varies according to the mother's diet. The energy content of breastmilk varies, but has been estimated to be about 700 kcal/L. The content of proteins and carbohydrates is relatively stable, while the fat content has large variations. The fatty acid composition and concentrations of most vitamins reflect the maternal intake, while the concentrations of most minerals are not affected by the maternal diet, except for selenium and iodine.

Breastmilk has protective properties. It contains a number of specialised components, including factors with anti-microbial and anti-inflammatory properties as well as constituents boosting the maturation of the infant's immune system. This benefits health in childhood and most likely also later in life. The milk antibodies are targeted against potential pathogens and other antigens to which the mother has been exposed. Moreover, maturation of the infant's immune system is influenced by contact with the immune-modulating factors in breastmilk as well as dietary and microbial constituents in the infant's gut. Different components in breastmilk facilitate the establishment of a beneficial intestinal microbiota, which is important for induction of a balanced mucosal immune system. Through all these mechanisms, breastfeeding represents an ingenious immunologic integration of mother and child.

Nutrients in infant formula

If breastfeeding is not possible or if there is a need for more milk in addition to breastmilk, infant formula is recommended until the child is 12 months of age.

Infant formula fulfills the infant´s established nutritional needs, but does not provide maternal antibodies and innate defence factors or immunity-promoting components. The majority of the infant formulas on the Norwegian market are cow's milk-based.

Data from a national dietary survey among infants (Spedkost, 2006) showed that at 6 months of age, 43% of the infants in Norway had been introduced to infant formula, and 36% used it regularly. At 1 year of age, 43% of the infants received infant formula regularly.

Infant formulas in Norway are subject to EU regulations that cover the composition, labelling, marketing and distribution of the product. The regulations give minimum and maximum limits for nutrients for infant formulas and include some of the provisions of the WHO Code[1].

Contaminants and exogenous microbes in breastmilk and infant formula

Breastmilk, as a reflection of the mother's body, contains low concentrations of a mixture of different contaminants. Only the most prevalent contaminants in breastmilk have been

[1]International Code of marketing of Breast-Milk substitutes.

determined chemically and even fewer have been studied in humans with regard to impact on early life health.

The main focus of the present benefit and risk assessment of breastmilk are contaminants which are included in the Stockholm convention on Persistent Organic Pollutants (POPs)[2]. They can be divided into the three main groups; pesticides (DDT and HCB), other halogenated organic pollutants (dioxins and dioxin-like PCBs, non dioxin-like PCBs, brominated flame retardants (PBDE), perfluorinated compounds (PFOS/PFOA)) and heavy metals (lead, mercury and cadmium).

In the identification and characterisation of negative health effects, combined exposures to multiple contaminants[3] from breastmilk have to some extent been taken into consideration, as several of the cohorts have been investigating the impact on health outcomes of PCBs and dioxins in combination with DDT or HCB and some in combination with mercury. Additionally, it should be noted that the contaminants studied may be considered as markers for the combined exposure of multiple contaminants, since their occurrences are often correlated.

Metal concentrations in both breastmilk and infant formula (e.g. mercury and lead) are generally low and not at levels associated with concern.

Due to national and international restrictions and bans on use, the levels of dioxins, PCBs, and pesticides (like DDTs and HCB) have declined substantially (more than 60%) in the environment and in humans the last three decades. Compared to DDTs, HCB, dioxins and PCBs, the concentration of PBDEs in breastmilk in Norway increased until approximately year 2000, after which a decline has been observed. The fluorinated surfactants PFOS and PFOA have shown a similar time trend as the PBDEs.

There are limited Norwegian data on levels of persistent organic pollutants in infant formula, but the levels reported are generally much lower than in breastmilk.

Some contaminants which do not accumulate in the food chain may also be relevant in both breastmilk and infant formula. Substances from food packaging materials, e.g. phthalates, may be present in both breastmilk and infant formula, as well as process-generated substances such as acrylamide, PAHs, furan and 3-MDCP. The hormone active substance bisphenol A (BPA) used in plastic has recently been banned in infant feeding bottles in EU and Norway. Occurrence data in breastmilk and infant formula for these substances in Norway are scarce.

The main difference between the contaminants in breastmilk and those provided by infant formula or bottle-feeding is that breastmilk generally contains higher levels of persistent organic pollutants, while most of the unwanted substances imposed by infant formula and bottle-feeding have a shorter half-life.

Infant formula may contain microbial contamination of concern, which may lead to diarrhea and in severe cases bacteraemia and meningitis. *Cronobacter* spp. (formerly *Enterobacter sakazakii*) is a rare cause of invasive infection with high death rates in newborn infants. Possible outbreak from microbiological hazards in infant formula itself or due to

[2]The Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) is a global treaty administered by the United Nations Environment Programme (UNEP) to protect human health and the environment from chemicals, and first entered into force in 2004 (Stockholm Convention on POPs 2004 http://www.chm.pops.int). The criteria for being included in SC are persistence, bioaccumulation, potential for long-range transport, and adverse effects.

[3]In popular terms often referred to as the “cocktail effect”.

contaminated water is an issue in developing countries, but no such outbreaks have been registered in Norway.

Methodological approach to this benefits and risk assessment

The *benefit* assessment is based on positive health effects reported in systematic reviews and meta-analyses published within the last 10 years. This implies that VKM has not conducted its own specific literature search to reveal the epidemiological studies that have examined positive health effects of breastmilk, but summarises and discusses the health outcomes described in the included systematic reviews and meta-analyses[4].

In addition, some other reviews or single studies of high quality have been included if recent publications of relevance to the benefit assessment have appeared, or the above described reviews and meta-analyses have not commented on diseases/conditions of interest. In the VKM conclusions, most emphasis has been put on two recent systematic reviews; one from Nordic Nutrition Recommendations (2013) and one from WHO (2013).

As a basis for the *risk* assessment to identify and characterise possible negative effects of contaminants in breastmilk, a full scale systematic literature search for single studies investigating such effects associated with exposure to contaminants in breastmilk was conducted.

It was set as an absolute inclusion criterion that the studies should provide good breastfeeding data and be able to differentiate effects of postnatal exposure from prenatal exposure.

The systematic literature search resulted in 46 studies which were rated for quality (A-B-C) according to a predefined set of criteria. Of these, no studies were categorised as A, while 24 studies qualified for category B. The category C-studies were not considered further. The negative health effects of contaminants in breastmilk in this report are thus based on 24 papers from 10 cohorts conducted in seven different countries (USA, Canada, Faroe Isles, Spain, Germany, the Netherlands and Slovakia). No such data on impact on infant and child health of exposure to contaminants via breastmilk was available from Norway. Only the contaminants PCBs, dioxins, DDT/DDE, HCB and mercury were investigated in the included studies.

Grading

There are several methods in use for grading evidence in systematic literature reviews. The grading of evidence used in this benefit and risk assessment is similar to the grading system used by the World Cancer Research Fund (WCRF) in the report Food, Nutrition, Physical Activity and the prevention of Cancer: a global perspective from 2007.

[4]The included systematic reviews and meta-analyses are: Breast-feeding: A Commentary by the ESPGHAN Committee on Nutrition (including a previous WHO-report from 2007, and two other systematic reviews from 2005 and 2007). The influence of maternal, fetal and child nutrition on the development of chronic disease in later life from the Scientific Advisory Committee on Nutrition in UK from 2011. Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th Nordic Nutrition Recommendations from 2013. Long-term effects of breastfeeding: A systematic review from WHO also from 2013.

In short, the grades for the evidence are[5]:

- Convincing
- Probable
- Limited – suggestive
- Limited – no conclusion

When grading the evidence of the literature on positive health effects associated with breastmilk, VKM has graded the sum of the work by others, some of whom have not graded their results and some using grading, but various grading systems. An element of "best judgement" is thus unavoidable from our side, as there to our knowledge are no international guidelines for grading on the basis of reviews and meta-analyses.

Breastfeeding data in meta-analyses and reviews for positive health effects

Few of the meta-analyses, reviews or single studies used as a basis for the benefit assessment enabled differentiation of health benefits of breastmilk in a long-term dose-dependent manner. Although some of the studies support conclusions that prolonged and exclusive breastfeeding up to 6 months of age stimulates protective effects better than shorter duration, the optimal length of exclusive and total duration of breastfeeding remains to be settled.

Norwegian contaminant exposure estimates and tolerable intake levels

Exposure estimates for Norwegian infants were done for PCB-153, DDE, HCB, dioxins and dl-PCBs. These compounds were the only contaminants where sufficient occurrence data in Norwegian breastmilk were available. Furthermore, these compounds were investigated in the included studies on possible negative health effects associated with contaminants in breastmilk.

The cumulative amounts pr kg body weight were estimated by combining contaminant concentrations in breastmilk from Norwegian women with mean consumption of breastmilk, mean fat concentration in breastmilk and the mean body weight in children.

In general, the risks from exposure to contaminants have been assessed by international risk assessment bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or European Food Safety Authority (EFSA). Tolerable intakes have been set for some contaminants (e.g. dioxins and dl-PCBs (dioxin-like PCBs)), DDE, HCB and some fluorinated compounds).

For other contaminants, EFSA could not establish tolerable intakes due to lack of data (e.g. PBDEs and ndl-PCBs (non dioxin-like PCBs)). However, VKM has previously used a guidance value and used this for sum of six ndl-PCBs.

As long as the exposure from breastmilk is below tolerable intakes, exposure in infants is considered not to be of concern. It is important to note that tolerable intake values are values presumed to be safe. They are not equivalent to levels above which negative health effects are

[5]More detailed criteria given in section 10.1.

likely to occur. Safety margins are incorporated in the values, and exceeding the tolerable intake merely results in a reduced safety margin.

The exposure estimates indicate that the DDE and HCB exposure via breastmilk to infants is lower than the tolerable daily intakes (TDIs). The guidance value for ndl-PCBs is based on maternal exposure in cohorts were the infants were breastfed. Exposure estimations in Norwegian women indicate that at least 95% of Norwegian infants are exposed to ndl-PCBs below a level which can be considered tolerable.

Exceeding the tolerable intake in infants does not necessarily imply that the concentration in the infant body reaches a level of concern, and therefore the tolerable intakes of dioxins and dl-PCBs are not directly applicable for infants. The tolerable intake of dioxins and dl-PCBs has been set to ensure that the contaminant concentration in the mother (the maternal whole body concentration, expressed per kg body weight) is below the highest concentration that is considered safe to the foetus. This is the body concentration associated with intake similar to the tolerable intake. Due to the rapid growth of the infant a dilution of contaminants takes place with a slower increase in body concentration.

The dietary intake of dioxins and dl-PCBs, as recently estimated in the Norwegian MoBa cohort, was below the tolerable weekly intake (TWI[6]) for more than 97% of the participating pregnant women. This indicates a low risk associated with prenatal exposure among infants in Norway.

Contaminant exposure in Norwegian infants in comparison with infants in included cohorts addressing negative health effects

The Norwegian levels of PCB-153 and DDE in breastmilk are substantially lower than in most of the cohorts included in the risk part of the assessment. However, Norwegian mothers continue partial breastfeeding longer than women in most of the studies addressing negative health effects. Even when being breastfed for 2 years, the accumulated amount of contaminants in Norwegian children will not reach the maximum levels of the included cohorts. Furthermore, the highest body concentration in Norwegian infants is reached later in infancy in comparison with the included cohorts.

Additional considerations

The present benefit and risk assessment has included studies that have separated measures of pre- and postnatal exposure and associations with the health outcomes. Effects from prenatal exposure can be assessed by e.g. investigating association between infant and/or maternal exposure levels at birth and health outcomes later in life. However, if postnatal exposure is also of importance, it may influence the associations with prenatal exposure. Further, associations between postnatal exposure and health outcome may also be influenced by prenatal exposure. Although such influence can be adjusted for in statistical analyses, residual influence cannot be ruled out. Focusing on studies separating associations with pre-and postnatal exposure was in view of VKM the best approach available for this benefit and risk assessment.

Many of the studies on negative effects on neurodevelopment included in the present assessment found an association between *prenatal* contaminant exposure[7] and increased risk –

[6]The TWI for dioxins and dl-PCBs is 14 pg TEQ/kg body weight/week.
[7]Mostly twice or more than current Norwegian exposure.

an effect not observed by postnatal exposure. Hence, assuming equal sensitivity in the pre- and postnatal period is probably a conservative approach which most likely overestimates the risk when the highest body concentration is reached as late in infancy as 12 months, as in Norway.

Benefit and risk assessment by health outcome

The literature grading forms the basis of the benefit and risk assessment. Furthermore, the evaluation of the benefits and possible risks related to breastmilk is based on Norwegian data on prevalence of breastfeeding and concentrations of persistent organic pollutants in breastmilk.

The health outcomes described in the included papers for positive and negative health effects are related to the following diseases or conditions:

- Neurodevelopment
- Immune response-associated diseases including infections, asthma/wheezing and allergy, coeliac disease, inflammatory bowel disease (Crohn's disease and ulcerative colitis) and type 1 diabetes.
- Growth, overweight and obesity
- Outcomes related to the metabolic syndrome such as blood-pressure, serum-cholesterol, type 2 diabetes and cardiovascular disease
- Malignant diseases (cancer)
- Sudden infant death syndrome
- Thyroid parameters
- Sexual maturation

The VKM conclusions are organised according to health outcomes, starting with those outcomes that have been studied from both a benefit and risk point of view.

Neurodevelopment - IQ and cognitive development

Based on systematic reviews and meta-analyses published within the last 10 years, VKM finds that the evidence is *convincing* for an enhancing effect of breastmilk on neurodevelopment. The optimal length of exclusive and partial breastfeeding which stimulates the positive effect remains to be settled.

The grading of the scientific evidence for negative health effects from persistent organic pollutants in breastmilk on neurodevelopment is based on seven cohorts, with findings of increased risk in three cohorts (Dutch, German and Canadian cohorts). The findings were transient in the Dutch and German cohorts, whereas no follow up study from the Canadian cohort was available.

The evidence is *limited suggestive* for a negative effect from persistent organic pollutants in breastmilk on neurodevelopment at exposure levels in these cohorts. The length of exclusive and partial breastfeeding associated with the transient negative effects in some studies remains to be settled. As to mercury, this compound was only investigated in one cohort and VKM concludes that the evidence for an effect of exposure from breastmilk is *limited and no conclusion* can be drawn.

A specific confounder challenge for interpretation of studies on neurodevelopment was the lack of HOME score measurements. HOME score measurements were used in 7 of the 14 papers investigating associations between POP's in breastmilk and neurodevelopment, including the two papers which reported transient findings.

The estimated cumulative amounts per kg body weight of contaminants in infants in the Dutch, German and Canadian cohorts were higher than currently in Norway, both prenatally, early postnatally and at 1 year of age. Even with a breastfeeding duration of 2 years, the cumulative amounts of contaminants in Norwegian infants will not reach similar peak levels as the infants in the included cohorts. Furthermore, the highest cumulative amount received by present-day Norwegian infants is reached later in infancy than in infants in the included cohorts. Taking the methodological challenges and the additional considerations into account, the following conclusion is drawn:

VKM concludes that the benefits of breastmilk clearly outweigh the possible risk of impaired neurodevelopment from contaminants in breastmilk.

Immune response-associated diseases

Based on recent systematic reviews and meta-analyses, VKM finds that the grading of the evidence on the benefit side is *convincing* for a protective effect of breastmilk on infections, at least as long as the child is breastfed.

The evidence is *limited and no conclusion* can be drawn for a possible protective effect of breastmilk on asthma and wheezing, allergies and atopic dermatitis.

The grading of the scientific evidence for negative health effects from persistent organic pollutants in breastmilk on immune response-associated diseases is based on four cohorts, with findings of increased risk in three cohorts (Faroe Island 3, Dutch and Slovakian cohorts). The evidence is considered *limited and no conclusion* can be drawn for a negative effect from persistent organic pollutants in breastmilk on thymus weight, vaccine antibody titre, respiratory infections, asthma and wheezing. VKM notes that although the evidence is limited and inconclusive, this is mainly due to the scarcity of studies and disparate endpoint measurements. All studies investigating immunological endpoints reported some effect.

In the risk assessment of immune response-associated diseases other than infections, a major challenge was the heterogeneity within each main group of outcomes, e.g. immune response- or allergy-related diseases, where all five papers included in reality measured widely different outcomes, and different parameters of similar outcomes.

The Faroe 3 cohort had approximately 9-fold higher concentrations of PCBs than the present-day Norwegian levels. The Slovakian and Dutch cohorts had about 3 to 4 times higher mean breastmilk levels than the present levels in Norway. In the INMA (Menorca) cohort DDE was addressed, and the mean levels were 100-fold higher than in Norway.

The estimated cumulative amount per kg body weight of contaminants in infants in the Faroese, Slovakian and Dutch cohorts were higher than current Norwegian levels, both prenatally, early postnatally and at 1 year of age. Even with a breastfeeding duration of 2 years, the cumulative amounts of contaminants in Norwegian infants will not reach similar peak levels as the infants in the included cohorts. Furthermore, the highest cumulative level in present-day Norwegian infants is reached later in infancy than in infants in the included cohorts.

Four of the five studies investigating association between persistent organic pollutants in breastmilk and immunological parameters also found an association between *prenatal* contaminant exposure and immune response-associated diseases. The nervous- and immune systems undergo substantial development after birth, and have been assumed to be sensitive to chemical exposures also in the postnatal period. In the absence of knowledge, a conservative approach would be to assume that the infant is as sensitive as the foetus.

Taking the methodological challenges and the additional considerations into account, the following conclusion is drawn:

VKM concludes that the benefits of breastmilk in terms of defence against infections clearly outweigh the possible risk of reduced resistance to infections from contaminants in breastmilk, at least as long as the child is breastfed.

No conclusion can be drawn on other immune response-associated diseases due to inconclusive results on the benefit side and few and disperse studies on the risk side.

Growth, overweight and obesity

Based on recent systematic reviews and meta-analyses, VKM finds that the evidence is *convincing* for a protective effect of breastmilk on risk of overweight and obesity in childhood. The optimal length of exclusive and partial breastfeeding associated with the reduction in risk remains to be settled.

Grading the evidence on the risk side, VKM concludes that the evidence is *limited and no conclusion* can be drawn for a possible association between persistent organic pollutants in breastmilk and impaired growth in children. The grading of the scientific evidence for negative health effects from persistent organic pollutants in breastmilk on growth was based on three cohorts, with negative findings in one, pointing towards reduced (and not increased) weight with increasing PCB exposure (Faroe Island 2). Infants in the Faroese cohorts have historically had substantially higher levels of contaminants in blood both at birth as well as early and late in the nursing period in comparison with Norwegian infants.

Taking the methodological challenges and the additional considerations into account, the following conclusion is drawn:

VKM concludes that the reduced risk of overweight and obesity associated with breastfeeding clearly outweighs the possible risk presented by contaminants in breastmilk.

Thyroid parameters

VKM concludes that the evidence for association between postnatal exposure to contaminants and parameters related to thyroid hormones is very *limited and no conclusion* can be drawn.

Type 1 and 2 diabetes, high blood pressure, Crohn's disease, ulcerative colitis, coeliac disease, childhood cancer and sudden infant death syndrome

VKM concludes that the evidence is *probable* for a protective effect of breastmilk on later risk of type 1 diabetes, type 2 diabetes and high blood pressure. The optimal length of exclusive or partial breastfeeding which protects remains to be settled.

VKM concludes that the evidence is *limited suggestive* for a protective effect of breastmilk on the risk of Crohn's disease, ulcerative colitis, coeliac disease, childhood cancer and sudden infant death syndrome.

No studies investigating these health outcomes in relation to contaminants in breastmilk were identified.

Overall conclusions on benefit and risk assessment of breastmilk to infant health in Norway

Taking the present-day levels of contaminants in breastmilk and the long duration of breastfeeding (12 months) in Norway into account, VKM concludes that:

- The benefits of breastmilk clearly outweigh the possible risk of impaired neurodevelopment from contaminants in breastmilk.
- The benefits of breastmilk in terms of defence against infections clearly outweigh the possible risk of reduced resistance to infections from contaminants in breastmilk, at least as long as the child is breastfed.
- The reduced risk of overweight and obesity associated with breastfeeding clearly outweighs the possible risk presented by contaminants in breastmilk.
- As regards the beneficial effects of breastmilk on risk of type 1 and type 2 diabetes and high blood pressure, the evidence suggests a *probable* beneficial effect later in life. There are no studies investigating these health outcomes in relation to contaminants in breastmilk.
- No conclusion can be drawn on other immune response-associated diseases due to inconclusive results on the benefit side and few and disperse studies on the risk side.

Following a comprehensive assessment of scientific literature on the positive health effects of breastmilk and concentrations in breastmilk of compounds representing possible health hazards, and given current knowledge about concentrations of contaminants in Norwegian breastmilk and breastfeeding duration in Norway, VKM concludes that the benefits associated with breastmilk clearly outweigh the risk presented by current levels of contaminants in breastmilk. This conclusion is not affected by whether a child is exclusively or partially breastfed up to the age of 6 months and partially breastfed up to 12 months of age.

Utvidet sammendrag

Introduksjon

Denne nytte- og risikovurderingen av morsmelk og miljøgifter i morsmelk ble initiert av Vitenskapskomiteen for mattrygghet (VKM). Det overordnede målet er å gi en balansert vurdering av fordelene ved morsmelk opp mot potensielle risikoer ved eksponering for miljøgifter i morsmelk, med fokus på norske forhold. Hensikten er å bidra til et grunnlag for beslutningstakere som gir anbefalinger om lengde på fullamming og delamming.

Sammensetningen av morsmelk er tilpasset den nyfødtes behov. Forutsatt at mor har dekket sine ernæringsbehov under svangerskapet og i ammeperioden, dekker morsmelk alle de ernæringsmessige behovene barnet har de første månedene, med unntak av vitamin D. Morsmelk inneholder også en rekke spesialiserte komponenter som vekstfaktorer og substanser med antimikrobielle og antiinflammatoriske egenskaper samt spesielle immunologiske komponenter som stimulerer modningen av barnets eget immunsystem. Morsmelkerstatning dekker de etablerte ernæringsmessige behovene, men inneholder ikke de spesialiserte beskyttelsesfaktorene som bare finnes i morsmelken.

Studier de siste fire tiårene har vist at fordi forurensende kjemikalier akkumulert i miljøet oppkonsentreres i næringskjeden, så finnes de også i våre kropper - spesielt i fettrikt vev, og dermed også i morsmelk. Nivåene av fettløselige, tungt nedbrytbare kontaminanter i fosteret, det nyfødte barnet og i morsmelken gjenspeiler i stor grad den akkumulerte mengden av disse kontaminantene i mors kropp.

Dette innebærer at morsmelk i tillegg til næringsstoffer og beskyttende immunologiske faktorer som har en positiv effekt på spedbarns helse, også kan inneholde kontaminanter. Særlig de fettløselige, tungt nedbrytbare kontaminantene akkumuleres hos barnet under amming. Dette er bakgrunnen for en debatt blant eksperter om anbefalt ammelengde, til tross for allment bred enighet om at amming er gunstig for barnet.

Amming i Norge

Ammeforekomsten er høyere i Norge enn i de fleste andre europeiske land. Undersøkelser viser at 80 % av barna blir ammet ved seks måneders alder og 46 % ved 12 måneder. Gjennomsnittlig ammelengde er ca. ti måneder.

Norske helsemyndigheter anbefaler at spedbarn fullammes i seks måneder og ammes videre til barnet er minst ett år. Imidlertid viser det seg at kun et mindretall av norske mødre fullammer i seks måneder. Andelen av barn som fullammes avtar raskt fra tre måneder og oppover, og ved seks måneder er det kun 9 % av barna som fullammes.

Det gjennomsnittlige inntaket av morsmelk hos et spedbarn som fullammes øker fra ca. 700 ml per dag ved en måneds alder til ca. 850 ml per dag ved seks måneder. Studier viser at inntak av morsmelk ikke er veldig forskjellig mellom barn som fullammes og barn som delammes de første fire månedene. Fra syv måneders alder og oppover er det gjennomsnittlige morsmelkinntaket estimert til å være ca. 500 ml per dag.

Amming anbefales ikke dersom mor har visse særskilte metabolske sykdommer eller infeksjoner, og bruk av enkelte farmasøytiske preparater utgjør også en kontraindikasjon for amming.

Næringsstoffer og immunologiske komponenter i morsmelk

De positive helseeffektene av morsmelk er særlig knyttet til morsmelkens innhold av næringsstoffer og immunologiske komponenter.

Forutsatt at mor har dekket sine ernæringsmessige behov, vil et spedbarn som fullammes de første seks månedene av livet ha alle de etablerte ernæringsmessige behovene dekket, med unntak av vitamin D. På verdensbasis er det derfor slik at anbefalt daglig inntak av næringsstoffer for spedbarn tar utgangspunkt i konsentrasjonene av de ulike næringsstoffene i morsmelk, multiplisert med det gjennomsnittlige morsmelkinntaket.

Sammensetningen av næringsstoffer i morsmelk varierer avhengig av laktasjonsfase, tid på dagen og i løpet av selve ammingen. Enkelte av næringsstoffene varierer også med mors kosthold. Energiinnholdet i morsmelk varierer, men har blitt anslått til å være ca. 700 kcal per liter. Innholdet av proteiner og karbohydrater er relativt stabilt, mens det er store variasjoner i fettinnholdet. Fettsyresammensetning og konsentrasjon av de fleste vitaminene reflekterer mors inntak, mens konsentrasjoner av mineraler stort sett ikke påvirkes av mors kosthold, med unntak av selen og jod.

Morsmelken bidrar med stoffer som har beskyttende egenskaper i barnet. Den inneholder en rekke spesialiserte immunologiske komponenter, inkludert faktorer med antimikrobielle og antiinflammatoriske egenskaper, så vel som substanser som stimulerer modningen av barnets eget immunsystem. Disse egenskapene er ikke bare positive for helsen i barndommen, men mest sannsynlig også senere i livet. Antistoffer i morsmelken er rettet mot potensielle patogener og antigener som mor har vært eksponert for. I tillegg blir modningen av barnets immunsystem påvirket av immunmodulerende faktorer i morsmelk og av komponenter fra stedegne bakterier og matrester i barnets tarm. Ulike substanser i morsmelk fremmer etableringen av en gunstig tarmflora (mikrobiota) hos barnet, noe som er viktig for induksjon av et balansert immunsystem både i slimhinnen og ellers i kroppen (immunologisk homeostase). Gjennom alle disse mekanismene representerer morsmelk og amming en enestående immunologisk integrering av mor og barn.

Næringsstoffer i morsmelkerstatning

Hvis amming ikke kan gjennomføres, eller dersom det er behov for mer melk i tillegg til morsmelk, anbefales morsmelkerstatning til barnet er 12 måneder.

Morsmelkerstatning dekker spedbarnets etablerte ernæringsmessige behov, men tilfører ikke barnet morens antistoffer og naturlige forsvarsfaktorer og heller ikke de andre komponentene i morsmelken som stimulerer barnets immunsystem. De fleste morsmelkerstatningene på det norske markedet er kumelksbaserte.

Data fra en nasjonal kostholdsundersøkelse blant spedbarn (Spedkost, 2006-2007) viste at ved seks måneders alder har 43 % av norske spedbarn blitt introdusert til morsmelkerstatning og 36 % brukte morsmelkerstatning som drikke. Ved ett års alder brukte 43 % av norske spedbarn morsmelkerstatning jevnlig.

Morsmelkerstatninger i Norge er underlagt EUs regelverk som dekker sammensetning, merking, markedsføring og distribusjon av produktet. Forskrift for morsmelkerstatning gir minimums- og maksimumsgrenser for næringsstoffer og inkluderer flere av bestemmelsene i WHO-koden[8].

[8]Internasjonal kode for markedsføring av morsmelkerstatninger.

Kontaminanter og eksogene mikrober i morsmelk og morsmelkerstatning

Morsmelken gjenspeiler mors konsentrasjoner av kontaminanter og inneholder derfor lave konsentrasjoner av forskjellige forurensninger. Bare de vanligste miljøgiftene er analysert i morsmelk, og enda færre er blitt studert i mennesker med hensyn til helseeffekter i spedbarn og barn.

I denne nytte- og risikovurdering av morsmelk har hovedfokuset vært på kontaminanter som er inkludert i Stockholmkonvensjonen om tungt nedbrytbare organiske miljøgifter (POPs)[9]. De kan deles inn i tre hovedgrupper: plantevernmidler (DDT og HCB), andre halogenerte organiske miljøgifter (dioksiner og dioksinliknende PCB, ikke-dioksinliknende PCB, bromerte flammehemmere (PBDE), perfluorerte forbindelser (PFOS / PFOA)) og tungmetaller (bly, kvikksølv og kadmium).

Ved identifisering og karakterisering av negative helseeffekter er kombinerte eksponeringer fra flere kontaminanter[10] i morsmelk til en viss grad tatt hensyn til, da det i flere av de inkluderte kohortene er undersøkt helseeffekter av PCB og dioksiner i kombinasjon med DDT eller HCB, og noen i kombinasjon med kvikksølv. I tillegg bør det bemerkes at de kontaminantene som er undersøkt kan anses som markører for en kombinert effekt av flere kontaminanter, ettersom forekomsten av flere kontaminanter som regel er korrelert.

Konsentrasjonen av metaller i både morsmelk og morsmelkerstatning (for eksempel kvikksølv og bly) er generelt lav, og nivåene gir generelt sett ikke grunnlag for bekymring

På grunn av nasjonale og internasjonale restriksjoner og forbud mot bruk, har nivåene av dioksiner, PCB og plantevernmidler (som DDT og HCB) falt betydelig (mer enn 60 %) i miljøet og i mennesker de siste tre tiårene. I motsetning til nivåene for dioksiner, PCB, DDT og HCB, så økte konsentrasjonen av PBDE i morsmelk i Norge fram til ca. år 2000, hvoretter en nedgang er observert. De perfluorerte forbindelsene PFOS og PFOA, som brukes blant annet til overflatebehandling, har vist en lignende tidstrend som PBDE.

Det finnes begrenset med norske data om nivåer av tungt nedbrytbare organiske miljøgifter i morsmelkerstatning, men de rapporterte nivåene er generelt mye lavere enn i morsmelk.

Noen forurensninger som ikke akkumuleres i næringskjeden kan også være relevante både i morsmelk og i morsmelkerstatning. Stoffer som finnes i matemballasje, for eksempel ftalater, kan være til stede både i morsmelk og morsmelkerstatning. Det samme gjelder for prosessfremkalte stoffer som akrylamid, PAH, furan og 3-MDCP. Det hormonaktive stoffet bisfenol A (BPA), som brukes i plast, har nylig blitt forbudt i tåteflasker i EU og i Norge. Forekomstdata i morsmelk og morsmelkerstatning for disse stoffene finnes ikke i Norge.

Den største forskjellen mellom kontaminanter i morsmelk og de som tilføres barnet via morsmelkerstatning eller flaske, er generelt at morsmelk inneholder høyere nivåer av tungt nedbrytbare organiske miljøgifter, mens de fleste av de uønskede stoffene i morsmelkerstatning og flaske har en kortere halveringstid.

Morsmelkerstatning kan inneholde uønskede mikroorganismer som kan gi barnet diaré, og i alvorlige tilfeller bakteriemi og meningitt. *Cronobacter* spp. (tidligere *Enterobacter sakazakii*) er en sjelden årsak til invasiv infeksjon med høy dødelighet hos nyfødte spedbarn.

[9]Stockholmkonvensjonen (SC) for tungt nedbrytbare organiske miljøgifter er en global avtale som administreres av FNs miljøprogram (UNEP) for å beskytte menneskers helse og miljøet mot kjemikalier. Stockholmkonvensjonen trådte i kraft første gang i 2004 (Stockholmkonvensjonen om organiske miljøgifter 2004 http://www.chm.pops.int). Kriteriene for å bli inkludert i SC er persistens, bioakkumulering, potensiale for spredning over store avstander, og alvorlige helseeffekter.

[10]Populært kalt «cocktaileffekten».

Mulig utbrudd fra mikroorganismer i morsmelkerstatning i seg selv, eller på grunn av forurenset vann, er et problem i utviklingsland, men slike utbrudd har ikke vært registrert i Norge.

Metodisk tilnærming i denne nytte og risikovurderingen

Nyttevurderingen av morsmelk er basert på positive helseeffekter rapportert i systematiske kunnskapsoppsummeringer og metaanalyser som er publisert i løpet av de siste ti årene. Dette innebærer at VKM ikke har gjennomført et eget litteratursøk for epidemiologiske studier som har undersøkt positive helseeffekter av morsmelk, men oppsummerer og diskuterer helseutfall som er beskrevet i de inkluderte systematiske kunnskapsoppsummeringene og metaanalysene. I tillegg er noen andre relevante, nyere kunnskapsoppsummeringer eller enkeltstudier av høy kvalitet inkludert.

I VKMs konklusjoner har det blitt lagt mest vekt på to nye systematiske kunnskapsoppsummeringer, én fra de nye nordiske næringsstoffanbefalingene (2013), og én fra WHO (2013).

Risikovurderingen av kontaminanter i morsmelk er basert på negative helseeffekter rapportert i studier fra kohorter som ble funnet gjennom et fullskala systematisk litteratursøk. Det ble satt som et absolutt inklusjonskriterium at studiene skulle ha gode ammedata og være i stand til å skille effekten av postnatal eksponering fra prenatal eksponering.

Det systematiske litteratursøket og eksklusjonskriteriene resulterte i 46 studier som ble vurdert for kvalitet (A-B-C) i henhold til et sett av forhåndsdefinerte kriterier. Av disse ble ingen studier kategorisert som A, mens 24 studier kvalifiserte for kategori B. Kategori C-studier ble ikke vurdert nærmere. De negative helseeffektene fra kontaminanter i morsmelk som er beskrevet i denne rapporten er dermed basert på 24 artikler fra ti kohorter gjennomført i syv forskjellige land (USA, Canada, Færøyene, Spania, Tyskland, Nederland og Slovakia). Ingen studier om helseeffekter på spedbarn og barn relatert til eksponering for kontaminanter via morsmelk var tilgjengelig fra Norge. Kun kontaminantene PCB, dioksiner, DDT/DDE, HCB og kvikksølv var undersøkt i de inkluderte studiene.

Gradering av evidens

Det finnes flere systematiske metoder for å gradere vitenskapelig evidens. Graderingen som brukes i denne nytte- og risikovurderingen er lik det systemet som brukes av World Cancer Research Fund (WCRF) i rapporten Food, Nutrition, Physical Activity and the prevention of Cancer: a global perspective fra 2007. De ulike graderingskategoriene er:

- Overbevisende (convincing)
- Sannsynlig (probable)
- Begrenset – indikerende (limited, suggstive)
- Begrenset – ingen konklusjon (limited, no conclusion)

I graderingen av evidensen for de positive helseeffektene forbundet med morsmelk har VKM gradert summen av arbeidet fra andre. Noen av de inkluderte kunnskapsoppsummeringene og metaanalysene som omhandler positive helseeffekter fra morsmelk har ikke gradert sine egne resultater. Andre bruker gradering, men ulike systemer. Et element av "beste skjønn" er

dermed uunngåelig fra VKM sin side, da det så vidt vi vet, ikke finnes internasjonale retningslinjer for gradering av kunnskapsoppsummeringer og metaanalyser.

Ammedata i kunnskapsoppsummeringer og metaanalyser som undersøker positive helseeffekter

Få av metaanalysene, kunnskapsoppsummeringene eller enkeltstudiene som brukes som grunnlag for nyttevurderingen muliggjør differensiering av helseeffekter ut fra dose-respons og fra måned til måned, og få ser på helseeffekter av langvarig amming. Selv om noen av studiene støtter en konklusjon om at fullamming fram til seks måneders alder har mer beskyttende effekter enn kortere amming, kan man ikke ut fra disse fastslå hva som er optimal lengde for fullamming eller optimal total ammelengde.

Beregnet kontaminanteksponering fra morsmelk i Norge og tolerable inntak

Eksponeringsestimater for norske spedbarn er gjort for PCB-153, DDE, HCB, dioksiner og dioksinliknende PCB (dl-PCB). Det er kun for disse kontaminantene at det finnes tilstrekkelige forekomstdata i norsk morsmelk. Disse stoffene er også undersøkt i de inkluderte studiene om mulige negative helseeffekter forbundet med kontaminanter i morsmelk.

Barnas akkumulerte mengder av kontaminanter per kg kroppsvekt er estimert ved å kombinere konsentrasjonene av kontaminantene i morsmelk fra norske kvinner med gjennomsnittlig inntak av morsmelk, gjennomsnittlig fettkonsentrasjon i morsmelk og gjennomsnittlig kroppsvekt hos barn.

Risiko forbundet med eksponering fra kontaminanter generelt er vurdert av internasjonale risikovurderingsorganer som Joint FAO/WHO Expert Committee on Food Additives (JECFA) eller European Food Safety Authority (EFSA). Tolerable inntak er satt for enkelte kontaminanter (for eksempel dioksiner og dl-PCB, DDE, HCB og noen fluorerte forbindelser).

For andre kontaminanter har ikke EFSA kunnet etablere tolerable inntak på grunn av manglende data (for eksempel PBDE og ikke-dioksinliknende PCB (ndl-PCB)). Imidlertid har VKM tidligere brukt et veiledende nivå for tolerabelt inntak, og brukt dette for summen av seks ndl-PCB.

Så lenge eksponeringen fra morsmelk er lavere enn tolerable inntak, anses eksponering for å være trygg. Det er likevel viktig å merke seg at selv om de tolerable inntaksverdiene er verdier som anses som trygge, vil ikke overskridelser av tolerable inntak nødvendigvis medføre negative helseeffekter. I de tolerable inntakene er det inkludert sikkerhetsmarginer, og en overskridelse av tolerabelt inntak resulterer derfor kun i en redusert sikkerhetsmargin.

Estimatene for norske barn tyder på at DDE- og HCB-eksponering via morsmelk er lavere enn tolerabelt daglig inntak (TDI). Veiledende nivå for ndl-PCB er basert på mors eksponering fra kohorter der barna er ammet. Eksponeringsberegninger for norske kvinner tyder på at minst 95 % av norske spedbarn har et eksponeringsnivå for ndl-PCB som er under det nivået som kan anses som trygt.

Overskridelse av tolerabelt inntak i spedbarn betyr ikke nødvendigvis at konsentrasjonen i spedbarnets kropp når et nivå som er til bekymring, og derfor er ikke det tolerable inntaket for dioksiner og dl-PCB direkte anvendbart for spedbarn. Det tolerable inntaket av dioksiner og dl-PCB er satt for å sikre at konsentrasjonen hos mor (mors kroppskonsentrasjon, uttrykt per

kg kroppsvekt) er lavere enn den høyeste konsentrasjonen som regnes som trygt for fosteret. Dette representerer kroppskonsentrasjonen som er forbundet med inntak likt med det tolerable. På grunn av spedbarnets raske vekst vil kontaminantkonsentrasjonen i barnet fortynnes, og dette gir en langsommere økning i konsentrasjonen i kroppen.

Inntaket av dioksiner og dl-PCB fra kosten ble nylig estimert i den norske Mor og Barn kohorten (MoBa), og var lavere enn det tolerable ukentlige inntaket (TWI[11]) hos mer en 97 % av de gravide kvinnene som deltok. Dette tyder på lav risiko forbundet med prenatal eksponering blant spedbarn i Norge.

Eksponering for kontaminanter i norske barn sammenliknet med barn i de inkluderte kohortene hvor negative helseeffekter undersøkes

De norske nivåene av PCB-153 og DDE i morsmelk er vesentlig lavere enn i de fleste av kohortene som danner grunnlaget for risikovurderingen. Imidlertid fortsetter norske mødre med delamming lenger enn kvinner i de fleste av de inkluderte studier som undersøker negative helseeffekter. Selv med en ammelengde på to år, vil den akkumulerte mengden av kontaminanter i norske barn ikke nå opp til maksimumsnivået i spedbarna i de inkluderte kohortene. Videre vil norske barn nå den høyeste akkumulerte mengden senere i spedbarnsalderen enn barna i de inkluderte kohortene.

Andre hensyn

I denne nytte- og risikovurderingen er det kun inkludert studier som har skilt på helseeffekter fra prenatal- og postnatal eksponering for kontaminanter. Effekter fra prenatal eksponering kan vurderes ved for eksempel å undersøke sammenhenger mellom spedbarnets og/eller mors eksponeringsnivåer ved fødselen og helsemessige konsekvenser senere i livet. Hvis den postnatale eksponeringen påvirker helsen, så kan imidlertid dette også påvirke assosiasjoner med den prenatale eksponeringen. Dessuten kan assosiasjoner mellom postnatal eksponering og helseeffekter bli påvirket av den prenatale eksponeringen. Selv om dette kan justeres for i statistiske analyser, kan det ikke utelukkes at det fremdeles finnes konfunderende faktorer som ikke er justert for.

Mange av de inkluderte studiene som undersøkte negative effekter på utviklingen av nervesystemet, fant en sammenheng mellom prenatal kontaminanteksponering og økt risiko, men ingen konsistent sammenheng med postnatal eksponering. Det å anta at barnet er like sensitiv i pre- og postnatal periode er trolig en forsiktig tilnærming som mest sannsynlig overvurderer risikoen for barn som når den høyeste kroppskonsentrasjonen først nås ved 12 måneders alder, som i Norge.

Nytte- og risikovurdering av helseeffekter

Graderingen av litteraturen danner grunnlaget for denne nytte- og risikovurderingen. Videre er evaluering av fordeler og mulig risiko knyttet til morsmelk basert på norske ammedata og konsentrasjoner av tungt nedbrytbare organiske miljøgifter i morsmelk.

[11]TWI for dioksiner og dl-PCB er 14 pg TEQ/kg kroppsvekt/uke.

Helseutfallene som er beskrevet i de inkluderte studiene for positive og negative helseeffekter er knyttet til følgende sykdommer eller tilstander:

- utvikling av nervesystemet
- immunrespons-assosierte sykdommer, inkludert infeksjoner, astma/pustevansker og allergi, cøliaki, inflammatorisk tarmsykdom (Crohns sykdom og ulcerøs kolitt) og type 1 diabetes
- vekst, overvekt og fedme
- helseutfall relatert til metabolsk syndrom, for eksempel blodtrykk, serum kolesterol, type 2 diabetes og hjerte-karsykdom
- kreft
- krybbedød
- skjoldbrukskjertel-parametere
- kjønnsmodning

VKMs konklusjoner er organisert etter helseutfall, og starter med de helseutfallene som har blitt studert for både fordeler (nytte) og risiko.

Utvikling av nervesystemet – IQ og kognitiv utvikling

Basert på systematiske kunnskapsoppsummeringer og metaanalyser publisert i løpet av de siste ti årene finner VKM at evidensen er *overbevisende* for at morsmelk fremmer utviklingen av nervesystemet. Den optimale lengden på fullamming og delvis amming som stimulerer den positive effekten kan ikke fastsettes.

Graderingen av evidensen for negative helseeffekter fra tungt nedbrytbare organiske miljøgifter i morsmelk på utvikling av nervesystemet er basert på syv kohorter, med funn av negativ effekt på utvikling av nervesystemet i tre kohorter (nederlandsk, tysk og kanadisk). Funnene var forbigående i den nederlandske og den tyske kohorten, mens det fra den kanadiske kohorten ikke foreligger noen oppfølgingsstudie.

VKM konkluderer med at evidensen er *begrenset indikerende* for en negativ effekt fra tungt nedbrytbare organiske miljøgifter i morsmelk på utviklingen av nervesystemet, gitt eksponeringsnivåene i disse tre kohortene. Hvilken ammelengde, fullamming eller delamming, som er assosiert med de forbigående negative effektene på utvikling av nervesystemet kan ikke fastslås. Kvikksølv er bare undersøkt i én av de inkluderte kohortene, og VKM konkluderer med at evidensen for en effekt av eksponering av kvikksølv fra morsmelk er begrenset, og at det ikke kan trekkes noen konklusjon for kvikksølv.

Manglende målinger av såkalt "HOME score" var en særlig utfordring i tolkningen av resultatene for utvikling av nervesystemet. "HOME score"-målinger ble brukt i syv av de 14 studiene som undersøkte sammenhenger mellom tungt nedbrytbare organiske miljøgifter i morsmelk og utvikling av nervesystemet, inkludert de to studiene som rapporterte forbigående funn.

Den estimerte akkumulerte mengden kontaminanter per kg kroppsvekt i barn i de nederlandske, tyske og kanadiske kohortene var høyere enn for norske barn, både prenatalt, tidlig postnatalt og ved ett års alder. Selv med en ammelengde på to år vil den akkumulerte mengden av kontaminanter i norske barn ikke nå opp til maksimumsnivået i spedbarna i de inkluderte kohortene. Videre vil norske barn nå den høyeste akkumulerte mengden senere i spedbarnsalderen enn barna i de inkluderte kohortene.

Metodiske utfordringer og andre hensyn er tatt i betraktning når VKM kommer fram til følgende konklusjon:

VKM konkluderer med at de helsemessige fordelene ved morsmelk på utvikling av nervesystemet klart oppveier mulig risiko for negativ påvirkning fra kontaminanter i morsmelk.

Immunrespons-assosierte sykdommer

Basert på systematiske kunnskapsoppsummeringer og metaanalyser, finner VKM at gradering av evidensen er *overbevisende* for en beskyttende effekt av morsmelk på infeksjoner, i alle fall så lenge barnet ammes.

Evidensen er *begrenset og ingen konklusjon* kan trekkes for en mulig beskyttende effekt av morsmelk på astma/pustevansker, allergi og atopisk dermatitt.

Graderingen av evidensen for negative helseeffekter fra tungt nedbrytbare organiske miljøgifter i morsmelk på immunrespons-assosierte sykdommer er basert på fire kohorter, med funn som viser økt risiko i tre kohorter (Færøysk kohorte 3, nederlandsk og slovakisk kohorte). Evidensen for negative effekter på tymusvekt, vaksineindusert antistofftiter, luftveisinfeksjoner, astma og pustevansker fra tungt nedbrytbare organiske miljøgifter i morsmelk er *begrenset og ingen konklusjon* kan trekkes. VKM understreker at evidensen er mangelfull på grunn av få studier og fordi de studiene som finnes har brukt ulike mål for helseutfall. Alle studier som har undersøkt immunologiske helseutfall rapporterer imidlertid en eller annen effekt.

I risikovurderingen av andre immunrespons-assosierte sykdommer enn infeksjoner, har det vært en stor utfordring å sammenlikne resultatene fordi alle de fem studiene som har studert immunresponsutfall i realiteten enten har målt vidt forskjellige endepunkter, eller målt ulike parametere for like utfall.

Kohorte 3 fra Færøyene hadde omtrent ni ganger høyere konsentrasjoner av PCB i morsmelk enn dagens norske nivåer. De slovakiske og nederlandske kohortene hadde omtrent tre til fire ganger høyere nivåer enn dagens nivå i Norge. I den spanske INMA kohorten fra Menorca ble DDE undersøkt, og gjennomsnittsnivået av DDE i morsmelk var 100 ganger høyere enn i Norge.

Den estimerte akkumulerte mengden kontaminanter per kg kroppsvekt i barn i de færøyske, slovakiske og nederlandske kohortene var høyere enn for norske barn, både prenatalt, tidlig postnatalt og ved ett års alder. Selv med en ammelengde på to år, vil den akkumulerte mengden av kontaminanter i norske barn ikke nå samme maksimumsnivå som spedbarna i de inkluderte kohortene. Videre vil norske barn nå den høyeste akkumulerte mengden senere i spedbarnsalderen enn barna i de inkluderte kohortene.

Fire av fem studier som har undersøkt sammenhenger mellom tungt nedbrytbare organiske miljøgifter i morsmelk og immunologiske parametere har funnet en sammenheng mellom prenatal eksponering og immunrespons-assosierte sykdommer. Nervesystemet og immunsystemet gjennomgår en betydelig utvikling etter fødselen, og er antatt å være følsomme for kjemisk eksponering også etter fødselen. Ettersom vi foreløpig har mangelfull av kunnskap om sensitive vinduer, vil det være en konservativ tilnærming å anta at det fødte barnet er like følsomt som fosteret.

Metodiske utfordringer og andre hensyn er tatt i betraktning når VKM kommer fram til følgende konklusjon:

VKM konkluderer med at de helsemessige fordelene ved morsmelk når det gjelder forsvar mot infeksjoner klart oppveier mulig risiko fra tungt nedbrytbare organiske miljøgifter i morsmelk for nedsatt motstand mot infeksjoner, i alle fall så lenge barnet blir ammet.

Ingen konklusjon kan trekkes for andre immunassosierte sykdommer på grunn av sprikende resultater i studier som har undersøkt positive helseeffekter fra morsmelk, og få studier med sprikende resultater i studier som har undersøkt negative helseeffekter fra kontaminanter i morsmelk.

Vekst, overvekt og fedme

Basert på systematiske kunnskapsoppsummeringer og metaanalyser publisert i løpet av de siste ti årene, finner VKM at evidensen er *overbevisende* for at morsmelk beskytter mot overvekt og fedme i barndommen. Den optimale lengden på fullamming og delvis amming som beskytter kan ikke fastsettes.

Graderingen av evidensen for negative helseeffekter fra tungt nedbrytbare organiske miljøgifter i morsmelk på vekstutvikling hos barn er basert på tre kohorter med negative funn i én kohorte; denne antyder redusert (og ikke økt) vekt med økende PCB-eksponering (kohorte 2 fra Færøyene). Spedbarna i de færøyske kohortene har historisk sett hatt et betydelig høyere nivå av miljøgifter i blodet både ved fødsel, samt tidlig og senere i ammeperioden sammenlignet med norske spedbarn. Graderingen av evidensen for negative helseeffekter fra tungt nedbrytbare organiske miljøgifter i morsmelk på vekstutvikling hos barn er *begrenset, og ingen konklusjon* kan trekkes.

Metodiske utfordringer og andre hensyn er tatt i betraktning, når VKM kommer fram til følgende konklusjon:

VKM konkluderer med at redusert risiko for overvekt og fedme ved amming klart oppveier en mulig risiko fra kontaminanter i morsmelk.

Skjoldbrukskjertel-parametere

VKM konkluderer med at evidensen for en sammenheng mellom postnatal eksponering for miljøgifter og parametere knyttet til skjoldbruskkjertelhormoner er svært *begrenset, og ingen konklusjon* kan trekkes.

Type 1 og 2 diabetes, høyt blodtrykk, Crohns sykdom, ulcerøs kolitt, cøliaki, kreft hos barn og krybbedød

VKM konkluderer med at evidensen er *sannsynlig* for at morsmelk har en beskyttende effekt på senere risiko for type 1 diabetes, type 2 diabetes og høyt blodtrykk. Den optimale lengden på fullamming og delvis amming som gir slik positiv effekt kan ikke fastsettes.

VKM konkluderer med at evidensen er *begrenset indikerende* for at morsmelk har en beskyttende effekt på senere risiko for Crohns sykdom, ulcerøs kolitt, cøliaki, kreft hos barn og krybbedød.

Ingen studier som undersøker disse helseutfallene for kontaminanter i morsmelk ble identifisert.

Konklusjoner i nytte og risikovurdering av morsmelkinntak – betydningen for helse hos norske barn

Tatt i betraktning dagens nivåer av kontaminanter i morsmelk i Norge, og en lang ammeperiode (12 måneder), konkluderer VKM med at:

- de helsemessige fordelene ved morsmelk på utvikling av nervesystemet klart oppveier mulig risiko for redusert utvikling av nervesystemet fra kontaminanter i morsmelk
- de helsemessige fordelene ved morsmelk når det gjelder forsvar mot infeksjoner klart oppveier mulig risiko for nedsatt motstand mot infeksjoner fra kontaminanater i morsmelk, i hvert fall så lenge barnet blir ammet
- redusert risiko for overvekt og fedme ved amming klart oppveier en mulig risiko fra kontaminanter i morsmelk
- evidensen er *sannsynlig* for at morsmelk har en beskyttende effekt på senere risiko for type 1 diabetes, type 2 diabetes og høyt blodtrykk. Ingen studier som undersøker disse helseutfallene for kontaminanter i morsmelk ble identifisert
- ingen konklusjon kan trekkes for andre immunassosierte sykdommer på grunn av sprikende resultater i studier som har undersøkt positive helseeffekter fra morsmelk, og få studier med sprikende resultater i studier som har undersøkt negative helseeffekter fra kontaminanter i morsmelk

Etter en helhetlig vurdering av den vitenskapelige litteraturen for positive helseeffekter av morsmelk og potensielle negative helseeffekter fra kontaminanter i morsmelk, kontaminantnivået i norsk morsmelk og ammelengde typisk for norske barn, konkluderer VKM med at de helsemessige fordelene forbundet med morsmelk klart oppveier mulig risiko som dagens nivåer av kontaminanter i morsmelk kan representere. Denne konklusjonen gjelder uansett om barna fullammes eller delammes fram til seks måneders alder, og delammes opp til 12 måneder.

Abbreviations

3-MCPD – 3-Monochloropropane-1,2-diol

AA – Arachidonic acid

AAP – American Academy of Pediatrics

AFSSA – French Agency for Food, Environmental and Occupational Health and Safety (now ANSES)

AI – Adequate intake

ALA – Alfa linolenic acid

Bayley Scales of Infant Development – See Appendix 7

BMD – Bench mark dose

BMDL – Bench mark dose lower confidence limit

BMI – Body mass index

Body burden – The total amount of a particular chemical present in the body

BPA – Bisphenol A

BSID – Bayley Scales of Infant Development – See Appendix 7

bw – Body weight

CI – Confidence interval

COT – Committee on Toxicity, UK

DALY – Disability-adjusted life year

DDE – Dichlorodiphenyldichloroethane is one of the more common breakdown products of DDT

DEHP – Di-2-ethylhexyl phthalate

DDT – Dichlorodiphenyltrichloroethane

DHA – Docosahexaenoic acid

DiBP – Di-isobutyl phthalate

dl-PCBs – Dioxin-like PCBs

DNA – Deoxyribonucleic acid

DnBP – Di-n-butyl phthalate

EER – Estimated energy requirement

EFSA – European Food Safety Authority

EPA – Environmental Protection Agency, USA

ESPGHAN – European Society of Paedriatic Gastroenterology, Hepatology and Nutrition

FAO – Food and Agriculture Organization of the United Nations

FFQ – Food frequency questionnaire

GALT – Gut-associated lymphoid tissue

GCI – General cognitive index

HCB – hexachlorobenzene

HCH – Hexachlorocyclohexane

Hg – Mercury

HOME score – See Appendix 7

HUMIS – Norwegian Human Milk Study

IARC – International Agency for Research on Cancer

IgA, IgE, IgG, IgM – Immunoglobulin A, E, G, M

IOM – Institute of Medicine, USA

IQ – Intelligence quotient

JECFA – Joint FAO/WHO Expert Committee on Food Additives

JMPR – Joint FAO/WHO Meeting on Pesticide Residues

K-ABC – Kaufman Assessment Battery for Children – See Appendix 7

Kaufman Assessment Battery for Children – See Appendix 7

LA – Linoleic acid

MALT – Mucosae-associated lymphoid tissue

MDI – Mental Development Index

McCarthy Scales of Children's Abilities – See Appendix 7

MD – Mean difference

ML – Maximum level

MOE – Margin of exposure

MRL – Maximum residue level

MSCA – McCarthy Scales of Children's Abilities – See Appendix 7

NAD – Nicotinamide adenine dinucleotide

NADP – Nicotinamide adenine dinucleotide phosphate

NALT – Nasopharynx-associated lymphoid tissue

ndl-PCBs – Non dioxin-like PCBs

NEC – Necrotising enterocolitis

NNR – Nordic Nutrition Recommendations

NOAEL – No observed adverse effect level

NOS – Neurological optimality score

NPN – Non-protein nitrogen

PAH – Polycyclic aromatic hydrocarbons

PBB – Polybrominated biphenyl

PBDEs – Polybrominated diphenyl ethers

PBPK – Physiologically based pharmacokinetic modelling

PCBs – Polychlorinated biphenyls (209 all together, 121, 153 and 180 are the most commononly analysed)

PCDDs and PCDFs – Dibenzo-p-dioxins and dibenzofurans (denoted dioxins)

PCP – Pentachlorophenol

PDI – Psychomotor development index

PFAS – Perfluoroalkylated substance

PFOS and PFOA – Perfluorooctane sulfonate and perfluorooctanoic acid

PICO – Population Intervention Comparison Outcome

POPs – Persistent organic pollutants

PTDI – Provisional tolerable daily intake

PUFA – Polyunsaturated fatty acid

QALY – Quality-adjusted life year

QAT – Quality assessment tool

RCT – Randomised controlled trial

RE – Retinol equivalents

RNA – Ribonucleic acid

SACN – Scientific Advisory Committee on Nutrition, UK

SC – Secretory Component

SCF – Scientific Committee on Food, EU; now replaced by EFSA

SD – Standard deviation

SIDS – Sudden infant death syndrome

SIgA – Secretory IgA

TDI – Tolerable daily intake

TCDD – Tetrachlorodibenzo(p)dioxin

TE – Toxic equivalent

TEF – TCDD toxic equivalency factor

TEQ – sum of TCDD toxic equivalents (concentration of each dioxin, furan and dl-PCB multiplied with its corresponding TEF and then summarised)

TWI – Tolerable weekly intake

WHO – World Health Organization

ww – Wet weight

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Background

Since the 1970s, Norway has been considered to be a model for other countries in its support for breastfeeding. The breastfeeding prevalence in Norway is among the highest in Europe in spite of a high proportion of women being in employment. This is at least in part the result of good parental leave conditions, public guidelines and well organised measures undertaken to promote breastfeeding. For most Norwegians, both the message and the practice is that ”breast is best”. Having said that, only a minority of Norwegian children are breastfed *exclusively* for 6 months, as recommended by the health authorities.

Norway has adopted the World Health Organization (WHO) recommendations on exclusive breastfeeding for the first 6 months of the infant’s life. Thereafter, WHO recommends that breastfeeding continue for up to 2 years of age or beyond, while the Norwegian Directorate of Health recommends that breastfeeding continues for at least 12 months.

Nutritionally, breastmilk is adapted to the infant’s nutritional requirements and contains all the nutrients and substances the infant needs for optimal growth and development in the first 6 months, the exception being vitamin D. Equally important, breastmilk contains several immunological substances which protect the infant from infections and contribute to the development and maturation of the infant’s own immune system. The latter benefit might explain some of the positive long-term health effects observed in breastfed children.

However, over several decades, the discovery of environmental contaminants in breastmilk has led to an intermittent debate about how long exclusive or partly breastfeeding should be continued. Analyses of breastmilk samples from Norway show that whilst the levels of some contaminants are decreasing, exposure to other and new substances continues to rise. For example, the levels of several bioaccumulating environmental contaminants such as dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs), non dioxin-like PCBs (ndl-PCBs) and the pesticides dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDE), hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB) have decreased considerably since 1986. The concentration level of brominated flame retardants such as polybrominated diphenyl ethers (PBDE) in breastmilk increased until about year 2000, but now the level of most PBDE substances seems to have been stabilised or decreased. However, another group of environmental contaminants, fluorinated compounds, used as surfactants (such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)), continues to show increasing concentration levels in breastmilk. Thus, even if the concentration of most of the toxicants covered by the Stockholm convention[12] has decreased radically, there are new contaminants continuously entering the scene.

Even if the concentration levels of the most important persistent contaminants (such as PCBs and dioxins) have decreased radically, breastfed infants and children may still be exposed to higher levels of environmental contaminants per kilo body weight than the average adult. The exposure is often compared with internationally accepted tolerable intake levels for the different substances, and the exposure of some substances or substance groups in breastfed infants and children exceeds tolerable intake levels. The tolerable intake levels for these substances are however determined on the assumption of a life-long exposure. In the embryonic stage, accumulated contaminant levels in the mother will be of significance for the embryo’s exposure. Later, the infant and child’s growth will lead to a dilution of the body concentration of the contaminants. So the exceeding of tolerable intake levels in breastfed

[12]Stockholm Convention on Persistent Organic Pollutants is an international environmental treaty, signed in 2001 and effective from May 2004, that aims to eliminate or restrict the production and use of persistent organic pollutants (POPs).

infants and children is relatively short-termed, but takes place in a sensitive phase of the child's development.

The following reviews and reports were valuable background documents when starting this work:

- Infant and young child nutrition. Global strategy on infant and young child feeding (WHO, 2003b).
- Evidence of the long-term effects of breastfeeding. Systematic reviews and meta-analyses (WHO, 2007).
- Contaminants in human milk; Weighing the risks against the benefits of breastfeeding (Mead, 2008).
- Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants (Wigle et al., 2008).
- The influence of maternal, fetal and child nutrition on the development of chronic disease in later life (SACN, 2011).
- Breast-feeding: A Commentary by the ESPGHAN Committee on Nutrition (Agostoni et al., 2009).

Terms of reference

The Norwegian Scientific Committee for Food Safety has initiated a benefit and risk assessment of breastmilk with focus on the infant's health. The assessment shall be based on updated levels of environmental contaminants in Norwegian breastmilk. The assessment shall evaluate the nutritional and protective properties of breastmilk compared with possible diseases/conditions that can be caused by environmental contaminants (e.g. infections, cancer and cognitive development). The purpose is to evaluate the benefits and potential risks related to breastmilk based on Norwegian data on prevalence of breastfeeding and concentrations of environmental contaminants in breastmilk.

- The assessment shall review the positive characteristics of breastmilk. Several scientific reviews of breastmilk and infant health have been published the last years. The beneficial aspects of breastmilk shall therefore be based on these reviews and if necessary be updated with newer scientific studies.
- The assessment shall particularly focus on the duration of exclusive and partly breastfeeding and possible diseases/conditions that can be caused by environmental contaminants in breastmilk.
- The assessment shall give a short description of microbiological hazards and contaminant conditions that may influence the infant's health if breastmilk is replaced by infant formula.
- Possible data gaps and need for new surveillance shall be pinpointed.
- The assessment shall be based on relevant Norwegian and international studies and publications.

1 Introduction

From a nutritional and health point of view, breastmilk is the healthiest form of milk for newborns. The nutrient content in breastmilk is tailored for the needs of the newborn. The protein and fat concentrations seem to be adapted to the growth velocity of the infant. In addition to its content of perfectly balanced nutrients, breastmilk also contains a number of non-nutrient protective factors, including immunoglobulins and growth factors.

While recognizing the superiority of breastfeeding, regulatory authorities have worked to minimise the risks that might result from formula feeding. A 2001 WHO report found that infant formula prepared in accordance with applicable Codex Alimentarius[13] standards is nutritionally adequate and safe complementary food and a suitable breastmilk substitute. Nonetheless, with few exceptions, the WHO report recommended exclusive breastfeeding for the first 6 months of life for all infants (WHO, 2001). These conclusions were updated and confirmed in 2009 and 2012 (Kramer et al., 2009; Kramer and Kakuma, 2012).

In the wake of the WHO recommendation many countries, including Norway, and health organisations, have issued guidelines consistent with those of the WHO.

However, similar to the exchange of substances *in utero* between mother and foetus, breastmilk does not act as a sieve which only lets positive substances pass through to the child. The concentration of many substances in breastmilk reflects the levels of substances stored in the mother's body, especially in fat tissue, or exposure levels during the nursing period. Accordingly, smoking, alcoholic beverages, caffeinated drinks, drugs and lastly, a group of substances which will be examined in this report: environmental contaminants, may compromise the beneficial effects of breastmilk.

Humans are exposed daily to different types of pollutants. Both man-made chemicals and natural substances and their degradation products are present in water, soil, air and food. Accordingly, studies over the last four decades have shown that polluting chemicals have built up in the environment, bioaccumulated in the food chain, are present in our bodies, and consequently in breastmilk.

Contaminant levels in breastmilk largely reflect the mother's body burden of lipid-soluble contaminants. The contaminant concentrations may exceed the internationally set tolerable intake levels for some substances. This contributes to the discussion among experts agreeing that breastfeeding is beneficial, but disagreeing about the advisable length of breastfeeding.

Analyses of breastmilk in Norway show that levels of many environmental contaminants, such as PCBs, dioxins and insecticide/pesticides such as DDT, HCH and HCB have had a substantial decline since the mid-1980s. The concentration of PBDEs increased in human milk until about the year 2000, while the levels now seem to have levelled out or are declining. However, new contaminants are entering the scene. A group of substances whose concentrations in human milk have increased is fluorinated compounds, which are used as e.g. surfactants. Many of these more recent contaminants have bio- and physiochemical properties that differ from lipid-soluble persistent contaminants. The amount that passes into breastmilk varies between substances.

This report will perform a benefit and risk assessment of breastmilk. The aim is to provide a foundation for Norwegian decision-makers to reliably provide future advice on the length of breastfeeding taking the environmental contaminant perspective into due consideration. The

[13]UN agency under the direction of the Food and Agriculture Organisation (FAO) and the WHO.

report will attempt to have a broad approach when it comes to the totality of substances in breastmilk.

The varied diet of an adult human being provides some 100 000 different substances in a day, and approximately 60 of these are nutrients. The rest (non-nutrients) belongs to various groups of bioactive substances, many of which are beneficial, but, some are harmful. Recent research indicates that many of the non-nutrients are absorbed and can affect gene, cell or organ functions. A number of these substances passes the blood-placenta barrier and are excreted in breastmilk after birth. For many of these substances, their impact on health and disease risk has been poorly investigated.

Effective methods for assessing the risks of individual substances have been developed by Codex Alimentarius. National bodies (national scientific committees for food safety and health, including the Norwegian Scientific Committee for Food Safety (VKM) and international organisations such as the FAO/WHO's Joint Expert Committee on Food Additives and Contaminants (JECFA), as well as the European Food Safety Authority (EFSA) and other bodies), have conducted health risk assessments of many different chemical contaminants. However, contaminants are most often evaluated as single substances. Methods to assess effects of mixtures of substances are slowly emerging.

The overall aim of this report is to provide a balanced assessment of the contribution and impact of persistent toxic substances in the context of benefits from breastfeeding for newborn children in Norway. Levels of all contaminants in breastmilk should be reduced, and the ambitious goal for the Norwegian government is to have a “detoxified” community and environment by 2020 (NOU 2010:9, 2012).

1.1 Interpretation of the mandate – limitations

There are a number of aspects connected to breastfeeding that this assessment does not cover. They are important aspects that deserve attention in their own right, but are beyond the scope of the present work:

- psychological aspects of breastfeeding, neither from a child nor mother perspective.
- impact of breastfeeding on maternal physical health, e.g. impact on weight reduction or later risk of disease.
- drug or medicinal contamination of breastmilk, or pathogenic microorganisms such as bacteria or virus.
- classical animal or cell studies dealing with toxicity of the compounds apart from using the NOAEL/LOAEL/PTWI etc., i.e. values that are often derived from animal studies.

The focus will be on breastmilk and not on the alternative; infant formula. Thus, this assessment will not comprise a benefit- and risk assessment of infant formula, although infant formula and substances in infant formula will be discussed for comparison reasons.

2 Recommendations, prevalence, degree and duration of breastfeeding

2.1 Infant feeding recommendations and breastfeeding definitions

The Norwegian Directorate of Health recommends that infants are exclusively breastfed for 6 months, with a total breastfeeding duration of at least 12 months or more (Norwegian Directorate for Health, 2001). The recommendation includes possible reasons why a supplement to breastmilk may be needed, such as poor growth, the infant being hungry even after frequent breastfeeding, lactation difficulties or maternal perception/experience with breastfeeding. If there is a need for additional food after the age of 4 months, semi-solid food instead of infant formula is recommended. Complementary foods should be gradually introduced from the age of 6 months to cover the infant's need for energy and nutrients. The national recommendations for infant nutrition are currently being revised.

The systematic literature review which forms the basis for the 5th Nordic Nutrition Recommendations (NNR 2012), concludes that the recommendation about exclusive breastfeeding until 6 months of age from NNR 2004 is unchanged (Hornell et al., 2013).

The WHO recommends exclusive breastfeeding for 6 months and a total breastfeeding period for up to 2 years or beyond (WHO, 2001). Nutritionally adequate and safe complementary feeding should be started from the age of 6 months (for definitions, see Table 2.1). Before 2001, the WHO recommended that infants should be exclusively breastfed for 4 to 6 months. The revised recommendation was based on a systematic review which concluded that breastmilk contributed adequate amounts of nutrients for growth and development until 6 months (Kramer and Kakuma, 2002). As long as breastfeeding supports adequate growth, the notion is that nutritionally superior breastmilk should not be replaced by less nutritious complementary foods. The most recent systematic review on this issue was published by the Cochrane Library in 2012 (Kramer and Kakuma, 2012). It includes two controlled trials and 21 observational studies and concludes that exclusive breastfeeding for 6 months has advantages over exclusive breastfeeding for 3 to 4 months followed by partial breastfeeding. These advantages include a lower risk of gastrointestinal and respiratory infections for the infant. A reduced risk of allergic diseases through exclusive breastfeeding for 6 months versus 4 months was not shown in this review.

In 2007, 20 out of 24 European countries recommended exclusive breastfeeding for 6 months and 12 out of 24 recommended breastfeeding for up to 2 years (Cattaneo et al., 2010). Exclusive breastfeeding for 6 months is also recommended by the American Academy of Pediatrics (AAP), Scientific Advisory Committee on Nutrition, UK (SACN), Health Canada and National Food Agency in Sweden (Gartner et al., 2005; Health Canada, 2012; Livsmedelsverket, 2011; SACN, 2003)[14]. In 2012, the AAP affirmed its previous recommendation of exclusive breastfeeding for 6 months with a total breastfeeding period for one year or longer (Sicherer and Burks, 2008; Thygarajan and Burks, 2008). According to AAP, it is documented that exclusive breastfeeding for at least 3 to 4 months reduces the risk of allergic diseases both in low-risk and high-risk populations. When considering the most appropriate duration of exclusive breastfeeding, various health effects should be evaluated. In addition to nutrient adequacy and risk of infections, outcomes such as allergic diseases,

[14]The conclusions in SACN, 2003 are confirmed in SACN, 2011.

cognitive development, development of healthy eating habits and overweight and obesity should also be considered.

At present, there is conflicting evidence regarding timing of adding complementary foods after 4 months and the risk of allergic diseases (Sicherer and Burks, 2008; Thygarajan and Burks, 2008). A report from the European Food Safety Authority (EFSA) concluded that the introduction of complementary food to healthy, term infants between 4 and 6 months is safe and does not pose a risk for adverse health effects (short-term and also possible long-term effects) (EFSA, 2009b). The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on nutrition concludes that exclusive or full breastfeeding for about 6 months is a desirable goal. Complementary feeding should not be introduced to any infant before 17 weeks (4 months), and all infants should start complementary feeding by 26 weeks (Agostoni et al., 2008).

There is a need for randomised studies comparing the effect of introduction of complementary foods at different ages within the 4-6 month interval (Michaelsen et al., 2010).

Table 2.1: WHO criteria that define selected infant feeding practices[1].

Feeding practice	Requires that the infant receive	Allows the infant to receive	Does not allow the infant to receive
Exclusive breastfeeding	Breastmilk (including milk expressed or from a wet nurse)	ORS[2], drops, syrups (vitamins, minerals including cod liver oil, medicines)	Anything else
Predominant breastfeeding	Breastmilk (including milk expressed or from a wet nurse) as the predominant source of nourishment	Certain liquids (water and water-based drinks, fruit juice), ritual fluids and ORS[2], drops, syrups (vitamins, minerals, medicines)	Anything else (in particular non-human milk, food-based fluids)
Complementary breastfeeding	Breastmilk (including milk expressed or from a wet nurse) and solid or semi-solid foods	Anything else: any food or liquid including non-human milk and formula	NA[3]
Breastfeeding	Breastmilk (including milk expressed or from a wet nurse)	Anything else: any food or liquid including non-human milk and formula	NA[3]
Bottle-feeding	Any liquid (including breastmilk) or semi-solid food from bottle with nipple/teat	Anything else: any food or liquid including non-human milk and formula	NA[3]

[1]WHO. Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6-8 November 2007 in Washington D.C., USA.
[2]ORS=Oral rehydration solutions.
[3]NA=Not applicable.

If any solid foods or liquids are introduced, the infant is no longer considered exclusively breastfed according to the WHO definition. Cod liver oil is regarded as a vitamin supplement and accepted within the definition of exclusive breastfeeding. The term *full breastfeeding* is not used by WHO, but is seen in several publications and comprises exclusive and predominant breastfeeding. The term *partial breastfeeding* is used in several publications for breastmilk combined with formula/milk or complementary foods. The term *any breastfeeding* implies that breastmilk is given, regardless of whether additional foods or liquids are given.

2.2 Breastfeeding patterns in Norway

National surveys on infant and young child feeding practises were conducted in 1998-1999 and 2006-2007 (Spedkost and Småbarnskost). Spedkost consists of several cohort studies,

with surveys at 6 and 12 months of age. Cross-sectional studies were conducted in the same periods for 2-year old children (Småbarnskost).

Short description of the relevant national surveys:

- **6-month old infants**; Spedkost 2006-2007 is based on a semi-quantitative food frequency questionnaire. The study was conducted in 2006, and a total of 1986 6-month old children participated (participation rate: 67%) (Øverby et al., 2008).

- **1-year old children**; Spedkost 2006-2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1635 1-year old children participated (participation rate: 57%) (Øverby et al., 2009).

- **2-year old children**; Småbarnskost 2007 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1674 2-year olds participated (participation rate: 56%) (Kristiansen et al., 2009).

- **6-month old infants**; Spedkost 6 months is based on a semi-quantitative frequency questionnaire. The study was conducted in 1998, and a total of 2383 6-month old children participated (participation rate: 80%) (Lande, 2003).

- **1-year old children**; Spedkost 12 months is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 1999, and a total of 1932 1-year olds participated (participation rate: 66%) (Lande and Andersen, 2005b)**.**

- **2-year old children**; Småbarnskost 1999 is based on a semi-quantitative food frequency questionnaire. In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 1999, and a total of 1720 2-year old children participated (participation rate: 58%) (Lande and Andersen, 2005a).

- **The Norwegian Mother and Child Cohort Study (MoBa)** is a nation-wide pregnancy cohort that in the years 1999-2009 included more than 100 000 pregnancies. The participation rate was 39%. (Meltzer et al., 2008). The mother reported breastfeeding length and intensity both at 6 and 18 months after birth (Haggkvist et al., 2010).

- **The HUMIS study** is a birth cohort study that recruited 2500 mother-child pairs in the years 2002-2009 by visiting health personnel. Human milk was collected approximately one month after delivery and environmental toxicants was measured in a subset of 1000 women. The families filled in further questionnaires when the children were 6, 12 and 24 months of age (Eggesbo et al., 2009).

Figure 2.1 presents data from Spedkost 2006-07. Almost all mothers initiated breastfeeding, 80% breastfed at 6 months and 46% at 12 months. After 12 months, breastfeeding rates

decreased rapidly, and 8% breastfed at 18 months and 4% at 24 months (Kristiansen et al., 2009). Breastfeeding rates at 12 months increased from 36% in 1998 to 46% in 2007.

In 2007, 46% of the mothers breastfed exclusively for 4 months. This is the same percentage as in 1998. Furthermore, in 2007, 9% breastfed exclusively for 6 months, while the corresponding figure was 7% in 1998. The data from Spedkost are supported by other national studies where information on breastfeeding prevalence has been collected, i.e. the Norwegian Mother and Child Cohort Study (Haggkvist et al., 2010).

Spedkost 2006-2007 showed that mean breastfeeding frequency at 6 months was 6.7 times per day, and 3.5 times per day at 12 months.

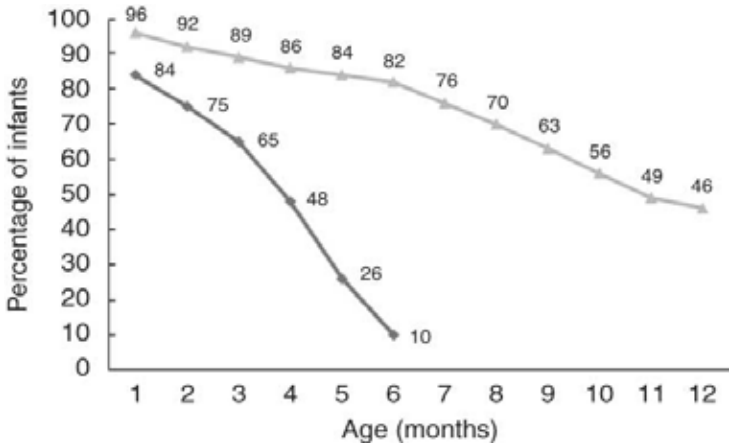

Figure 2.1: Exclusive breastfeeding (◆) in Norway during the first 6 months of life and breastfeeding (▲) during the first year of life (n=1490). Source: Kristiansen *et al*., 2010.

2.2.1 Historical data and trends

From an evolutionary perspective, breastmilk has been the prerequisite for survival of the offspring. Through history it has provided the primary source of nutrition for newborns before they are able to eat and digest other foods.

A mother is able to produce milk as long as the milk-producing glands are regularly stimulated. Thus, it has not been uncommon in some ethnic groups to breastfeed well into childhood. Infants are born with a sucking reflex allowing them to extract the milk from the nipples of the breasts, as well as an instinctive behaviour known as rooting with which they seek out the nipple. If a mother could not, or would not breastfeed, a wet nurse was generally necessary for survival of the infant until approximately a century ago.

Breastfeeding practice is sensitive to socio-demographic factors, cultural perceptions and health care practices and hence breastfeeding prevalence may change rapidly. In Norway, we have records of breastfeeding prevalence covering the three largest hospitals from 1860 to 1980, see Figure 2.2 (Liestol et al., 1988). Until 1920, breastfeeding was common in most groups of society. About 90% of the mothers breastfed for at least 3 months, and 50% for at least a year. There was some variation between socio-economic groups, as breastfeeding was more common among low-income and less educated families, which is quite the opposite of the trend seen in Norway today. Breastfeeding was more common in rural than in urban areas, which is also contrary to the present trend. In 1920 the breastfeeding prevalence started to decline and breastfeeding was increasingly replaced by formula feeding. In 1967

breastfeeding rates reached an all-time low level, as only 31% of Norwegian women breastfed for 3 months. The same trend was seen in many developed countries.

The increase in infant formula use in the 1930s had a number of reasons. It became more common to give birth in hospital. In hospitals, mothers were routinely separated from their infants between the feedings (Helsing and Haggkvist, 2012; Liestol et al., 1988). Mothers were also subjected to strict feeding regimes where they should breastfeed their infants only 4-6 times during the day and not at night. This resulted in many mothers having problems with producing sufficient breastmilk for their infants, as breastmilk production is self-regulating and "demand driven". Only a sufficiently high feeding frequency will stimulate the breast to produce sufficient quantities of milk. Information about breastfeeding was not included in medical personnel's curriculum or in their instruction manuals, or the information given was not likely to promote lactation. When problems arose and breastfeeding failed, the solution was to use bottled milk. The quality of breastmilk was also being questioned by pediatricians, who doubted that it could cover the infant's energy needs. while they warned against frequent feeding since the infant's digestive system needed to rest (Helsing and Haggkvist, 2012).

The infant food industry developed a number of products as substitutes for breastmilk, and these were welcomed by many health workers who thought that modern women were not able to breastfeed their child (Helsing and Haggkvist, 2012). The producers of infant formulas became increasingly aggressive in their marketing. Maternity wards had posters that informed mothers about the benefits of using these products and health workers were handing out free samples of infant formulas. This kind of marketing probably contributed to the decrease in breastfeeding both in developed and developing countries. The consequences were serious, in particular in developing countries where diluted infant formulas and the use of contaminated water led to under-nutrition, diseases and the deaths of many children. In 1981, the World Health Assembly adopted the International Code of Marketing of Breast-Milk Substitutes. The aim of the Code is "*…to contribute to the provision of safe and adequate nutrition of infants, by the protection and promotion of breastfeeding, and by ensuring the proper use of breastmilk substitutes, when these are necessary, on the basis of adequate information and through appropriate marketing and distribution*" (WHO, 1981).

For several reasons, the rates of breastfeeding began to rise around 1970. As a reaction to the medical trend in child rearing, many women had a desire for a more natural behaviour. The Norwegian mother-to-mother breastfeeding support group "Ammehjelpen" was started in 1968 and strongly reinforced this continuing change of attitudes. Gradually, hospital routines improved and the aggressive marketing of infant formula halted. After 1980, a plateau was reached, and 77% of Norwegian women breastfed for 3 months, 55% for 6 months and 10% for 12 months (Liestol et al., 1988).

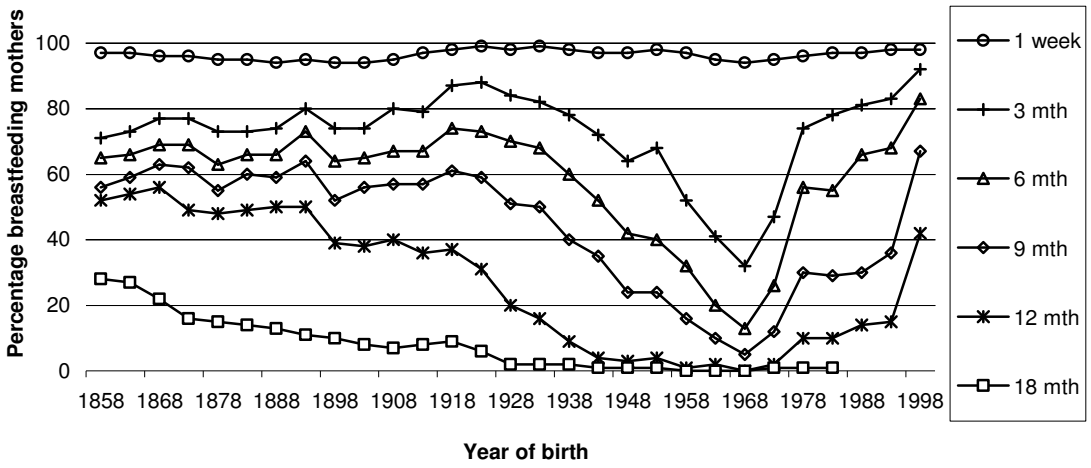

Figure 2.2: Distribution of durations of breastfeeding in Norway, 1860-1998, for all children born in subsequent 5-year groups. Source: Liestol *et al*., 1988 and adapted by Elisabet Helsing.

2.2.2 Breastfeeding in Norway compared with other countries

Socio-demographic and life style factors influence breastfeeding practice. Higher education, higher age, no-smoking, living in urban areas and being married are all factors associated with longer duration of exclusive breastfeeding and total breastfeeding duration. Low birth weight is associated with shorter breastfeeding duration. Infants with asthma or allergy among family members are breastfed longer compared with infants from families with no heredity of asthma or allergy (Lande et al., 2003).

Information about breastfeeding practices among non-western immigrants living in Norway is limited. Available data indicate that rates of exclusive breastfeeding often are low (Madar et al., 2009).

The EU project *Protection, promotion and support of breastfeeding in Europe: a blueprint for action* collected data about breastfeeding prevalence in Europe (Cattaneo et al., 2010). Breastfeeding rates for European countries are presented in Figures 2.3-2.5. The lack of standardised definitions, methods, age groups and indicators across countries makes comparisons difficult. According to Figure 2.3 the rate of *exclusive* breastfeeding at 6 months in Norway seem to be among the lowest in Europe. This is probably due to a rigorous survey method for assessing breastfeeding rates in the Spedkost survey. On the other hand, Norway has the highest rate of *any* breastfeeding at 6 and 12 months.

A slight increase in breastfeeding rates was reported for most European countries in a survey where two points in time were available (Cattaneo et al., 2010). However, the most recent breastfeeding statistics from Sweden demonstrates a clear downward trend in breastfeeding of infants born in 2010 (not presented in the Figures) http://www.socialstyrelsen.se/publikationer2010/2010-8-2.

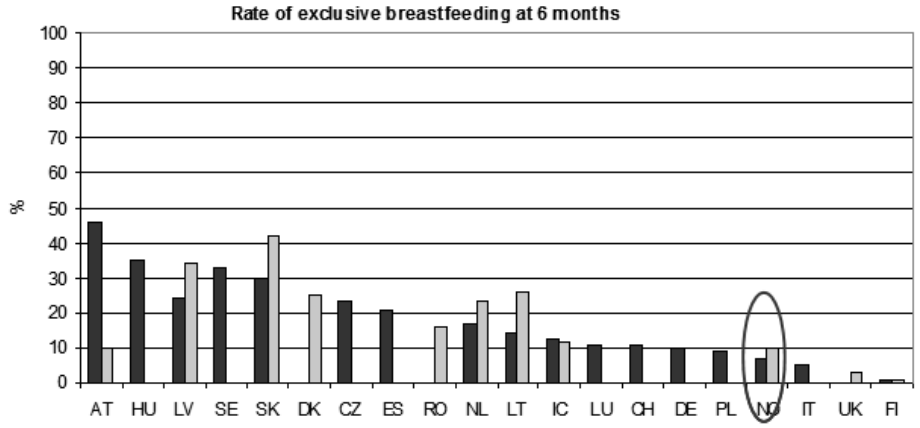

Figure 2.3: Rates of exclusive breastfeeding at 6 months in Europe: 1998-2002 and 2003-2007 . Based on Cattaneo *et al*., 2010 and data from Spedkost 2006-2007.

AT: Austria, HU: Hungary, LV: Latvia, SE: Sweden, SK: Slovakia, DK: Denmark, CZ: Czech Republic, ES: Spain, RO: Romania, NL: Netherland, LT: Lithuania, IC: Iceland, LU: Luxembourg, CH: Switzerland, DE: Germany, PL: Poland, NO: Norway, IT: Italy, UK: United Kingdom, FI: Finland, EE: Estonia, BG: Bulgaria, PT: Portugal, BE: Belgium.

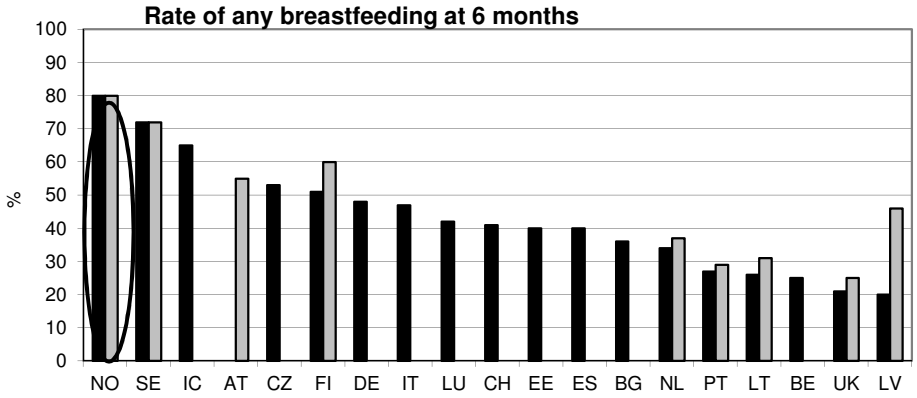

Figure 2.4: Rates of any breastfeeding at 6 months in Europe: 1998-2002 and 2003 2007 . Based on Cattaneo *et al*., 2010 and data from Spedkost 2006-2007.

NO: Norway, SE: Sweden, IC: Iceland, AT: Austria, CZ: Czech Republic, FI: Finland, DE: Germany, IT: Italy, LU: Luxembourg, CH: Switzerland, EE: Estonia, ES: Spain, BG: Bulgaria, NL: Netherland, PT: Portugal, LT: Lithuania, BE: Belgium, UK: United Kingdom, LV: Latvia.

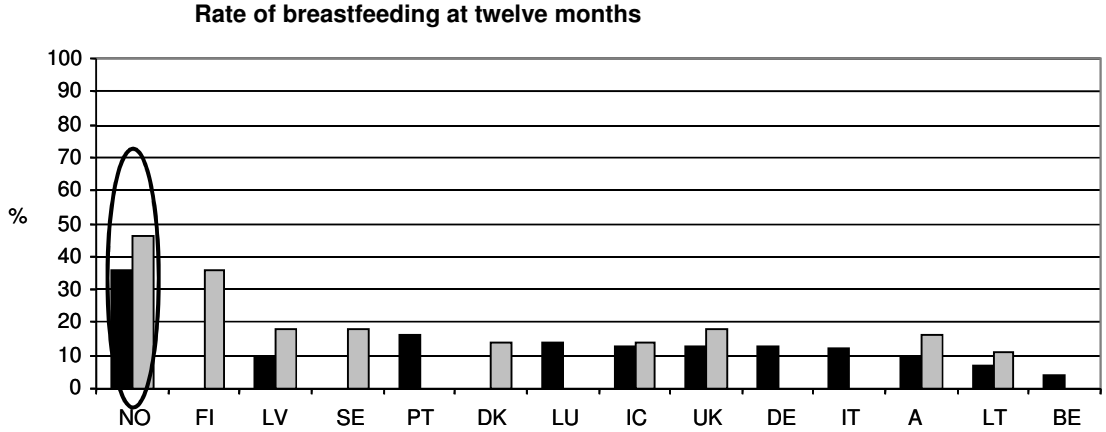

Figure 2.5: Rates of any breastfeeding at 12 months in Europe: 1998-2002 and 2003-2007 . Based on Cattaneo *et al*., 2010 and data from Spedkost 2006-2007.

NO: Norway, FI: Finland, LV: Latvia, SE: Sweden, PT: Portugal, DK: Denmark, LU: Luxembourg, IC: Iceland, UK: United Kingdom, DE: Germany, IT: Italy, AT: Austria, LT: Lithuania, BE: Belgium.

2.3 Breastmilk volume and consumption

Precise and accurate measurements of breastmilk consumption are difficult to obtain because the quantity ingested is not directly observable. Several methods for measuring breastmilk consumption have been applied. The most common method for measuring milk consumption or production has been test-weighing, a procedure in which the infant or the mother is weighed before and after each feeding (Butte N. et al., 2002; da Costa et al., 2010). The introduction of isotope tracer methods to measure breastmilk consumption represents a substantial improvement over test-weighing. However, it is difficult to apply in field conditions and unsuitable for large group studies (da Costa et al., 2010).

In this report, the mean consumption of breastmilk per day is based on results from 28 different studies at different months in developed countries and was presented in a publication from WHO (Butte N. et al., 2002). The studies presented were conducted in presumably well-nourished populations from developed countries in the 1980s to1990s. In most of these studies, breastmilk consumption was assessed using the 24-hour test-weighing method, while the 12-hour test-weighing method and the deuterium dilution method were used in a few cases.

Mean breastmilk consumption of exclusively breastfed infants increased from 699 g/day at one month to 854 g/day at 6 months. Longitudinal studies reported no marked increase in breastmilk volume after 3 months (Reilly et al., 2005). The mean coefficient of variation was 16% in exclusively breastfed infants compared with 34% in partially breastfed infants. Milk intakes among partially breastfed hovered around 675 g/day in the first 6 months and 530 g/day in the next 6 months. From Tables 2.2-2.4 it appears that the total breastmilk consumption in exclusively breastfed children compared with partially breastfed does not differ as much as expected. Possible reasons for this may be that complementary foods are introduced gradually and in small quantities, and that children with higher energy needs signal need for more food and therefore are over-represented among those given complementary foods.

Table 2.2: Breastmilk consumption of exclusively breastfed infants 0-6 months of age from developed countries (g/day). Source: Butte *et al*., 2002.
http://www.who.int/nutrition/publications/infantfeeding/9241562110/en/).

Age, months	1	2	3	4	5	6
Mean, weighted for sample size	699	731	751	780	796	854
Pooled SD[1]	134	132	130	138	141	118
N	186	354	376	257	131	93
Number of study groups	11	14	17	13	10	8

[1]SD=standard deviation.

Table 2.3: Breastmilk consumption of partially breastfed infants 0-6 months of age from developed countries (g/day). Source: Butte *et al*., 2002.
http://www.who.int/nutrition/publications/infantfeeding/9241562110/en/).

Age, months	1	2	3	4	5	6
Mean, weighted for sample size	611	697	730	704	710	612
Pooled SD[1]	129	150	149	184	194	180
N	116	227	241	251	163	380
Number of study groups	3	7	9	8	8	15

[1]SD=standard deviation.

Table 2.4: Breastmilk consumption of partially breastfed infants 7-12 months of age from developed countries (g/day). Source: Butte *et al*., 2002.
http://www.who.int/nutrition/publications/infantfeeding/9241562110/en/**).**

Age, months	7	8	9	10	11	12
Mean, weighted for sample size	569	417	497	691	516	497
Pooled SD[1]	188	226	249	233	215	238
N	251	123	154	5	6	48
Number of study groups	11	8	11	1	1	4

[1]SD=standard deviation.

Results from a more recent 12 country study on breastmilk consumption, using a standardised stable isotope methodology, are presented in Figure 2.6 (da Costa et al., 2010). This dataset did not distinguish between exclusive and partial breastfeeding and included mothers from both developed and developing countries. The figure depicts similar consumption of breastmilk as in the WHO report by Butte *et al*., although the levels tend to be higher in the second half of infancy. A longitudinal study of the adequacy of breastmilk consumption during exclusive breastfeeding using double-labelled water method found that the mean breastmilk consumption was 923 g/day (SD: 122) at around 15 weeks and 999 g/day (SD: 146) when the child was around 26 weeks old (Nielsen et al., 2011).

The volumes presented in Figure 2.6 correspond with the findings from a randomised intervention trial (Cohen et al., 1994). To evaluate whether there are any advantages of complementary feeding prior to 6 months, low-income primiparous mothers who had exclusively breastfed for 4 months were randomly assigned to one of three groups: 1) continued exclusive breastfeeding to 6 months (n=50), 2) introduction of complementary foods from 4 months onwards without any instruction on breastfeeding frequency from 4 to 6 months (n=47) and 3) introduction of complementary foods from 4 months onwards with instruction to maintain the same high frequency of breastfeeding from 4 to 6 months (n=44) (Figure 2.7).

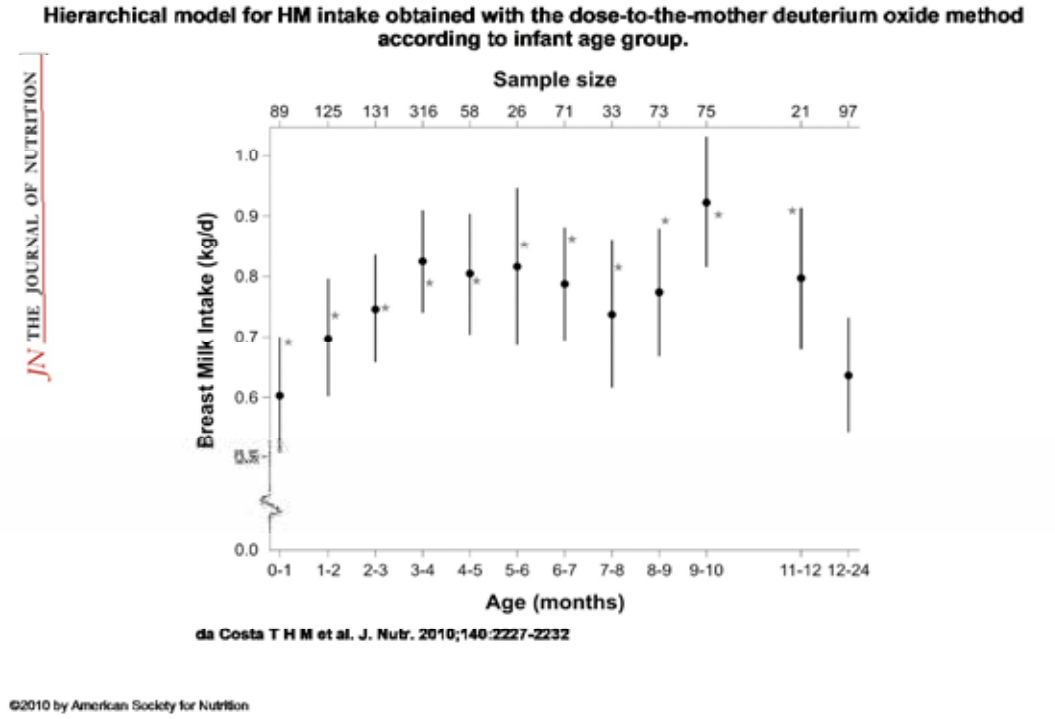

Figure 2.6: Results from a 12 country study on breastmilk consumption (human milk=HM). Source: da Costa *et al*., 2010.

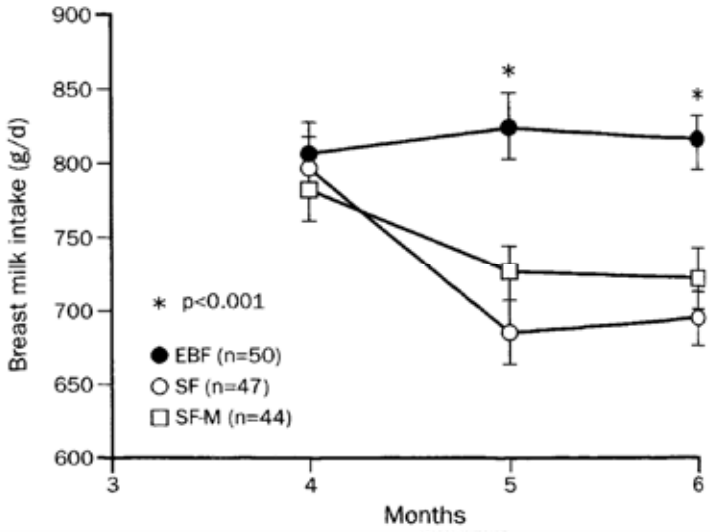

Figure 2.7: Breastmilk intake of Honduran infants from 4 to 6 months (mean +/-SEM). Source: Cohen *et al.*, 1994.

EBF=exclusive breastfeeding.
SF=introduction of complementary foods from 4 months with *ad libitum* nursing from 4-6 months.
SF-M=introduction of complementary foods from 4 months with maintenance of baseline nursing frequency from 4-6 months.

At 4 months, breastmilk consumption averaged 797 (SD=139) g/day (no difference between groups). Between 4 and 6 months consumption was unchanged in EBF infants (+6 g), but

decreased in the SF (-103 g), and SF-M (-62 g) groups (p>0.001). Change in total energy and infant weight and lengths did not differ significantly between the groups, and were comparable to breastfed infants in an affluent US population. According to the authors, the results indicate that breastfed infants self-regulate their total energy intake when other foods are introduced. The introduction of solid foods in this period will result in replacement of breastmilk.

No similar study on breastmilk consumption in Norwegian infants has been conducted.

2.4 Relative contraindications to breastfeeding

There are a few conditions where breastfeeding is or may be contraindicated; among these are some metabolic disorders, infections and drug use. These contraindications are summarised in AAP's Policy statement 2012: Breastfeeding and the use of human milk (AAP, 2012).

Microbial contaminants in human milk

Analyses of milk samples from healthy Norwegian milk-donors have revealed bacteria in about 10% of the samples; the majority being *Staphylococcus epidermidis* (Lindemann et al., 2004).

A few cases have been reported where breastmilk has been suggested as source for Group B *Streptococcus* (GBS) or Klebsiella pneumonia disease in preterm infants (Godambe et al., 2005).

A prospective, population-based study was performed in Italy to investigate GBS late-onset disease (LOD; disease from day 7-89) in premature and mature infants in the period 2003-2010. F2007 milk samples were analysed from the mothers of 45 infants (11 preterm, 34 term) diagnosed with LOD. Of these, two and nine milk samples were positive in premature and mature infants respectively. One and two GBS mastitis cases were diagnosed in the mothers of premature and mature infants, respectively. The authors concluded that "most mothers of neonates with LOD are identified at diagnosis with anogenital GBS carriage. It seems unlikely that GBS infected milk is a source of LOD in the absence of mastitis" (Berardi et al., 2013).

Mothers with human T-cell lymphotropic virus type I or II, untreated brucellose or HIV (in developed, but not in developing countries), untreated tuberculosis or active herpes simplex lesions on the breast should not breastfeed. Mothers with varicella five days before, through two days after delivery, as well as mothers acutely infected with H1N1 influenza virus, should be isolated from their infants but can provide expressed breastmilk for feeding. Full-term infants may receive breastmilk from a cytomegalovirus seropositive mother, while there are specific guidelines for preterm infants (AAP, 2012).

Metabolic disorders

The metabolic disorder classic galactosemia represents a condition where breastmilk is contraindicated, whereas children with phenylketonuria may alternate between modified formula and breastmilk (AAP, 2012).

Medications

There are a limited number of agents that are contraindicated in a nursing mother, and an appropriate substitute can usually be found. Every potential drug should however be carefully considered with respect to safety and balanced against the benefits to the infant. Specific literature exists on this topic (Hale, 2012).

Alcohol, tobacco smoke and drugs

Regarding use of alcohol, ingestion should be minimized and limited to an occasional intake, but not more than 0.5 g alcohol per kg bw, and the mother should not nurse until a minimum of two hours after intake. In Norway it is recommended to wait three hours after intake of maximum one unit alcohol (Helsedirektoratet, 2011).

Maternal smoking is not necessarily a contraindication to breastfeeding but should be discouraged because of its association with e.g. increased incidence of sudden infant death syndrome. If smoking is continued, it should be restricted to after the breastfeeding (Helsedirektoratet, 2011).

Maternal substance abuse is not a categorical contraindication, but depends on the type of drug.

2.5 Summary of recommendations, prevalence, degree and duration of breastfeeding

The WHO and Norwegian health authorities recommend that infants are exclusively breastfed for 6 months with a total duration of at least two years and one year, respectively. This is a public health recommendation which should be adjusted in individual consultations. Only a minority of Norwegian mothers breastfeeds exclusively for 6 months. However, breastfeeding prevalence is higher in Norway than in most European countries, with 80% of the mothers breastfeeding when the child is 6 months and 46% when the child is 12-months old. At 4 and 6 months of age 46% and 9% of infants are exclusively breastfed. This is a low rate compared with results from other European countries and is probably partly due to differences in the methodology used in the surveys. Mean breastmilk consumption in exclusively breastfed infants increases from 699 ml/day at age one month to 854 g/day at age 6 months. Breastmilk consumption in partially breastfed infants is approximately 675 g/day during the first 6 months and 530 g/day in the next 6 months, demonstrating only minor differences between exclusively and partially breastfed infants. Published reviews on breastmilk consumption are largely based on studies using test-weighing methods. Results from some recent studies using the isotopic methods find somewhat higher breastmilk consumption both in exclusively and partially breastfed infants. There are a few conditions where breastfeeding is or may be contraindicated, among these are some infections, metabolic disorders and drug use.

3 Content of nutrients and immunological components in breastmilk

3.1 Breastmilk composition

The composition of breastmilk is not uniform. The concentration of many of its constituents changes during the lactation period and differ among individuals, also when samples are collected and analysed under controlled, defined conditions (Lawrence and Lawrence, 2010a).

Breastmilk production can be divided into three phases that differ in the composition and volume of milk produced: colostrum (first to approximately seventh day postpartum), transitional milk (<14 days postpartum) and mature milk (≥14 days postpartum) (Lawrence and Lawrence, 2010a). Colostrum is the fluid secreted by the mammary gland immediately following parturition. During the colostral period, rapid changes occur in milk composition. The concentrations of fat and lactose increase while those of protein and minerals decrease. Transitional and mature milk also exhibits variability, but to a much smaller extent than in early lactation (IOM, 1991).

Breastmilk composition can also vary during the day and from the beginning to the end of a feeding. This is most pronounced for fat; the fat concentration in the hindmilk (the breastmilk after several minutes of feeding) has been shown to be approximately 2-3 times that of the foremilk (the breastmilk at the onset of the feeding) (Saarela et al., 2005). Other factors which may influence breastmilk composition are breastfeeding routines (frequency of feeding), parity, age, maternal diet and other maternal characteristics (Prentice, 1996). The extent to which the maternal diet influences the nutrient concentrations in breastmilk varies between nutrients. The nutrients most affected by low maternal intakes and stores are the B vitamins thiamine and riboflavin, vitamin B_6 and B_{12}, vitamin A and iodine (Allen, 2005). The quantity of fat in breastmilk has not been shown to be influenced by maternal fat intake, but it has been shown repeatedly that the nature of the fat consumed by the mother will influence the fatty acid composition of the breastmilk (Lawrence and Lawrence, 2010a).

Assessment of the composition of breastmilk is associated with methodological challenges both due to normal variation in the composition and to analytical challenges (IOM, 1991). To obtain accurate results, one must apply proper sampling, extraction, handling, and storage procedures as well as a sensitive and selective detection system. Large variations reported for many milk constituents may reflect improper sampling and/or analytic inaccuracies in addition to true biologic variance.

3.2 Estimating adequacy of nutrient intake in infants

An infant who is exclusively breastfed the first 6 months of life has been shown to have all nutritional needs covered, with the exception of vitamin D (Butte N. et al., 2002). The recommended daily intake for infants 0-6 months are for most nutrients equal to the actual intakes of the various nutrients of breastfed infants, as described in international recommendations (IOM, 2006). The mean nutrient intake data of healthy, full-term, exclusively breastfed infants is termed Adequate Intake (AI) which is derived by the following formula: mean intake of breastmilk multiplied by mean nutrient concentrations (Butte N. et al., 2002; IOM, 2006). The Nordic Nutrition Recommendations (NNR) from 2012 (NNR Project Group, 2013) recommend exclusive breastfeeding the first 6 months, and

therefore do not provide specific nutrient recommendations for infants below 6 months. NNR 2012 states: "recommendations for single nutrients are not given for infants <6 months. If breastfeeding is not possible, infant formula formulated to serve as the only food for infants should be given (…). If complementary feeding has started at 4-5 months, the recommended intakes for 6 to 11-month old infants should be used". Estimated nutrient needs for infants 7 to 11 months of age are usually determined by extrapolating from the intake measures of the first 6 months. New Norwegian recommendations for infant nutrition are currently under preparation.

3.3 Nutrient concentrations in breastmilk

The concentrations of nutrients in breastmilk presented here are mainly based on NNR (NNR Project Group, 2013) and the US Institute of Medicine's (IOM) "Nutrition during lactation" (IOM, 1991). The same figures from IOM were also used in the more recent book "Breastfeeding – a guide for the medical profession" (Lawrence and Lawrence, 2010a). Although the data for breastmilk nutrient concentrations stem from relatively old research, it was out of the scope for the present report to update the figures through a systematic literature review.

Breastfed infants have a special capacity to self-regulate milk intake. Thus, the total intake of each nutrient is difficult to assess. In the following section selected nutrients are presented with their main physiological function, followed by concentrations and factors influencing the concentrations in breastmilk and the recommended intake of the nutrients for infants in Norway. The nutritional status of infants in Norway is mentioned for those nutrients where such information exists.

Tables 3.1-3.3 give an overview of the estimated nutrient concentrations per liter breastmilk.

Table 3.1: Estimates of the concentration of macronutrients in mature breastmilk[1] and daily recommended intake for infants[2].

Nutrient	Amount in breastmilk, per L, ± SD[3]	Daily recommended intakes (6-11 months)
Energy, kJ[3] **Kcal**	2887[3] 690	See Table 3.4
Protein, g[4]	7-8[4]	7-15% of total energy intake
Fat, g Fatty acids (% of total fat)[5] 12:0 tauric 14:0 myristic 16:0 palmitic 18:0 stearic 18:1 oleic 18:2, n-6 linoleic 18:3, n-3 linolenic C_{20} and C_{22} polyunsaturated	39.0 ± 4.00 5.8 8.6 21.0 8.0 35.5 7.2 1.0 2.9	30-45% of total energy intake
Lactose, g	72.0 ± 2.50	45-60% of total energy intake

[1]Data from Institute of Medicine, unless otherwise indicated. Bold indicate that the nutrient is described in the text (IOM, 1991).

[2]Data from Nordic Nutrient Recommendations (NNR Project Group, 2013).
[3]SD=standard deviation.
[3]Data from Butte *et al*. 2002. Values were given in kJ/g, and are converted by using a density of milk of 1.03 g/ml. Standard deviation not reported; range 2678-206 kJ/L and 639-824 kcal/L.
[4]Data from Lonnerdal, 2003.
[5]Data from Lawrence & Lawrence, 2010, Table A-1.

Table 3.2: Estimates of the concentration of vitamins in mature breastmilk[1] and daily recommended intake for infants 6-11 months of age[2].

Nutrient	Amount in breastmilk, per L, ± SD[3]	Daily recommended intakes 6-11 months of age
Vitamin A, RE[4], µg	450-600[5]	300
Vitamin D, µg	0.55 ± 0.10	10
Vitamin E, α-TE[6]	2.3 ± 1.0	3
Vitamin K, µg	2.1 ± 0.1	-
Thiamin, mg	0.21 ± 0.04	0.4
Riboflavin, mg	0.35 ± 0.03	0.5
Niacin, NE[7]	1.50 ± 0.20	5
Vitamin B_6, mg	93 ± 8	0.4
Folate, µg	85 ± 37	50
Vitamin B_{12}, µg	0.42[8]	0.5
Biotin, µg	4 ± 1	n.a.
Pantothenic acid, mg	1.80 ± 0.20	n.a.
Vitamin C, mg	40 ± 10	20

[1]Data from Institute of Medicine unless otherwise indicated (IOM, 1991).
[2]Data from Nordic Nutrient Recommendations (NNR Project Group, 2013).
[3]SD=standard deviation.
[4]RE, retinol equivalents; 1RE=1 µg retinol=12 µg β-carotene.
[5]Data from Nordic Nutrient Recommendations (NNR Project Group, 2013). Standard deviation not reported.
[6] α-TE, α-tocopherol equivalents; 1 α-TE=1 mg RRR a-tocopherol.
[7]NE, niacin equivalents; 1 NE=1 mg niacin=60 mg tryptophan.
[8]Data from IOM (IOM, 1998). Standard deviation not reported.

Table 3.3: Estimates of the concentration of minerals and trace elements in mature breastmilk[1] and daily recommended intake for infants 6-11 months of age[2].

Nutrient	Amount in breastmilk, per L, ± SD[3]	Daily recommended intakes 6-11 months of age
Calcium, mg	280 ± 26	540
Phosphorus, mg	140 ± 22	420
Magnesium, mg	35 ± 2	80
Sodium, mg	180 ± 40	n.a.
Potassium, mg	525 ± 35	1100
Chloride, mg	420 ± 60	n.a.
Iron, mg	0.3 ± 0.1	8
Zinc, mg	1.2 ± 0.2	5
Selenium, µg	20 ± 5	15
Iodine, µg	110 ± 40	50
Copper, mg	0.25 ± 0.03	0.3
Manganese, µg	6 ± 2	0.08
Fluoride, µg	16 ± 5	n.a.

[1]Data from Institute of Medicine (IOM, 1991).
[2]Data from Nordic Nutrient Recommendations (NNR Project Group, 2013).
[3]SD=standard deviation.

3.3.1 Energy

Energy in the diet is used for basal metabolism, growth and physical activity. The energy content in breastmilk has been reported to be in the range of 639 to 824 kcal/L, and Butte *et al*. used a value of 690 in their report from 2002 (Butte N. et al., 2002).

Proteins, carbohydrates and lipids are the sources of energy in breastmilk. Protein and carbohydrate concentration change with duration of lactation but they are relatively stable between women at any given stage of lactation (Butte N. et al., 2002). Lipid concentrations may vary to a greater extent between both individual women and populations, which accounts for the variation observed in the energy content in breastmilk (Butte N. et al., 2002).

The NNR estimated energy requirement (EER) for infants is based on infants' energy consumption assessed by the double-labelled water method (Table 3.4) (NNR Project Group, 2013). The Norwegian Directorate for Health points out, however, that the reference values for energy intake have no practical meaning for breastfeeding infants who are following a normal growth curve (Norwegian Directorate for Health, 2001).

Table 3.4: Reference values for estimated average daily energy requirements for infants 6-12 months of age assuming partial breastfeeding (NNR Project Group, 2013).

Age, months	Average daily energy requirements kJ/kg body weigh	
	Boys	Girls
6	339	342
12	337	333

3.3.2 Protein

The protein constituents of breastmilk serve diverse functions. Besides providing essential amino acids for growth, they provide protective factors (e.g. immunoglobulins, lysosomes and lactoferrin), they are carriers of vitamins and minerals (e.g. folate, vitamin D, vitamin B_{12} and iron-binding proteins) and of hormones (e.g. thyroxin and corticosteroid-binding proteins), and they entail enzymatic activity (e.g. amylase and bile-salt stimulated lipase) and growth factors (e.g. insulin-like growth factor, epidermal growth factor, and prolactin) (Lonnerdal, 2003; Picciano, 2001). Breastmilk protein concentration is high early in the lactation period (14-16 g/L), and decreases to 8-10 g/L at 3-4 months of lactation and 7-8 g/L at 6 months and later (Lonnerdal, 2003). About 25% of the nitrogen in human milk is non-protein nitrogen (NPN) compared with only 5% NPN in bovine milk (Lawrence and Lawrence, 2010b). The concentration of protein in human milk is lower than in most other animal milks, which ranges from about 32 g/L (cow's milk) to about 114 g/L (polar bear's milk), perhaps reflecting the lower growth rates in humans compared with other mammals (Helsing and Heggkvist, 2008).

Recommended protein intake in infants is based on the factorial method. The calculation is based on estimates of the need for maintenance and growth, the efficiency of conversion from dietary protein to body protein and intra-individual variation in growth (NNR Project Group, 2013). The NNR does not provide recommendations for protein intake in infants the first 6 months. It is stated that the infant's protein requirement will be covered when adequate breastmilk (in terms of energy) is provided (NNR Project Group, 2013). For infants 6-11 months, the NNR estimates that 1.1 g protein/kg body weight is adequate.

3.3.3 Fats

Fats are among the most variable constituents in breastmilk. The breastmilk fatty acids are crucial not only for energy to support growth but also for the synthesis and development of retinal and neural tissues (Picciano, 2001). The polyunsaturated fatty acids (PUFA) linoleic acid (LA) (18:2 n-6) and α-linolenic acid (ALA) (18:3 n-3) are essential fatty acids that cannot be synthesised in the human body and must be consumed as part of the diet. The n-6 and n-3 fatty acids represent each their PUFA "family" which can be desaturated and elongated. Infants have a reduced capacity to transform n-3 fatty acid to docosahexaenoic acid (DHA) (22:6 n-3) and n-6 fatty acids to arachidonic acid (AA) (20:4 n-6). These are of special importance for infant development and are present in breastmilk (Aggett et al., 1991).

The total fat concentration in breastmilk varies from 30 to 70 g/L and the corresponding energy contribution is approximately 45% to 55% of total energy intake (Michaelsen, Larsen

et al., 1994; Picciano, 2001). However, some studies indicate that the variation might be even larger, depending on a variety of different parameters as illustrated in Figure 3.1. There are both inter- and intra-individual variations.

Figure 3.1: Some parameters that may influence the fat concentration measured in breastmilk: [a]Higher fat content during day/evening compared with morning/night (Kent et al., 2006), [b]mother's body fat (Nommsen et al., 1991), [c]fat concentration positively associated with pregnancy weight gain (Michaelsen, Larsen et al., 1994) (see also Figure 3.2) and [d]greater energy content in milk of male infant mothers (Powe et al., 2010), and [e]gestational age (Molto-Puigmarti et al., 2011).

As an example, in a study by Michaelsen *et al*. the fat concentration at 5 months varied between 20 g/L and 70 g/L, and at 4 months the variability was significantly associated with maternal weight gain during pregnancy (Figure 3.2). The median fat concentration, however, was 39 g/L and is hence at the same level as reported by others (Michaelsen, Larsen et al., 1994).

In the Norwegian Human Milk Study (HUMIS) mean and median fat concentration in breastmilk samples was 34g/L[15] (n=295) (Polder et al., 2009). This is also in accordance with the EFSA opinion on PBDEs in food (EFSA, 2011c).

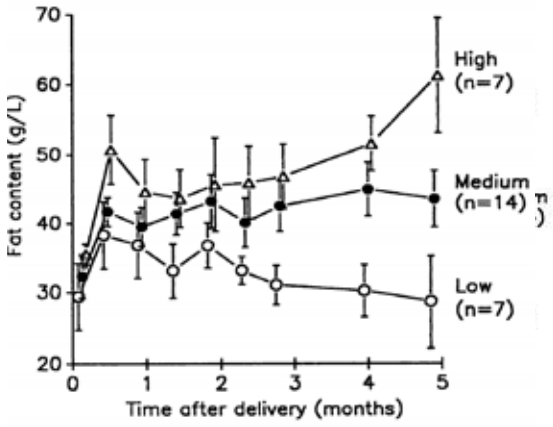

Figure 3.2: Fat concentration in human milk in relation to time after delivery in three groups of mothers with different weight gain during pregnancy: low (<11.2 kg), medium or high (>16.8 kg). Error bars indicate ± SEM. The association between weight gain and fat concentration in the milk was significant at 4 months only. Source: Michaelsen *et al*., 1994.

[15]Calculated from the value of 3.5 g fat/100 g breastmilk which was given in the reference, using a conversion factor of 1.03 g milk per ml.

It has also been documented that the percent of maternal body fat seems to be a determinant for the fat concentration in breastmilk. A study from the USA showed that milk fat concentration was positively correlated with maternal skinfold thickness in late lactation (6-12 months), but not in the early lactation period (Nommsen et al., 1991).

The hindmilk secreted towards the end of a feed contains more fat than that of the foremilk. This seems to be due to the fact that number of fat containing droplets (milk fat globules) increase as the feed progresses (Mizuno et al., 2009).

The fatty acids in breastmilk may originate either from recent dietary fatty acid intake, from fatty acids released from maternal adipose tissue or from *de novo* synthesis or further metabolism of dietary fatty acids in the maternal liver (Innis, 2007). While the maternal diet has little impact on the total amount of fat in breastmilk, the levels of the various unsaturated fatty acids are to a certain extent dependent on the maternal diet (Innis, 2007). The levels of n-6 and n-3, particularly LA, ALA and DHA in breastmilk vary widely within and among populations, and they are readily changed by the maternal dietary intake of the respective fatty acids (Innis, 2007). The role of DHA in the infant diet has received particular attention because of its critical roles in the development of the visual and neural systems (Innis, 2007). A range of 0.1-1.0 g DHA/100 g milk has been documented across various population groups with varying intakes of fish and fish oils, which are the main sources of DHA (Innis, 2007).

NNR does not give recommendations for fat intake for infants under 6 months of age, when exclusive breastfeeding is recommended. For infants 6-11 months, it is recommended that the proportion of total fat should contribute to between 30 and 45% of the total energy intake (NNR Project Group, 2013). NNR further recommend that the total amount of PUFA should constitute 5-10% of the total energy intake, including at least 1% of the energy from n-3 fatty acids. The optimal ratio of n-6/n-3 fatty acids is not known for the various age groups.

3.3.4 Carbohydrates

The principal carbohydrate in breastmilk is lactose, which is an important source of energy. The concentration is about 72 g/L, which is more than in most other mammal milks (IOM, 1991). Lactose is one of the most stable constituents of breastmilk, with most of the variability caused by maternal individuality (Picciano, 2001). The concentration of unbound oligosaccharides in breastmilk is 0.5-1.0 g per 100 g (Bode, 2006).

NNR states that a minimum of 130 g glycaemic carbohydrates per day will cover the needs for glucose for the brain and avoid ketosis, and thus gives this as an average requirement for glycaemic carbohydrates (NNR Project Group, 2013).

3.3.5 Vitamins and minerals

With the exception of vitamin D, breastmilk normally contains sufficient vitamins for an infant, unless the mother herself is deficient (Butte N. et al., 2002). The infant needs exposure to sunlight to generate endogenous vitamin D, otherwise, a supplement is necessary to ensure adequate vitamin D status (NNR Project Group, 2013). Fat-soluble vitamins are generally transported into the breastmilk as part of the fat fraction, and the breastmilk concentration of these compounds are not easily affected by changes in the maternal diet (Lawrence and Lawrence, 2010b). Water-soluble vitamins move with ease from serum to milk, thus their dietary fluctuation is more apparent (Lawrence and Lawrence, 2010a). Most levels of minerals in breastmilk are not influenced by maternal intake, the exceptions being iodine and selenium (IOM, 1991).

3.3.5.1 Fat-soluble vitamins

Vitamin A

Vitamin A is a generic term for a group of compounds possessing the biological activity of retinol. The term includes retinol, retinal, retinyl esters, retinoic acid and substances considered to be pro-vitamin A because they can be transformed into retinol (expressed as retinol equivalents (RE))[16] (NNR Project Group, 2013). Among the pro-vitamin A compounds, β-carotene has the highest potential vitamin A activity. Vitamin A is involved in a variety of functions such as vision, maintenance of epithelial surfaces, immune competence, growth, development and reproduction. Inadequate intake of vitamin A may lead to reduced immune function, blindness and increased risk of death (NNR Project Group, 2013). Vitamin A intake above the recommended intake has been associated with embryonic malformations, reduced bone mineral density and increased risk of hip fracture (NNR Project Group, 2013).

The vitamin A concentration in breastmilk in developed countries is 450-600 RE/L (NNR Project Group, 2013). The concentration is influenced by the maternal nutritional status. NNR does not provide recommendations for vitamin A intake for infants but states that the average intake (based on an estimated daily consumption of 0.75 L breastmilk per day) will correspond to 350-450 RE/day (NNR Project Group, 2013). Recommended intake for infants 6-11 months is 300 RE/day (NNR Project Group, 2013). High intake of retinol may cause negative health effects, but it is unlikely that breastmilk concentrations reach toxic levels due to high vitamin A intake by the mother (Allen and Haskell, 2002). The Scientific Committee for Food (SCF) has established a tolerable upper intake level (UL) for vitamin A at 800 µg RE for 1 to 3-year old children (SCF, 2002).

Vitamin D

Vitamin D is a steroid-like molecule that exists in two forms: vitamin D_3 (cholecalciferol) synthesised in the skin and vitamin D_2 (ergocalciferol) found in yeast. Factors affecting formation of vitamin D from sunlight exposure include the amount of skin pigmentation, age, season, degree of pollution and latitude. Dietary sources are fish, fish oils, vitamin D-containing supplements and fortified foods. Either form of vitamin D is taken up by the liver and hydroxylated to 25-hydroxyvitamin D (25-OH-D), which is commonly used for assessing vitamin D status. 25-OH-D is further converted into the physiologically active form 1,25-dihydroxyvitamin D in the kidney. This hormone is necessary for ensuring that the concentration of calcium and phosphate in plasma is kept within narrow limits, through regulating calcium absorption in the intestine and by stimulating release of calcium from bone. Vitamin D is thus essential for a normal bone metabolism (NNR Project Group, 2013). The vitamin probably also plays a role in cell differentiation. Inadequate sun exposure or vitamin D intake may lead to rickets in children and osteomalacia (soft bones) in adults. It has been hypothesised that vitamin D deficiency may play a role in the development of several chronic conditions (Nasjonalt råd for ernæring, 2006).

A systematic literature review conducted by NNR concluded that there is convincing evidence that combined supplementation with vitamin D and calcium in adults is associated with reduced total mortality (NNR Project Group, 2013). The literature review further found suggestive evidence for an inverse association between vitamin D and some types of cancer (colorectal and breast cancer), and probable evidence for an inverse association between vitamin D status and increased coronary vascular disease risk (NNR 2012). The evidence for

[16] 1 retinol equivalent=1 µg retinol.

an association between vitamin D and type 1 and type 2-diabetes is limited and inconclusive (NNR Project Group, 2013).

Breastmilk contains very low concentrations of vitamin D (assessed as 25-OH-D) (0.1 to 1.0 µg/L) (Picciano, 2001). This is not sufficient to prevent rickets even if the mother takes a vitamin D supplement (NNR Project Group, 2013). Newborns have a store of vitamin D which depends on the mother's vitamin D status. However, infants born in countries at high latitudes, or in places where sun exposure is restricted for cultural or other reasons, are likely to be born with low vitamin D stores due to low maternal vitamin D status. During the first 6 weeks of life there is a rapid fall of serum 25-OH-D concentrations, to a range where there is a high risk of developing rickets (NNR Project Group, 2013).

To ensure adequate levels of vitamin D, NNR recommends that all infants from the first weeks of age receive a supplement of 10 µg/day (NNR Project Group, 2013).

According to Spedkost 2006-2007, 80% of the 6-month old infants were given vitamin D supplements, whereas this was the case for 67% of the 12-months old (Øverby et al., 2008; Øverby et al., 2009). A study from Oslo found that most of the ethically Norwegian 1-year old infants had adequate vitamin D status. Among breastfed infants, 34% had low serum levels of vitamin D (serum 25-OH-D levels <50 nmol/L) and among those not receiving breastmilk, 20% had low levels (Holvik et al., 2008). Madar *et al*. found that 47% of 6-weeks old infants living in Norway with mothers born in Pakistan, Turkey or Somalia, had sub-optimal vitamin D status (serum 25-OH-D <25 nmol/L) (Madar et al., 2009). Exclusively breastfed infants with no supplements had lowest vitamin D status, with an average 25-OH-D level of only 11 nmol/L. Breastfed infants who also received vitamin D supplements had an average level of 33.5 nmol/L 25-OH-D. The infants who received infant formula in addition to breastmilk, had an average level of 64.5 nmol/L 25-OH-D (Madar et al., 2009).

Vitamin E

Vitamin E is a generic term for four tocopherols and four tocotrienols, however, alpha-tocopherol is the only form which is recognised to meet human requirements (NNR Project Group, 2013). The main biological function of alpha-tocopherol has been suggested to be its antioxidant activity. In addition, several other important biological functions, including modulation of cell signalling and gene expression, are ascribed to vitamin E (NNR Project Group, 2013). Approximately 83% of the total vitamin E in breastmilk is present as α-tocopherol. One α-tocopherol equivalent (α-TE) equals 1 mg α-tocopherol. The concentration of tocopherols, which is high in colostrum (8 mg/L) decreases and stabilises in mature breastmilk (2-4 mg/L) (IOM, 1991). The NNR for tocopherol intake for infants is based on the relationship to the intake of PUFA and is set at a rate of at least 0.6 α-TE/g total PUFA with a mean intake of PUFA corresponding to 5% of the energy intake (NNR Project Group, 2013).

Vitamin K

Vitamin K is a group of fat-soluble vitamins which are essential for the modification of certain proteins, in particular proteins that are required for blood coagulation and bone metabolism. Maternal dietary intake has little influence on the breastmilk vitamin K concentration. The breastmilk concentration of vitamin K ranges from 0.85 to 9.2 µg/L (IOM, 1991). Based on this, NNR gives a recommended intake to infants at about 2 µg/kg bw/day (NNR Project Group, 2013).

3.3.5.2 Water-soluble vitamins

Thiamine

Thiamine (vitamin B_1) functions as a coenzyme in the metabolism of carbohydrates and proteins. There are large variations in the thiamine concentration in breastmilk between individuals and over the lactation period (IOM, 1991). Thiamine in breastmilk reflects the maternal intake, but seems to reach a plateau (Lawrence and Lawrence, 2010b). The average concentration in mature milk has been estimated to 0.2 mg/L (IOM, 1991). Recommended thiamine intake is given in relation to the energy intake, and is set to 0.10 mg/MJ for infants under 12 months (NNR Project Group, 2013).

Riboflavin

Riboflavin (vitamin B_2) is a central component of the cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which plays a key role in metabolism of energy, fat, carbohydrates and proteins. The riboflavin concentration in breastmilk is largely a reflection of the maternal diet and is about 0.35 mg/L in mature milk (IOM, 1991). Recommended riboflavin intake is also related to total energy intake, and is set to 0.14 mg/MJ (NNR Project Group, 2013).

Niacin

Niacin (also called vitamin B_3), in the form of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), participates in many biological redox reactions related to metabolism of energy, fats, carbohydrates and proteins. The niacin concentration in breastmilk is largely dependent on maternal intake, with an estimated average level at 1.5 mg/L (IOM, 1991). The recommended intake for infants and children above 6 months is 1.6 NE[17]/MJ (NNR Project Group, 2013).

Pyridoxine

Pyridoxine (vitamin B_6) functions as a coenzyme in the metabolism of fat, carbohydrates and proteins. The vitamin B_6 concentration in breastmilk varies with maternal B_6 status and intake, and besides vitamin D, it is the vitamin which is most likely to be deficient in the breastmilk (Lawrence and Lawrence, 2010b). In infants, vitamin B_6 deficiency may adversely influence growth (Butte N. et al., 2002). In well-nourished populations, breastmilk appears to maintain normal vitamin B_6 status in most exclusively breastfed infants during the first 6 months of age. The risk of vitamin B_6 inadequacy increases beyond 6 months (Butte N. et al., 2002). Breastmilk vitamin B_6 concentration is on average 93 mg/L in mature milk (IOM, 1991). The Nordic recommendation for vitamin B_6 intake is 0.015 mg/g protein (NNR Project Group, 2013).

Folate

Folate (also known as vitamin B_9) is essential for the production and maintenance of new cells and for DNA and RNA synthesis. It is especially important during periods of rapid cell

[17]NE, niacin equivalents; 1 NE=1 mg niacin=60 mg tryptophan.

division and growth, and is essential for production of normal red blood cells and to prevent anaemia. The folate concentration in breastmilk is relatively independent of maternal folate status (Picciano, 2001). A longitudinal study in Norwegian infants (84% breastfed at 6 months), showed increasing concentrations of serum folate from birth up to 6 months of age, with higher values than reported in older children or adults (Hay et al., 2008). At 6 months of age, serum folate was similar in the breastfed and the non-breastfed group, but exclusively breastfed infants had the highest concentrations, and serum folate was positively associated with exclusive breastfeeding. Between 6-24 months of age, the serum folate levels declined, probably due to the introduction of complementary foods. The authors concluded that their results support the view that exclusive breastfeeding for 6 months maintains adequate folate nutrition. The average breastmilk concentration of folate is about 85 µg/L (IOM, 1991). The Nordic recommendation for folate intake for infants is 5 µg/kg bw/day (NNR Project Group, 2013).

Vitamin B_{12}

Vitamin B_{12} plays a key role in normal functioning of the brain and nervous system and in the formation of blood. Vitamin B_{12} is also involved in DNA synthesis and regulation and fatty acid synthesis. The concentration of vitamin B_{12} in breastmilk from well-nourished mothers is about 0.4 µg/L (IOM, 1998). Severe vitamin B_{12} deficiency has been reported in infants nursed by mothers who were strict vegetarians (vegans), and evidence suggests that vitamin B_{12} deficiency early in infancy may cause lasting neurodisability (Picciano, 2001). The longitudinal study reported in the folate section also assessed vitamin B_{12} status (Hay et al., 2008). They found that vitamin B_{12} status in breastfed infants declined from birth to 6 months, and thereafter increased. Breastfed infants had lower B_{12}-status than non-breastfed infants. The authors concluded that low vitamin B_{12} status is a normal finding in breastfed infants and that further studies are required to determine the mechanisms for this finding (Hay et al., 2008). The Nordic recommendation for infants' vitamin B_{12} intake is 0.05 µg/kg bw/day (NNR Project Group, 2013).

Vitamin C

Vitamin C, or ascorbic acid, is an antioxidant and a cofactor in several enzymatic reactions, including several collagen synthesis reactions. The average vitamin C concentration in breastmilk is about 30 mg/L (IOM, 1991). It has been reported that the vitamin C concentration in mature breastmilk levels off at 50-60 mg/L if daily maternal intake is equal to or exceeds 100 mg. When maternal vitamin C intake is relatively low, increases in intake are associated with increased breastmilk concentrations (IOM, 1991). NNR recommends a vitamin C intake of 20 mg/day for infants 6-11 months (NNR Project Group, 2013).

3.3.6 Minerals

Calcium

Adequate calcium intake is important for normal bone mineralisation. The calcium concentration in breastmilk is on average 280 mg/L (IOM, 1991). The concentration is fairly constant during lactation and is not influenced by maternal diet (Butte N. et al., 2002). NNR recommend an intake of calcium of 540 mg/day for infants 6-11 months (NNR Project Group, 2013).

Iron

Iron is a component in a number of proteins, including enzymes involved in energy metabolism and immune functions, and haemoglobin. The concentration of iron in breastmilk decreases rapidly in early lactation and stabilises in mature milk at around 0.3 mg/L (IOM, 1991; Picciano, 2001). The iron concentration in breastmilk is unaffected by maternal iron status or diet (Butte N. et al., 2002). Iron deficiency is a main cause of anaemia. Iron deficiency anaemia in 6-24 months infants has been related to poorer cognitive and neuropsychological development (Lozoff, 2007). Iron deficiency anaemia is relatively common in Norwegian infants, with one study showing a prevalence of 10% among 12-month old children (iron deficiency anaemia defined as Hb <110 g/L in combination with ferritin <15 µg/L) (Hay et al., 2004).

The iron concentration in breastmilk is relatively low, and although the bioavailability and absorption is high, it is insufficient to meet the infant's requirements (Picciano, 2001). Normal weight, full-term infants are born with a body iron store sufficient to supply their needs for approximately 6 months (Butte N. et al., 2002). Thereafter, additional iron from complementary foods or supplements is needed to avoid iron deficiency (Butte N. et al., 2002). The time between delivery and cutting the cord from the placenta has significant impact on the newborn's blood volume and thereby the newborn's iron stores (McDonald and Middleton, 2008).

NNR does not provide recommendations for iron intake for infants less than 6 months, but states that breastmilk and infant formulas have been shown to cover the nutritional need for iron. NNR's recommendation for infants 6-11 months is 8 mg iron per day. As the iron concentration in cow's milk is low, it is recommended to use infant formula instead of cow's milk up to 12 months.

Zinc

Zinc is a component in a number of enzymes which take part in the maintenance of the structural integrity of proteins and in the regulation of gene expression. The concentration of zinc in breastmilk declines from 4-5 mg/L in early lactation to 1-2 mg/L 3 months postpartum and to about 0.5 mg/L at 6 months (Butte N. et al., 2002). The average concentration is estimated to be 1.2 mg/L (IOM, 1991).

Zinc concentrations in breastmilk are only marginally influenced by maternal zinc intake or status (Butte N. et al., 2002). The zinc concentration in breastmilk is insufficient to cover the infant's requirement, and prenatal body stores contribute to adequate zinc status during the first 6 months (Butte N. et al., 2002). Zink deficiency is related to impaired growth and reduced immune function. No Norwegian studies on zinc status in infants have been identified, but a Danish study showed that mean serum zinc concentration did not change significantly between two and 6 months, however, serum zinc concentrations then fell significantly between 6 and 9 months (Michaelsen, Samuelson et al., 1994). NNR's recommendation for zinc intake is 5 mg/day for infants 6-11 months (NNR Project Group, 2013).

Selenium

Selenium is a component of selenoproteins, several of which are enzymes with antioxidant functions. The selenium concentration in breastmilk is high in early lactation and decreases as lactation progresses, and reflects maternal selenium intake (IOM, 1991). Mean values in

mature milk (10 to 30 µg/L) differ geographically both within and between countries (IOM, 1991). Breastmilk selenium concentration is positively correlated with the infant's plasma concentration and the selenium-containing enzyme glutathione peroxidase (Picciano, 2001). NNR's recommendation for selenium intake is 15 µg/day for infants 6-11 months (NNR Project Group, 2013).

Iodine

Iodine is an essential component of the thyroid hormones which are involved in the regulation of various enzymes and metabolic processes that control growth and nervous system development. Severe iodine deficiency during fetal development and early childhood is associated with physical and cognitive impairment (Melse-Boonstra and Jaiswal, 2010).

Concentrations of iodine in breastmilk correlate directly with maternal intakes, with an estimated average concentration of 110 µg/L (IOM, 1991). Results from the Norwegian Mother and Child Cohort Study showed that among pregnant women who were not taking iodine containing supplements, 80% had an intake below the daily recommended intake (Haugen et al., 2008). A validation study among pregnant women showed that among those not taking iodine supplement,median urinary iodine excretion was only 110 µg/24 hours (Brantsaeter et al., 2007). Median urinary iodine excretion in pregnant women below 150 µg/day is regarded as insufficient (WHO et al., 2007). The iodine status of Norwegian infants has not yet been studied. NNR's recommendation for iodine intake is 50 µg/day for infants 6-11 months (NNR Project Group, 2013).

3.4 Immunological components in breastmilk

Knowledge about the immunological properties of mammalian milk can be traced back to 1892, when Paul Ehrlich showed that newborn mice were protected against the toxic effects of phytotoxins if they were fed milk from an immunised mouse (Ehrlich, 1892). Almost 80 years ago, Grulee *et al*. published a study on the morbidity and mortality of 20 000 infants, showing that infants who received breastmilk substitute had significantly higher risk of infections (respiratory, gastrointestinal or unclassified) as well as higher mortality rates than exclusively or partially breastfed children (Grulee et al., 1935).

Numerous studies over the last decades have contributed to our present knowledge of the immunological potential of breastmilk. The active components not only possess innate and adaptive defence properties, but may also aid the nursed infant's immune development, including generation of immunological tolerance with appropriately controlled inflammatory reactions. Together, such immunoregulatory properties may explain some of the long-term beneficial health effects observed in breastfed children.

Breastmilk can be regarded as a communication vehicle between the maternal immune system and the infant, actively instructing the newborn's immune and metabolic systems as well as the establishing of a beneficial commensal microbiota, while at the same time conferring multiple means of protection from pathogens (Field, 2005). Among such bioactive constituents are anti-microbial components, factors with anti-inflammatory properties, substances important for the active maturation of the infant's own immune system and factors promoting immunological tolerance (see Figure 3.3).

All these components depend on the mamamalian species, both in terms of concentration and characteristics that are determined by the genetic and environmental impact. For instance, the concentration of the anti-microbial protein lactoferrin is approximately 10 times higher in

human milk than in cow's milk (Fox and McSweeney, 1998; Goedhart and Bindels, 1994; Miranda et al., 2004). Similarly, there are striking species differences in milk lipids, both with regard to concentrations and the content of fatty acids in triglycerides (Devle et al., 2012). Likewise, the oligosaccharides in human milk differ from those in animal milk (Jenness, 1979; Jensen, 1995; Miller et al., 2000). Also the membranes of milk fat globules show species differences in protein and enzyme constituents (Pallesen et al., 2007; Pallesen et al., 2008).

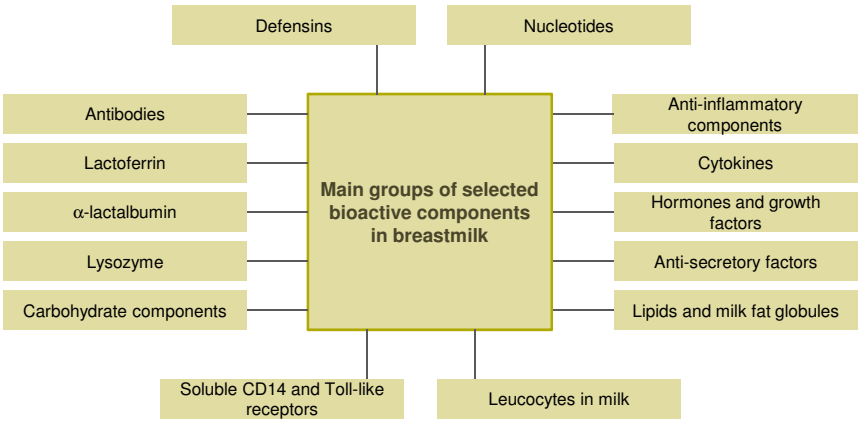

Figure 3.3: Main groups of selected bioactive components in breastmilk.

3.4.1 Selected immunological breastmilk components

Antibodies

The antibody defence system provided by human milk is well characterised and is of major importance to prevent infectious diseases in the newborn and infant and guides the early establishment of a balanced mucosal microbiota. This system will be described in more detail than other immunological components of breastmilk because we have extensive knowledge about its cellular-molecular functions and because it is quite distinct for the human species and represents a unique immunological integration between mother and child.

Antibodies are soluble proteins called immunoglobulins (Ig) because they are responsible for humoral immunity – that is, protection performed against antigens in blood and body fluids, including external secretions. Microbes and other foreign elements that can stimulate the immune system are collectively called *antigens*, and during the birth process the newborn begins to encounter such stimuli. Most of these immunological challenges take place at mucosal surfaces such as in the gut and airways.

The immune system of the newborn is immature, and at birth it is not prepared to meet the contaminated external environment, including the mother's vaginal and fecal microbiota. In humans, transplacental transfer of maternal antibodies confers systemic immune protection in blood and tissues over the first 2 to 3 months of life. However, these antibodies are of the immunoglobulin G (IgG) class which provides a type of defence that is proinflammatory in that it may activate complement (an enzyme system) and various phagocytes. Such immune reactions require energy and may cause symptoms such as fever, pain and loss of appetite.

Through breastmilk, the newborn is provided with another type of antibody that normally does not cause inflammation, namely immunoglobulin A (IgA) which is the predominant

antibody class in human colostrum and mature milk as well as in other external secretions. Locally produced IgA antibodies are actively exported to mucosal surfaces and are then known as secretory IgA (SIgA). The epithelial "IgA pump" is driven by a receptor-mediated mechanism (Figure 3.4 and Box 1.) which also transports small amounts of immunoglobulin M (IgM) antibodies to the mucosal surface. SIgA is mainly responsible for a humoral protection process called immune exclusion – initiating a first line of defence which uses the exported antibodies to bind bacteria and other potentially harmful compounds at the mucosal surface. SIgA antibodies coat pathogens to prevent them from invading the mucosal wall, and they form foreign cell fragments or macromolecules to regulate their passage into the body.

Newborn babies produce little or no SIgA during the first vulnerable months of life, so the only significant source of such antibodies during this period is breastmilk. Milk SIgA helps protect the gut and upper airways until the immune system of the infant has matured, and it is the most prominent and best studied bioactive component of human milk. In developed countries, the child's ability to produce SIgA is quite variable, being completed between 1 and 10 years of age. Infants in developing countries often establish secretory immunity much earlier, presumably because of greater exposure to immune-stimulating microbes.

In addition to their action of binding up troublesome antigens, SIgA antibodies protect the gut and upper airway mucosae by enhancing the barrier function of the epithelial constituting the surface lining. Although the mucosae of breastfed infants usually matures during the first months of life, the epithelial barrier function remains inadequate in some children for several years, and incomplete secretory immunity contributes to this delay.

Immune cells are diffusely woven into the fabric of the gut mucosae (*lamina propria*) rather than being restricted to one place, but there are also discrete structures for immune surveillance. Between the tiny villi that cover most of the intestinal tract are swollen domes called *Peyer's patches*. These regions, part of a larger system of *gut-associated lymphoid tissue,* or GALT, are covered by an epithelial-cell layer containing specialised M cells (the M stands for membrane or microfold) that constantly scan the stream of passing antigens in the gut lumen and transport them to the principal cell types of the immune system - B cells (from the bone marrow), T cells (from the thymus) and antigen-presenting cells (APCs) such as macrophages and dendritic cells. It is mainly in the GALT structures that mucosal immunity is induced and regulated.

What follows the identification of an antigen is a complicated ballet of cells, secreted molecular signals and migration of immune cells from one compartment of the body to another. The keys to the system are the APCs, the "decision makers" in the immune system. APCs process chunks of antigen brought in by M cells and then show the pieces, along with a selection of co-stimulatory signals, to so-called naïve (unexperienced) T cells, which have never met their cognate antigens before. Those specific T cells whose antigen receptors match one of the antigen pieces become primed or activated; they then release cytokines (hormone-like regulatory proteins) and growth factors that instruct B cells to proliferate, differentiate and become prepared for producing specialised mucosal IgA.

Activated T and B cells migrate from GALT structures to nearby lymph nodes (from the gut wall, to mesenteric lymph nodes) where they receive additional biological signals; most of those cells then enter the bloodstream (Box 1, Fig. 3.4). Many such cells will migrate ("home") to the *lamina propria* of the gut, but some also to the mammary glands of lactating mothers through a kind of chemical navigation system. This is the cellular-molecular basis for the immunological integration between mother and child.

At the secretory effector sites, depending on what sort of molecular "second signals" the B cells receive, they may undergo one last, or terminal, differentiation to become plasma cells, which produce antibodies in large quantity (about 10,000 molecules per second). However, very few B cells with IgA-producing capacity circulate in the blood of newborns, although this number is approximately 75 times higher after the first month of life, a period of continuous stimulation of GALT by microbial and other exogenous antigens.

One lactating mammary gland has on average the same antibody-producing capacity as one meter of adult intestine, and the large storage capacity for locally produced IgA in the mammary gland stroma, secretory epithelium and ducts explains the remarkable output of SIgA during feeding (Brandtzaeg, 1983). As illustrated in Fig 3.4, the breastfed child is supplied with maternal SIgA antibodies highly targeted against infectious agents (bacteria, viruses and fungi) and other exogenous antigens stimulating the mother's mucosae-associated lymphoid tissue (GALT and nasopharynx-associated lymphoid tissue, NALT), and comparable antigens are likely to be encountered by the infant (Brandtzaeg, 2003). Interestingly, the reactivity of SIgA antibodies may, in addition, reflect pathogens that the mother has been exposed to earlier in life.

SIgA is a remarkably stable antibody, being far more resistant to degradation by proteolytic enzymes than IgG antibodies which predominate in blood. Breastmilk SIgA concentrations are highest in colostrum and decrease in mature milk. Concentrations are particularly high in the breastmilk of mothers whose infants were born preterm. These phenomena illustrate how the composition of breastmilk varies over the course of lactation to meet the demands of the growing infant (Gross et al., 1981). The systemic humoral immunity of the newborn is well taken care of by the placental transfer of maternal IgG antibodies. However, these are catabolised at the same time as the maturation of the newborn's immune system develops gradually. Around 2-3 months of age, therefore, the systemic IgG antibody concentration of the baby is particularly low, highlighting the importance of a continuous supply of maternal SIgA from breastmilk until the infant's mucosal SIgA production has reached protective levels.

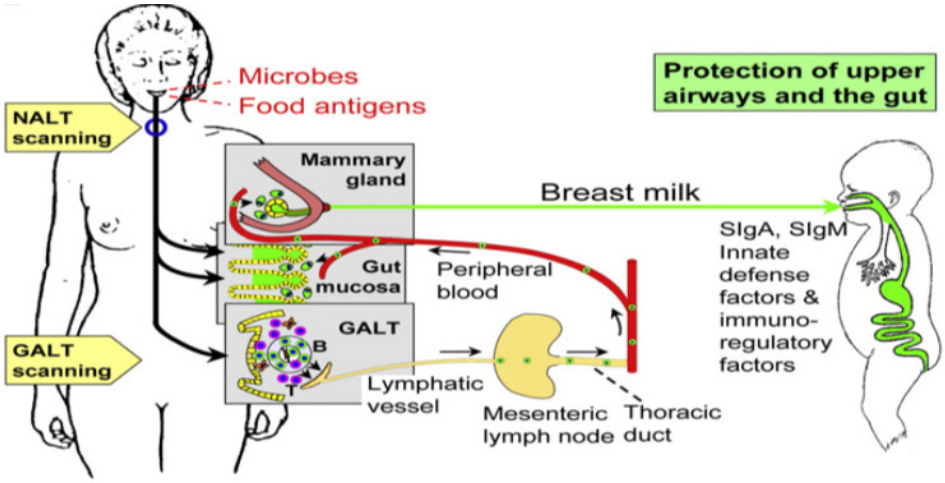

Figure 3.4: The enteromammaric and nasopharynx-mammaric links of activated antibody-producing cells. The figure illustrates the integration of mucosal immunity between mother and the newborn, with emphasis on migration of effector B cells (labelled B) from gut-associated lymphoid tissue, or GALT, via lymph and peripheral blood to the lactating mammary gland. Such distribution (arrows) beyond the gut of precursor B cells for local IgA-producing plasma cells is crucial to obtain export into breastmilk of secretory IgA (SIgA) (and smaller amounts of SIgM, not depicted) antibodies specific for enteric (and also airway) antigens; i.e. microorganisms and exogenous proteins stimulating GALT (and also nasopharynx-associated lymphoid tissue, or NALT). By this mechanism, the breastfed infant will receive relevant secretory antibodies directed against, for instance, the microorganism colonising its mucosae (reflecting the environment of the mother) and, hence, be better protected both in the gut and in the upper airways in the same way as the mother's mucosae are protected by similar antibodies (green areas) (adapted from Brandtzaeg, 2010b).

Box 1. Biology of the mucosal antibody system.

The mucosal IgA system constitutes the largest adaptive immune defence of our body in terms of the number of immune cells and quantities of antibodies produced. At mucosal surfaces these antibodies perform immune exclusion of exogenous antigens by inhibiting colonisation of pathogens and restricting the epithelial penetration of potentially harmful antigens such as dietary proteins and components of commensal bacteria (Brandtzaeg, 2010b). Lactating mammary glands are part of the integrated mucosal immune system, and milk antibodies reflect antigenic stimulation of mucosae-associated lymphoid tissue (MALT) both in the gut and airways (Brandtzaeg, 2003).

After stimulation in gut-associated lymphoid tissue (GALT), such as the Peyer's patches, naïve (unexperienced) B lymphocytes (also called B cells) become activated to memory/effector cells which migrate rapidly via lymphatics to mesenteric lymph nodes where some of them become further differentiated to precursors of antibody-producing plasma cells, or so-called plasmablasts. After reaching peripheral blood, the activated mucosal B cells, partially now being plasmablasts, are targeted for migration ("homing") particularly to the intestinal *lamina propria* by so-called gut-homing receptors. However, a fraction of the GALT-derived B cells will migrate from peripheral blood into distant secretory mucosae and glandular effector sites – notably also to the lactating mammary glands, referred to as "the enteromammaric link" (Figure 3.4). In this latter selective molecule-mediated extravasation from the blood circulation, GALT-derived B cells are joined by B cells that have been activated in nasopharynx-associated lymphoid tissue (NALT) such as the adenoids and palatine tonsils.

At all secretory effector sites, extravasated mucosal memory/effector B cells will undergo terminal differentiation to plasma cells (PCs) which mainly produce polymeric IgA (dimeres and some trimers), but also pentameric IgM. Polymeric antibody molecules contain a small polypeptide called "joining" (J) chain. In addition to stabilising the polymers by covalent bridges, the J chain facilitates their receptor-mediated transport by secretory epithelia. This external export of Ig polymers is mediated by the epithelial polymeric Ig receptor (pIgR), also known as membrane secretory component (SC). After the pIgR-mediated epithelial transport of locally produced IgA dimers/trimers and IgM pentamers to the luminal face of secretory epithelia, such as the lactating mammary glands, secretory (S)IgA and (S)IgM are released by apical cleavage of the pIgR – only the C-terminal receptor part remaining for degradation in the epithelial cell. The extracellular pIg-binding receptor domains are incorporated into the secretory antibodies as so-called bound SC, thereby providing protection against proteolytic degradation, particularly of SIgA where it becomes covalently integrated. Thus, the functional stability of SIgA antibodies in external secretions is remarkable (Brandtzaeg and Prydz, 1984; Brandtzaeg, 2010b; Brandtzaeg, 2011).

Lactoferrin

Lactoferrin is one of the major milk proteins. It is bactericidal against certain bacteria and has antiviral effects and acts against fungi such as *Candida albicans*. Like SIgA, lactoferrin decreases the risk of infections without the use of inflammatory mechanisms as it blocks production of proinflammatory cytokines. Lactoferrin is quite resistant to degradation in the gut and contributes to the maintenance of an effective intestinal barrier function. It promotes growth of beneficial bacteria and is immunostimulatory (Hanson, 2007; Hanson, 2004; Lonnerdal, 2003). Lactoferrin is an iron-binding protein that can bind two ferric ions at a time. It is believed that its ability to withhold iron from pathogens partly explains its anti-microbial ability.

α-Lactalbumin

α-Lactalbumin also exerts anti-microbial activity against bacteria and fungi (Lonnerdal, 2003). A more studied function of this protein is, however, its effect on malignant cells. After a specific reorganisation of the molecule, the so-called HAMLET is formed (Human α-lactalbumin Made LEthal to Tumor cells). In this form, it induces apoptosis of human malignant cells both *in vitro* and *in vivo* (Pavet et al., 2011; Svensson et al., 2000). It is not known whether this protein may partly explain why breastfeeding reduces the risk of maternal breast cancer. The suggested effect of human milk on childhood leukaemia may also be related to this protein.

Lysozyme

Lysozyme is a major part of the whey protein fraction of breastmilk (Lonnerdal, 2003). It may act alone or together with lactoferrin, SIgA and other anti-microbial components against gram-negative and gram-positive bacteria and probably also against viruses (Hosea Blewett et al., 2008).

Carbohydrate components

Carbohydrate components comprise oligosaccharides and also glycoproteins (e.g. lactoferrin, SIgA) and glycolipids (e.g. gangliosides). Oligosaccharides are produced by epithelial cells of the mammary gland. Because of their resistance to hydrolysis by gastrointestinal enzymes, they are present in the small intestine where they act against microbes by preventing them from attaching to the mucosal surface cells. Milk-derived oligosaccharides have been shown to protect infants from various pathogenic microbes including *E. coli*, Campylobacter species, *S. pneumonia* and *V. cholerae* (Hosea Blewett et al., 2008). This is yet another anti-microbial function of breastmilk components acting without inducing inflammation. Oligosaccharides may also act as prebiotics in the breastfed infant's gut by promoting the growth of beneficial bacteria, particularly bifidobacteria.

Fats and fatty acids

Fats and fatty acids in breastmilk (primarily medium-chain saturated, long-chain unsaturated fatty acids and monoacylglycerols) have been demonstrated to have antiviral, antiprotozoan and anti-microbial properties. The mechanisms are still not clear, but may be due to disruption

of the cell membrane of the microorganism or by changing intracellular pH (Hosea Blewett et al., 2008).

Cytokines

Cytokines are multifunctional glycoproteins involved in cell communication and immune system activation. They operate in networks and orchestrate the development and functions of the immune system. There are numerous cytokines as well as soluble receptors for cytokines in breastmilk (Garofalo, 2010). These cytokines have the potential to influence the maturation and development of immune cells in the infant (Brandtzaeg, 2010b). Some of the cytokines are considered as anti-inflammatory (such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10)), whereas others are proinflammatory (e.g. tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-8, IL-12). Also soluble receptors for cytokines, e.g. the receptor for TNF-α, may be anti-inflammatory.

In general, the concentration of cytokines varies widely among breastmilk samples, making it difficult to assess the roles of these factors (individually or together) in the development of the infant's immune system (Hosea Blewett et al., 2008). A recent review of published studies on the TGF-β levels in breastmilk suggested that this cytokine protects against allergy in breastfed infants and young children (Oddy and Rosales, 2010).

Hormones and growth factors

Hormones and growth factors are found in breastmilk in relatively high amounts. Most likely they act as signals from the mother to the infant, thereby having the potential to influence the developing immune system. Among the hormones are erythropoietin, prolactin, thyroid hormone and leptin, the latter being involved in appetite regulation. Growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF), growth hormone releasing factor (GH-releasing factor) and hepatocyte growth factor (HGF) are also present in breastmilk. Little is known about the activity of these components on the naïve immune system of an infant when ingested, but one suspect that they influence immune development and strengthen the barrier function of the gut epithelium (Hanson, 2004; Hosea Blewett et al., 2008).

Immune cells

Immune cells such as macrophages, neutrophils and lymphocytes are present with an activated phenotype in breastmilk after transepithelial migration from the stroma of the mammary gland. It is likely that the major role of neutrophils and macrophages in breastmilk is local defence of the mammary gland. Some maternal lymphocytes may be taken up through the gut wall of the infant, which is surprising because of the differences between the infant and the mother as to the tissue type molecules (HLA molecules). Interestingly, breastfed infants seem to be relatively tolerant to the mother's HLA molecules as shown after organ transplantation. The maternal lymphocytes may also have a function in compensating for the immature function of neonatal T cells and promote their maturation (Hanson and Korotkova, 2002). Experimental studies indicate that maternal lymphocytes may confer immunological information, such as an enhanced responsiveness to a vaccine (Hanson, 1998). Also B lymphocytes are present in breastmilk. Little is known about their potential role, but they may have an influence on the infant's immune system (Hosea Blewett et al., 2008).

3.4.2 Factors with anti-inflammatory properties

Inflammation provides amplification of systemic immune reactions, thereby aiding protection of the infant against invading pathogens. However, if this process is not controlled, it may cause harm to the healthy tissue. Breastmilk protects the infant from infection with no obvious unwanted effects, probably because its anti-inflammatory components such as SIgA antibodies ensure the development of effective and appropriate immune defence in the infant. Among the non-antibody components in breastmilk that may add to this effect are cytokines and their soluble receptors (IL-10, TGF-β, IL-1 receptor antagonist and soluble TNF-α receptors), lactoferrin, oligosaccharides and long-chain PUFAs (Hosea Blewett et al., 2008).

Also an excess of unoccupied cleaved IgA-transporting receptor (Box 1), so-called free secretory component (SC), is present in relatively large amounts in breastmilk (Brandtzaeg, 2002). Before membrane SC in phylogeny was exploited by the adaptive immune system as an epithelial polymeric Ig receptor, SC probably belonged merely to the innate immune system. Thus, both in its free and SIgA (or SIgM)-associated form, SC is adding to the efficiency of the secretory immune system.

3.4.3 Selected milk components important for the maturation of the infant's mucosal immune system

At birth, the structural components of the immune system are present both at the mucosal and systemic compartments, but adequate antibody responses need some time to develop due to the immaturity of the cellular elements involved. After contact with dietary and microbial constituents in the gut, the mucosal immune system will become more and more activated, depending on the environmental impact. Immune-modulating factors in breastmilk may contribute significantly to this development in the newborn (Brandtzaeg, 2010b). The presence of TGF-β, IL-6, IL-7 and IL-10 in breastmilk is of particular interest for immunological maturation and differentiation of IgA-producing cells. Interestingly, a fully breastfed infant has a thymus (which is crucial for the development of T cells) twice the size of a formula fed infant, possibly because breastmilk provides important signals to the infant's immune system such as cytokine IL-7. In breastfed children, the size of the thymus corresponds with the number of T cells, suggesting that breastmilk might have both a current and long-term immune-modulating effect on the developing cellular immune system (Jeppesen et al., 2004).

3.4.4 Establishment of the commensal intestinal microbiota

Before birth, the neonate is almost sterile. During a natural delivery, the principally sterile gastrointestinal tract is rapidly colonised with microbes from the mother's faeces and vagina. Colonisation of the newborn's mucosae with beneficial bacteria is what primarily drives the differentiation and balanced expansion of the immune system where homeostasis normally will prevail (Brandtzaeg, 2013). The indigenous gut microbiota is established during infancy, and this process is influenced by genetic, environmental, and dietary factors, as well as by the way of birth – whether vaginal or by caesarean section (Rautava and Walker, 2009).

The number of bacteria in the gut exceeds the total number of cells in our body by some ten times, and their total genes (the gut microbiome) are at least hundred times the human genome. The commensal bacteria communicate both with each other and with the host (Valeur et al., 2011). Among the dominating commensals of the gut in the early colonisation

process of healthy, vaginally delivered and breastfed infants are *Bifidobacterium* and *Enterobacteriaceae* (Eggesbo et al., 2011). These bacteria modulate the expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis and postnatal intestinal maturation (Brandtzaeg, 2003; Hooper et al., 2001; Neish, 2009).

Besides its importance for the general maturation of the child's adaptive defence mechanisms, exposure to microbes also aids the developing immune system to learn how to become tolerant to environmental constituents that it should not react against, i.e. together with relevant antigens the commensal microbiota prevents the infant from allergic and inflammatory diseases by its homeostatic anti-inflammatory effects (Brandtzaeg, 2010b). During this normal colonisation process, the mother provides protection against the microbes originating from herself by the earlier transplacentally transferred IgG antibodies and the breastmilk content of optimally targeted SIgA antibodies (the enteromammaric link, described in section 3.4.1).

Breastmilk influences the gut microbiota differently from infant formula (Collins et al., 2012; Diamond et al., 2011; Forsythe et al., 2012).

3.4.5 Induction of immunological tolerance

Mucosal immunity provides a first defence line that reduces the need for elimination of penetrating exogenous antigens by proinflammatory systemic immunity. The local immune system operates by two adaptive anti-inflammatory mechanisms:

- immune exclusion performed by SIgA and SIgM antibodies and
- immune suppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous antigens.

The latter strategy is called *mucosally induced tolerance* or simply "oral tolerance" when induced via the gut. These strategies may explain why persistent hypersensitivity to food proteins is relatively rare (except coeliac disease). Oral tolerance appears rather robust in view of the fact that more than a ton of food and drink may pass through the gut of an adult every year, including some 100 kg of protein - of which there is a daily absorption of 130-190 mg. After a meal, 3-10 ng/ml of the absorbed protein may be found circulating as intact molecules or in immune complexes.

The neonatal period is particularly critical, not only with regard to infections, but also in terms of immunological homeostasis and sensitisation to allergic disease. This is so because the epithelial barrier function and the immunoregulatory network are poorly developed. Notably, the development of immune homeostasis depends on the establishment of balanced mucosal microbiota as well as on adequate timing and dose of dietary antigens when first introduced (Brandtzaeg, 2010b).

Innocuous environmental proteins such as dietary antigens and components of the commensal microbiota can stimulate both production of SIgA antibodies and the induction of suppressive tolerance mechanisms via the gut or airways. Such mucosally induced hypo-responsiveness, that operates locally as well as systemically, probably explains why most individuals normally show no adverse immune reactions to persistent contact with harmless environmental and dietary proteins.

As an example, breastmilk contains SIgA antibodies to gluten which may partly explain the protective or delaying effect of breastfeeding on childhood coeliac disease shown in several

studies (Ludvigsson and Fasano, 2012). Similarly, breastmilk SIgA with reactivity against commensal bacteria may contribute to the apparent protective effect of breastfeeding on later development of inflammatory bowel disease (Gearry et al., 2010). Interestingly, receptors for milk SIgA antibodies on the infant's GALT epithelium can probably guide uptake of selected luminal antigens for induction of homeostatic immunity in the infant (Corthesy, 2010).

Successful induction of oral tolerance depends on the dose and timing of enteric exposure to potential allergens, immune-modulating microbial components and dietary factors, such as vitamin A and lipids. Strict allergen avoidance during pregnancy, lactation and early childhood to prevent food allergy in atopic families seems to be based on mythology rather than science (Brandtzaeg, 2010a). Small amounts of allergens continuously present in the gut may in fact be required to promote tolerance induction. Environmental antigens, including food proteins, present at low levels in breastmilk may contribute to oral tolerance induction in infancy (Brandtzaeg, 2010a). Also, some recent studies suggest that postponing solid foods beyond 4-6 months does not enhance immunological homeostasis but, on the contrary, may increase the risk of hypersensitivity to food (Fewtrell et al., 2011).

3.4.5.1 Prebiotics and probiotics in breastmilk

The high content of oligosaccharides in breastmilk serves as a prebiotic promoting the growth of beneficial lactic acid-producing bacteria (particularly bifidobacteria) and reducing anaerobic bacteria in the gut of breastfed infants. Probiotic bacteria of bifidobacterial species and lactobacilli can reportedly also occur in breastmilk (Abrahamsson et al., 2009), perhaps after being transported to the mammary glands from the gut lumen by dendritic cells (Donnet-Hughes et al., 2010). However, human milk contains a variety of bacterial genera, and the milk microbiome characterised by pyrosequencing of 16S ribosomal RNA genes is quite complex and not always stable over time in the same mother (Hunt et al., 2011). Notably, changes over lactation and an apparent impact of the mother's weight gain during pregnancy have been observed (Cabrera-Rubio et al., 2012). Thus, overweight mothers tended to have lower counts of *Bifidobacterium* group bacteria in their breastmilk which seemed to be related to higher levels of the suppressive cytokine TGF-beta2 and the anti-inflammatory endotoxin-inhibiting factor sCD14 as well as a lower level of the proinflammatory cytokine IL-6 (Collado et al., 2012). Altogether, the source of the milk microbiome and its potential effects on infant gut colonisation and neonatal immune homeostasis remain an intriguing research topic.

3.5 Summary of nutrients and immunological components in breastmilk

An infant who is exclusively breastfed the first 6 months of life has all its nutritional needs covered, with the exception of vitamin D, provided that the mother's diet fulfill all her nutritional needs during both pregnancy and the breastfeeding period. Therefore, the daily estimated requirement for each nutrient is derived from the nutrient's concentration in breastmilk multiplied with the average intake of breastmilk. Breastmilk is not a uniform fluid and the composition of nutrients varies by stage of lactation, the time of day and during a given feeding. The concentration of some nutrients also varies according to the mother's diet. The energy content of breastmilk varies, but has been estimated to about 690 kcal/L. The concentrations of proteins and carbohydrates are relatively stable, while the fat content has large variations. The fatty acid composition reflects the maternal diet. Breastmilk concentrations of most vitamins reflect maternal vitamin intake, while the concentrations of most minerals are not affected by the maternal diet, except for selenium and iodine.

The newborn infant is immunologically immature, but will through breastmilk be provided with maternal antibodies and innate defence factors as well as immunity-promoting components. This benefits health in childhood, and most likely, also later in life. The milk antibodies are targeted against potential pathogens and other antigens to which the mother's airway and gut mucosae have been exposed. These antibodies are mainly of the IgA class, being actively transported to the milk as secretory (S)IgA molecules, which are highly resistant to proteolytic degradation. The SIgA antibodies act at mucosal surfaces not only by inhibiting colonisation of pathogens, but are also dampening epithelial penetration of innocuous components such as dietary proteins and commensal bacteria. Contrary to circulating IgG antibodies which are potentially proinflammatory, SIgA functions without causing inflammation. Numerous other milk proteins, lipids, carbohydrate components, cytokines and immune cells may also contribute to the defence of the infant's mucosae. Moreover, maturation of the infant's immune system depends on contact with the immune modulating factors in breastmilk as well as dietary and microbial constituents in the infant's gut. Different components in breastmilk facilitate the establishment of a beneficial intestinal microbiota, which is decisive for induction of a balanced mucosal immune system, both with regard to the SIgA system and oral tolerance. Through all these mechanisms, breastfeeding represents an ingenious immunologic integration of mother and child.

4 Infant formula

Historical data and trends in the use of infant formula are presented in section 2.2.1. The sale of infant formula, at least to a certain degree, is inversely proportional to the prevalence and duration of breastfeeding in a population.

The recommended food for infants below 4 months who are not exclusively breastfed, is iron fortified, industrially produced infant formula (Norwegian Directorate for Health, 2001). The recommended age for introducing complementary food is 4 months for infants who receive infant formula. Exclusively breastfed infants who need extra food in the age period between 4 and 6 months, should in general be given semi-solid food (e.g. porridge) and not infant formula. However, if extra milk is needed besides breastmilk, infant formula is recommended and not regular cow's milk, up to the age of one year. (Norwegian Directorate for Health, 2001). The main reason for this recommendation is the risk of deficient iron intake due to low content of iron in cow's milk.

The majority of the infant formulas on the Norwegian market are cow's milk-based. The content of nutrients in infant formulas is adjusted to the physiological and nutritional needs of the growing infant. However, a number of immunologically protective/bioactive factors available in breastmilk will not be provided through infant formula. The Norwegian regulation of infant formula is based on the EU Directive for Infant Formula[18], and covers the composition, labelling, marketing and distribution of infant formula. The regulation has two objectives; ensuring that infant formulas are safe and cover the infants' nutritional needs and that breastfeeding is promoted and protected. The regulation gives minimum and maximum limits for nearly all nutrients in infant formulas and includes some of the provisions of the WHO Code (Norwegian Ministry of Health and Care Services, 2008).

4.1 Use of infant formulas in Norway

According to the Norwegian study Spedkost 2006-07, 18% of newborn infants received infant formula during the first week of life (Øverby et al., 2008). For most of the infants, this was a supplement to breastmilk since 98% of the infants were breastfed the first week. Infant formula or other milk products were introduced to 22% of infants below 3 months of age (Øverby et al., 2008). By 4 months of age, 36% and by 6 months 43% of the infants had been introduced to infant formula or other milk, as drink or in porridge (Table 4.1). Among the 36% of mothers who served infant formula by 6 months, the average frequency of serving the infant formula was 3.3 times per day (Øverby et al., 2008).

Table 4.2 shows the use of infant formula and cow's milk in the first year of life. Almost 60% of infants were introduced to infant formula as drink by the age of one year. Parents seem to largely follow the recommendation to delay the introduction of cow's milk. Spedkost 12 months also assessed the current diet of the child at 1 year of age, and found that 43% of the 1-year olds regularly consumed infant formula as drink.

[18]Commission Directive 2006/141/EC on infant formulae and follow-on formulae.

Table 4.1: Age at introduction of infant formula/other milk in the first 6 months of age, n=1986[1].

Infants's age at introduction	%	Cumulative %
< 3 months	22	22
3-3.9 months	6	28
4-4.9 months	8	36
5-5.9 months	5	41
6 months	2	43

[1]Adapted from Table 14 in Spedkost 6 måneder (Øverby et al., 2008).

Table 4.2: Age at introduction of infant formula/other milk in the first year of life (%), n=1635[1].

Infants's age at introduction	Infant formula as drink	Infant formula used in porridge	Cow's milk as drink	Cow's milk used in porridge	Cow's milk used for other cooking
< 6 months	34	30	<0.5	<0.5	1
6-9 months	21	20	4	4	19
10-12 months	8	2	50	20	47

[1]Adapted from Table 12 in Spedkost – 12 måneder (Øverby et al., 2009).

4.2 Content of nutrients in infant formulas

The composition of infant formula should serve to meet the particular nutritional requirements and to promote normal growth and development of the infants for whom they are intended (Koletzko et al., 2005). The nutrient concentration of formulas should not necessarily mimic the composition of breastmilk, since there are considerable differences in the bioavailability and metabolic effects of several of the nutrients (Koletzko et al., 2005).

The regulation of infant formula states that infant formula ready for consumption, shall contain no less than 60 kcal (250 kJ) and not more than 70 kcal (295 kJ) per 100 ml. It further defines minimum and maximum levels of nutrients (per 100 kJ or 100 kcal prepared formula) where applicable, as listed in Tables 4.3-4.5.

Regarding proteins, the regulation says that for an equal energy value the infant formula must contain an available quantity of each indispensable and conditionally indispensable amino acid at least equal to that contained in the reference protein, which is protein in breastmilk. The regulation states that the protein content needs to be kept between 1.8 and 3 g per 100 kcal. However, as noted in section 3.1, the protein content in breastmilk is adjusted to the changing requirements of the developing infant. Breastmilk protein concentration is high early in the lactation period (3.9 g/100 kcal), decreases to about 1.3 g/100 kcal in mature milk and further to 1 g/100 kcal at 6 months and later (Lawrence and Lawrence, 2010a; Lonnerdal, 2003). Protein levels in infant formulas are fixed and designed to meet protein needs when they are at their highest (in the first 1-2 months). The relationship between infants' protein intake and growth, satiety and regulation is complex, and more data and greater insight into

mechanisms have been called for before changing the current recommendations (Kalhan, 2009).

In addition to the nutrients in Table 4.5, the regulation also proposes levels of optional ingredients (e.g. taurine, fructooligosaccharides, galactooligosaccharides and various nucleotides).

The Norwegian Food Safety Authority monitors infant foods sold in Norway, including infant formulas. The project ”Analysis of nutrients in selected baby food products 2006/2008” analysed the concentrations of sugars, vitamins A and D, iron and calcium in five infant formulas from different producers (Gjevestad, 2007). The analysis showed that there was good correspondence between the values analysed and the amounts declared on the labels of the infant formulas.

Regarding sugars, the level of lactose per 100 g varied between 8.7 g and 11.7 g (based on declared information), which is above the minimum recommended level of 4.5 g/100 kcal. While the current regulation does not give any upper limit for lactose, the EU Scientific Committee on Food suggested in 2003 that the maximum limit of lactose should not exceed 10 g lactose/100 kcal (SCF, 2003). Four of the five formulas tested had a lactose level above this limit.

Levels of calcium in the formulas varied between 61 mg and 105 mg/100 kcal, and were within the limits of the regulation which says minimum 50 mg and maximum 140 mg/100 kcal. The iron concentrations varied between 0.8 mg to 1.1 mg/100 kcal which is also within the limits of 0.3 mg and 1.3 mg/100 kcal.

The concentration of vitamin A was between 68 µg and 100 µg/100 kcal, which was within the minimum and maximum limits of 60 µg and 180 µg/100 kcal. The analysed formulas’ concentrations of vitamin D varied between 1.8 µg and 2.7 µg/100 kcal while the limits are 1 µg and 2.5 µg/100 kcal. Based on results from this project, the Norwegian Food Safety Authority concluded that there was no need to take action against the enterprises in question (Gjevestad, 2007).

In a second phase of the project the content of vitamin A and D were tested in formulas at the end of their expiration date. The conclusion from the project was that there was no significant loss of vitamin A or D during shelf life (Bueso, 2009).

Table 4.3: Macronutrient concentrations in infant formula according to EU regulation.

Component	Required levels[1]		Nutrient labelling in a commonly used infant formula	Concentrations per L[3]
	Minimum	Maximum		
Energy, kcal/100 ml	60	70	67	650
Proteins				
Cow's milk proteins, g/100 kcal	1.8	3	1.8	15
Hydrolysed cow's milk protein, g/100kcal	1.8	3		
Soy protein isolates, g/100 kcal	2.25	3		
Total fat, g/100 kcal	4.4	6.0	5.3	33
Linoleic acid, g/100 kcal	0.3	1.2		
α-linolenic acid, mg/100 kcal	50	NS[2]		
Ratio linoleic/α-linolenic acid	5:1	15:1		
Lauric+mystiric acids, % of fat	NS	20		
Trans fatty acids, % of fat	NS	3		
Euric acid, % of fat	NS	1		
Total carbohydrates, g/100 kcal	9.0	14.0	11.1	74
Lactose, g/100 kcal	4.5	NS	11.1	

[1]Compositional requirements in regulation on infant formula and follow-on formula (Norwegian Ministry of Health and Care Services, 2008).
[2]NS=Not stated.
[3]Source: Norwegian Food Composition Table, 2001.

Table 4.4: Vitamin concentrations in infant formula.

Component	Required levels[1]		Nutrient labelling in a commonly used infant formula	Concentrations per L[7]
	Minimum	Maximum		
Vitamin A, µg RE/100 kcal[2]	60	180	101	590
Vitamin D, µg/100 kcal[3]	1	2.5	1.4	10
Thiamin, µg/100 kcal	60	300	112	400
Riboflavin, µg/100 kcal	80	400	209	900
Niacin, µg/100 kcal[4]	300	1500	879	5000
Pantotenic acid, µg/100 kcal	400	2000	939	
Vitamin B_6, µg/100 kcal	35	175	77	600
Biotin, µg/100 kcal	1.5	7.5	2.2	
Folate, µg/100 kcal	10	50	14	60
Vitamin B_{12}, µg/100 kcal	0.1	0.5	0.4	1
Vitamin C, mg/100 kcal	10	30	16	50
Vitamin K, µg/100 kcal	4	25	8	
Vitamin E, mg α-TE[5]/100 kcal	0.5[6]	5	1.3	8

[1]Compositional requirements in regulation on infant formula and follow-on formula (Norwegian Ministry of Health and Care Services, 2008).
[2]RE=all trans-retinol equivalent.
[3]In the form of cholecalciferol, of which 10 µg=400 i.u. of vitamin D.
[4]Preformed niacin.
[5]α-TE=d-alfa-tocopherol equivalent.
[6]0.5 mg α-TE/1 g linoleic acid (18:2 n-6); 0.75 mg α-TE/1 g α-linolenic acid (18:3 n-3); 1.0 mg α-TE/1 g arachidonic acid (20:4 n-6); 1.25 mg α-TE/1 g eicosapentaenoic acid (20:5 n-3); 1.5 mg α-TE/1 g docosahexaenoic acid (22:6 n-3).
[7]Source: Norwegian Food Composition Table, 2001.

Table 4.5: Concentrations of minerals, trace elements and other substances in infant formula.

Component	Required levels[1]		Nutrient labelling in a commonly used infant formula	Concentrations per L[4]
	Minimum	Maximum		
Minerals and trace elements				
Sodium, mg/100 kcal	20	60	25	150
Potassium, mg/100 kcal	60	160	101	640
Chlorine, mg/100 kcal	50	160	70	
Calcium, mg/100 kcal	50[2]	140	64	410
Phosphorus, mg/100 kcal	25[2]	90	36	200
Magnesium, mg/100 kcal	5	15	8	40
Iron (formula based on cows' milk protein and protein hydrolysate), mg/100 kcal	0.3	1.3	1.0	8
Iron (formula based on soy protein isolate), mg/100 kcal	0.45	2.0		
Zinc, mg/100 kcal	0.5	1.5	1.0	5
Copper, µg/100 kcal	35	100	77	0.4
Iodine, µg/100 kcal	10	50	19	
Selenium, µg/100 kcal	1	9	2	
Manganese, µg/100 kcal	1	100	22	
Fluoride, µg/100 kcal	NS	100		
Other substances				
L-carnitine, mg/100 kcal	1.2	NS[3]	1.5	
Choline, mg/100 kcal	7	50	18	
Inositol, mg/100 kcal	4	40	15	

[1]Compositional requirements in regulation on infant formula and follow-on formula (Norwegian Ministry of Health and Care Services, 2008).
[2]The calcium:phosphorus ratio shall not be less than 1 nor greater than 2.
[3]NS=not stated.
[4]Source: Norwegian Food Composition Table, 2001.

4.3 Summary of infant formula

Although breastmilk is the main recommended food for infants in Norway, infant formula is recommended under some conditions. If breastfeeding is not possible or if there is a need for more milk in addition to breastmilk, infant formula, and not cow's milk, is recommended until 12 months of age. Data from the national dietary survey among infants (Spedkost) showed that at 6 months of age, 43% of the infants had been introduced to infant formula, and 36% used it regularly. At 1 year of age, 43% of the infants were given infant formula regularly.

Infant formulas in Norway are subject to regulations which cover the composition, labelling, marketing and distribution of infant formula. Theregulations are in line with relevant EU directives. The regulations give minimum and maximum limits for nutrients in infant formulas and include some of the provisions of the WHO Code. Monitoring of the nutrient levels of some selected nutrients by the Norwegian Food Safety has not revealed any serious deviations neither from the allowed nor from the declared nutrient content.

The content of nutrients in infant formulas is adjusted to the physiological and nutritional needs of the growing infant. However, a number of immunologically protective and bioactive factors available in breastmilk are not provided through infant formula.

5 Contaminants and other undesirable compounds in breastmilk and infant formula

Compounds that can be hazardous to health may be present both in breastmilk and in infant formula. The purpose of this chapter is to describe the presence of specific groups of substances in breastmilk and infant formula, the ability of these substances to cause adverse health effects, and the exposure level which can be regarded as safe. Furthermore, concentrations in breastmilk and infant formula in Norway have been included when available; otherwise concentrations reported in Nordic or other European countries are given. This has been done to facilitate a comparison of concentrations in breastmilk and infant formula.

Environmental contaminants are mostly present in breastmilk as a result of man-made emissions from industry and other activity, but some also occur naturally. All contaminants which are present in breastmilk can in principle be present in formula, and vice versa. However, depending on the substance or class of substance, the concentrations found in breastmilk and infant formula can be quite different. Humans are exposed to low levels of an increasing number of chemicals, and taking improved analysing methodologies into account, a range of these substances may potentially be found at low levels in breastmilk and infant formula. This chapter has been restricted to descriptions of a) substances that were included as criteria in the literature search for negative health effects of breastfeeding (Table 7.4, substances described in sections 5.1 and 5.2), and b) substances for which there were available studies on occurrence in infant formula, based on findings in a literature search performed on contaminants in infant formula (literature search described in Appendix 6, substances described in sections 5.3 and 5.4). Possible microbiological hazards in infant formula are described in section 5.5.

The risks from exposure to contaminants have been assessed by international risk assessment bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the EFSA. The short description of contaminants considered relevant for breastmilk and infant formula is based on the most recent international risk assessments. For some substances a tolerable daily intake (TDI) has been set. A TDI is the amount of a substance, or substance group, which can be consumed safely throughout a person's lifetime without appreciable risk of adverse health effects[19].

Many of the contaminants discussed below accumulate in the body, causing detectable effects only when a certain concentration has been reached. Therefore, variations in daily intake have little significance and, as a result, tolerable intake levels are sometimes expressed on a weekly (TWI) or even monthly basis.

Tolerable intakes have been derived from studies in humans when sufficient quantitative data have been available. If that is not the case, data from studies in laboratory animals have been used. If the tolerable value has been derived from animal studies, data from the most sensitive animal species and the most critical effect have been used. Uncertainty factors (also called assessment factors) have been used to account for uncertainty in the extrapolation of the data derived from animals to humans and to account for variations among humans. Usually, a default factor of 10 is used for extrapolation from animals to humans and a factor of 10 is

[19]WHO (1994) has defined adverse effect as follows: ”A change in morphology, physiology, growth, development and life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase susceptibility to the adverse effects of other environmental influences. Decisions on whether or not any effect is adverse require expert judgement.”

used for variation among humans (100 altogether). Tolerable intakes derived from large-scale epidemiological studies encompassing the most sensitive segment of the population justify the use of small uncertainty factors. This approach for deriving tolerable intake levels assumes that the adverse health effects are of such a nature that there is a dose threshold for effect, i.e. that a certain amount of exposure must be reached before adverse health effects occur.

Two different methods have been used to express dose threshold: 1) the no adverse effect level (NOAEL), which is the highest dose without any adverse effect, or 2) benchmark dose lower bound (BMDL), which is the 95-percentile lower confidence limit of the benchmark dose (BMD). The BMD is a dose associated with adverse effect in 5% or 10% of the animals, derived from a modelled dose-response curve. The tolerable intake levels are derived by dividing the NOAEL or BMDL by the uncertainty factor (usually 100).

It follows from the above that the tolerable intake level is not a threshold for toxicity above which adverse or toxic effects will instantly occur. The level is conservative and has, as a general rule, built-in safety margins. Consequently, when the level is moderately exceeded, the safety margin is eroded.

Some chemical compounds are considered to be carcinogenic. In a large-scale programme spanning many years, the International Agency for Research on Cancer (IARC), an agency of the WHO, has assessed the carcinogenic potential of chemicals, work processes and exposures. IARC classifies chemicals and exposures according to the quality of scientific evidence of carcinogenicity. The most solid evidence comes from studies in humans that unambiguously show carcinogenic effects. Chemicals with evidence of carcinogenicity in laboratory animals are generally regarded as possibly or probably carcinogenic to humans, unless the mechanism is not relevant to humans. The ability of a substance to damage genetic material (to be genotoxic) is assessed as part of the basis for the classification. The classification system operates with the following groups:

Group 1: The agent is carcinogenic to humans.

Group 2A: The agent is probably carcinogenic to humans.

Group 2B: The agent is possibly carcinogenic to humans.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

Group 4: The agent is probably not carcinogenic to humans.

IARC does not assess carcinogenic potency. For carcinogenic substances that are toxic to genetic material and cause cancer via genotoxic mechanisms, it is not possible to identify a dose-threshold under which there is no risk. In other words, any exposure down to zero may result in a theoretical risk. In such cases, various methods have been used for quantitative extrapolation. In Norway and some other countries, such as the USA, linear extrapolation from a dose that causes cancer in a certain percentage of the laboratory animals down to a negligible lifetime risk, e.g. 10^{-5}, has been used. Methods like these most probably result in conservative risk estimates. In recent years, a margin of exposure (MOE) approach has been used. The MOE specifies the margin between current human exposure and the lower confidence level of the exposure causing e.g. cancer in 10% of the laboratory animals ($BMDL_{10}$). The assessment of margins considered to be tolerable is comparable to the assessments conducted using linear extrapolation.

5.1 Metals

5.1.1 Lead (Pb)

The human body is exposed to lead through diet, drinking water and air. The metal accumulates in several tissues and organs of the body, but first and foremost it is stored in bone. The bone lead stores can be mobilised together with calcium during pregnancy and lactation. Lead in blood is considered to be the best indicator of the concentration of lead in soft tissues, reflecting recent and, to some extent, past exposure. During pregnancy lead easily crosses the placenta. Long-term, low level exposure to lead is associated with impaired neurodevelopment in children. Both EFSA (2010) and JECFA (2011) identified developmental neurotoxicity and reduction of the Intelligence Quotient (IQ) in children up to the age of 7 as the most critical effect of lead exposure, and foetuses, infants and children are considered to be the most sensitive subgroups for lead exposure (EFSA, 2010b; JECFA, 2011). EFSA assessed the risk of reduction of IQ and chose a $BMDL_{01}$ of 12µg lead/L blood as reference point for the risk characterisation of lead to children. This concentration corresponds to an average daily dietary intake of lead of 0.5 µg/kg bw. According to EFSA and JECFA, a small IQ-decrease may be viewed as having a small impact for an individual child, but is considered important as a reduction in a population's IQ. Since no threshold for this critical lead-induced effect was found, a tolerable intake has not been established. It was concluded by EFSA that the MOEs were such that a possibility for effects from lead in some consumers, particularly in children from one to 7 years of age, cannot be excluded.

Generally, the concentration of lead in blood in European populations has decreased dramatically over the last three decades due to prohibition of use of lead in petrol, paint and seams of tinned food. The geometric mean or median concentrations of lead in blood in recent Norwegian studies are from 11 to 27 µg/L, which is in the same range as studies in most European countries the last 10 years. Median concentration of lead in blood in pregnant Norwegian women sampled in 2003-2004 was 11µg/L (VKM, 2013).

Data on lead in breastmilk samples from Norway have not been found. Swedish breastmilk samples have shown lead concentrations of 0.5 ± 0.3 µg/L (Hallen et al., 1995). Several studies indicate that breastmilk concentrations are less than 5% of the concentrations in mother's blood (Gulson et al., 1998). In their opinion on lead, EFSA used a mean lead concentration in breastmilk of 1.6 µg/L based on a study from Austria and concluded that the risk from lead in breastfed infants is likely to be low since their exposure is predicted to be below 0.5 µg/kg bw (EFSA, 2010b).

Both infant formula powder and water can contribute to the lead content in ready-to-drink infant formula. According to the EFSA-opinion, the concentration in ready-to-drink infant formula was calculated to be 2-4.7 µg/kg. Thus, infant formula might contain up to three times the lead concentration of breastmilk. In the EFSA opinion, lead exposure based on lower bound[20] assumptions in both average and high 3-month old infant consumers of infant formula was below 0.5 µg/kg bw, but may exceed this level, based on upper bound[21] estimates. Therefore, EFSA concluded that the possibility of a risk to infants consuming infant formula cannot be excluded.

[20]Lower bound: Values below LOD or LOQ are set to zero.
[21]Upper bound: Values below LOD or LOQ are set equal to the LOD or LOQ.

5.1.2 Mercury (Hg)

Mercury is released into the environment from both natural and anthropogenic sources. Once released, mercury undergoes a series of complex transformations and cycles between atmosphere, ocean and land. The three chemical forms of mercury are (i) elemental or metallic mercury (Hg^0), (ii) inorganic mercury (mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}) cations) and (iii) organic mercury. Methylmercury is by far the most common form of organic mercury in the food chain, and after oral intake, methylmercury is much more extensively and rapidly absorbed than mercuric and mercurous mercury. Seafood is a main dietary source of both inorganic mercury and methylmercury exposure.

Methylmercury

Methylmercury accumulates in the body and crosses the placenta- and blood-brain barriers. Total mercury in hair and blood are routinely used as biomarkers of methylmercury exposure.

Unborn children constitute the most vulnerable group for developmental effects of methylmercury exposure. EFSA recently reduced the TWI for methylmercury from 1.6 to 1.3 µg/kg bw/week, expressed as mercury, based on recent findings of neurodevelopmental effects in children at slightly lower methylmercury exposure than previously reported (EFSA, 2012a). EFSA calculated that mean exposure in Europe is below the TWI, whereas 95-percentile exposure is in the range of or exceeding the TWI. This was confirmed by biomonitoring data.

The mean concentrations of methylmercury in breastmilk in a limited number of European studies were used for exposure assessment in EFSA. EFSA found that based on mean concentrations of methylmercury in breastmilk, the dietary exposure to methylmercury for 3-month old infants (6.1 kg) with an average daily breastmilk consumption (800 ml), ranged from 0.09 to 0.62 µg/kg bw per week and for infants with high milk consumption (1200 ml) the dietary exposure ranged from 0.14 to 0.94 µg/kg bw per week. Since these exposures are below the TWI, the exposure to methylmercury from breastmilk was considered not of concern (EFSA, 2012a). Since the exposure was estimated based on limited data, higher exposure to breastfed children in Europe cannot be excluded. Data on methylmercury in breastmilk from Norway is not available.

Data on methylmercury in infant formula have not been found. In general, all mercury in other food groups than fish and other seafood is believed to be inorganic.

Inorganic mercury

The kidney is sensitive to inorganic mercury toxicity. Inorganic mercury is also toxic to the liver, the nervous system and the immune system, and is also a reproductive and developmental toxicant.

EFSA recently established a tolerable intake of inorganic mercury of 4.0 µg/kg bw/week, expressed as mercury (EFSA, 2012a). This was in line with the evaluation from the JECFA in 2010. EFSA found that based on mean concentrations of inorganic mercury in breastmilk in Europe, the dietary exposure to inorganic mercury for 3-month old infants (6.1 kg) with an average daily breastmilk consumption (800 ml), ranged from 0.17 to 1.29 µg/kg bw per week and from 0.25 to 1.94 µg/kg bw per week for infants with a high milk consumption (1200 ml). Since these exposures are below the TWI, this was not considered to be of concern (EFSA, 2012a). Since the exposure was estimated based on limited data, higher exposure to breastfed

children in Europe cannot be excluded. Data on inorganic mercury in breastmilk from Norway are not available.

Only two surveys reported consumption data for infants to EFSA, and mean middle bound[22] exposure from the diet, including milk formula, was 0.74 and 0.80 µg/kg bw/week, which is lower than the TWI.

5.1.3 Cadmium (Cd)

Cadmium is a heavy metal found as an environmental contaminant both through natural occurrence and from industrial and agricultural sources. Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. IARC has classified cadmium as a human carcinogen (Group 1). EFSA established a tolerable weekly intake (TWI) for cadmium of 2.5 µg/kg bw in 2009, and confirmed this in 2011 (EFSA, 2009a; EFSA, 2011e). The mean exposure for adults across Europe is close to, or slightly exceeding, the TWI.

According to EFSA, cadmium concentrations in human milk are 5-10% of levels in blood. A higher maternal cadmium exposure during pregnancy and lactation results in a higher fetal and infant exposure, although at lower concentrations than maternal levels. The intestinal absorption of cadmium during infancy is most likely considerably higher than in adults. No data on cadmium in breastmilk in Norway have been found. Swedish breastmilk samples have shown cadmium concentrations of 0.06 ± 0.04 µg/L (Hallen et al., 1995). Infant formula on the Swedish marked based on cow's milk contained 0.08 to 0.39 µg/L, whereas 1.17 µg/L was found in formula based on soy protein (Ljung et al., 2011).

A higher gastrointestinal absorption in combination with consumption of infant formula composed by ingredients with higher cadmium content than breastmilk (e.g. wheat or soy) may lead to increased internal cadmium exposure in these infants (Eklund and Oskarsson, 1999). Cadmium from drinking water would come in addition.

5.1.4 Other metals

In a recent paper from Sweden, the content of essential and toxic elements in infant formulas was analysed (Ljung et al., 2011). The daily intakes of manganese and molybdenum were much higher from infant formulas than the intake from breastmilk at the ages of 1 and 4 months. The daily intake of manganese from infant formula varied from ten up to several hundred times the intake of a breastfed infant. The authors criticise the acceptable upper limit of manganese in infant formula, arguing that it does not consider the increasing evidence of neurotoxicity in children. The arsenic concentration was higher in some hypoallergenic formulas for special medical purposes than in breastmilk and in infant formula based on cow's milk. Also aluminum has been shown to be present at higher concentrations in infant formula than in breastmilk, and particularly in soy-based formula. The aluminum intake from infant formula (in particular soy-based formula) may exceed tolerable weekly intake of 2 mg/kg bw set by JECFA in 2012 (JECFA, 2013).

[22]For samples below LOQ, the concentration has been set to half of the LOQ.

5.2 Halogenated organic compounds

A large number of substances in the group of chlorine-, fluorine-, or bromine-substituted organic compounds can represent a hazard to human health. This applies to polychlorinated dibenzodioxins and furans (PCDD/Fs denoted dioxins), PCBs, campheclor (toxaphene), DDT and its metabolites (DDD and DDE), chlordane, dieldrine, aldrin, endrin, heptachlor, HCB, chlorinated cyclohexane, brominated flame retardants such as PBDEs and fluorinated compound such as PFOS and PFOA. Some of these compounds occur as by-products of industrial production (PCDD/Fs), in products (PCBs and PBDEs) or from past use as pesticides. They are fat-soluble and persistent to degradation, they bioaccumulate and are biomagnified in the environment. They are found in the highest concentrations in organisms located high up in the food chain. Fat of animal origin, and in particular fat of marine origin, is the major exposure source. Most of these compounds are no longer in use. Cleaning of industrial emissions has been implemented, and as a result, the levels in the environment are generally declining.

The situation is different for the fluorinated compounds. These substances have been widely used for decades because of their water and oil repellent abilities, but this did not gain much attention until approximately 10 years ago. Although substances in this class are not fat-soluble, they are persistent and the highest concentrations are found in organisms high up in the food chain. A summary of the features of the health hazards represented by the most relevant compounds is given below.

5.2.1 Dioxins and polychlorinated biphenyls (PCBs)

Dioxins and PCBs are closely related groups of chlorinated organic compounds. They are fat-soluble and persistent to degradation, they bioaccumulate and are biomagnified in the environment. They are found in the highest concentrations in organisms located high up in the food chain. Fat of animal origin, and in particular marine fat, is the major exposure source.

The term ”dioxins” usually encompasses both the 75 chlorinated dibenzo-p-dioxins (PCDDs) and 135 chlorinated dibenzofurans (PCDFs). There are 209 different PCB congeners. The chemical properties and toxicological effects of dioxins and PCBs vary according to the number and positions of the chlorine atoms on the aromatic rings.

Of the 209 possible PCB congeners, 12 are included in the group of dioxin-like PCBs (dl-PCBs) and are evaluated together with the dioxins, since they share mechanism of action with the most toxic dioxins. The rest of the PCBs are referred to as non dioxin-like PCBs (ndl-PCBs).

5.2.1.1 Dioxins and dioxin-like PCBs

The toxicity of 17 dioxins and 12 dl-PCBs is related to binding and activation of the transcription factor Ah (aryl hydrocarbon) receptor, also known as the TCDD or dioxin receptor. These substances have been assigned toxic equivalency factors (TEF) in relation to 2,3,7,8-TCDD, which is the most potent dioxin congener and has a TEF of 1. The total amount of toxic equivalents (total TEQ) in a sample is calculated by multiplying the concentration of each congener with the associated TEF and then adding up the contributions from the different congeners. The total TEQ in a sample is an estimate of the total dioxin effect, which is a simplified method for making risk assessments of dioxin/PCB-mixtures. The WHO-TEFs were set in 1998 (WHO_{1998}-TEF) and revised in 2005 (WHO_{2005}-TEF).

Abnormal activation of the Ah-receptor may disrupt cell function by altering the transcription of a vast array of genes whose activities are involved in a number of processes, including growth regulation and development. The most significant hazardous effects on health resulting from chronic exposure to dioxins and dl-PCBs are impairment of the reproductive system, a weakened immune system, impairment of the endocrine system and neurotoxic and carcinogenic effects. Dioxins have been classified as carcinogenic to humans (Group 1) by IARC (IARC, 1997), but they are not genotoxic (JECFA, 2001; SCF, 2001). The critical effect used in risk assessments was the reproductive effects in rats. Risk assessments performed by SCF and JECFA took into account the large difference in biological half-life of TCDD between rats and humans (i.e. about one month versus 7.5 years), the insufficiency of the toxicological database, and limited knowledge about the variation in the biological half-lives in different population groups. The TWI established by SCF is 14 pg TE/kg bw/week (SCF, 2001). JECFA's assessment is comparable with that of SCF, except that JECFA expresses the tolerable intake level on a monthly basis (70 pg TE/kg bw/month) (JECFA, 2001).

Levels and trends for dioxins and dl-PCBs in breastmilk and infant formula

From 1986 to 2005 the concentration of dioxins and dl-PCBs in breastmilk from Norway decreased by almost 70% (Figure 5.1).

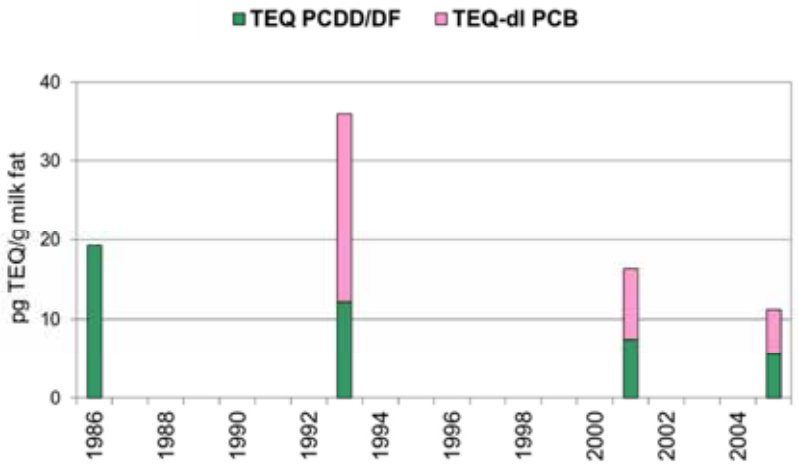

Figure 5.1: Dioxins and dl-PCBs in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breastmilk (pooled data from 10-50 mothers per year) Source: "Morsmelk og miljøgifter faktaark", Norwegian Institute of Public Health based on Becher *et al*., 1995; Becher *et al*., 2002; NILU, 2013; Stigum *et al*., 2005.

Individual breastmilk samples from HUMIS (n=27) collected in 2003-2005 are in the same range as the results from 2005 in Figure 5.1, and show in addition inter-individual variations in contamination levels from 4.6 to 18 pg WHO_{2005} TEQ/g lipid for dioxins and dl-PCBs. Mean level was 11 pg WHO_{2005} TEQ/g lipid (Stigum et al., 2005).

The maximum level (ML) of dioxins and dl-PCBs in infant formula is 0.1 pg WHO_{2005}-TEQ/g wet weight for dioxins and 0.2 pg WHO_{2005}-TEQ/g wet weight for the sum of dioxins

and dl-PCBs, as laid down in the EU regulation for MLs for dioxins, dl-PCBs and ndl-PCBs in foods[23].

The occurrence of dioxins and dl-PCBs in six samples of infant formula sold in Norway in 2008 showed a mean UB concentration of 0.37 pg WHO_{2005}-TEQ/g fat (range 0.17-0.55 pg WHO_{2005}-TEQ/g fat) (personal communication[24]). European samples of liquid and powder infant and follow-on formula showed upper bound levels maximally up to 0.06 pg WHO_{2005}-TEQ/g wet weight for the sum of dioxins and dl-PCBs (EFSA, 2013a). Provided a fat concentration of 3.5% in infant formula, the ML corresponds to 5.7 pg/g fat, whereas the highest upper bound level reported to the EU was 1.71 pg/g fat.

5.2.1.2 Non dioxin-like PCBs

The presence of non dioxin-like PCBs (ndl-PCBs) is often expressed as the sum of three PCB congeners (PCB-138, -153 and -180) or PCB-6 (PCB-28, -52, -101, -138, -153, -180), or as PCB-7, which in addition to PCB-6 includes PCB-118 (a dl-PCB). Sometimes the PCB concentration is expressed as total PCBs. PCB-153 is often used as an indicator of total PCB or PCB-6 because the correlation between PCB-153 and PCB-6 is usually high.

IARC has classified PCBs in Group 2A, i.e. probably carcinogenic to humans. There are reasons to believe that it is the dl-PCBs (and not the ndl-PCBs) that are responsible for the carcinogenic effect (EFSA, 2005a). In epidemiological studies, the most important adverse health effects associated with exposure from food and the environment were related to perinatal PCB exposure and the impairment of reproduction, including delayed development of the central nervous system and an impaired function of the immune system. It has not been possible to distinguish between the effects resulting from dioxins and dl-PCBs and the effects resulting from ndl-PCBs. This is because exposure to ndl-PCBs is normally highly correlated with exposure to dioxins and dl-PCBs. Furthermore, in many experimental studies the PCB test substance has been contaminated with dioxins. As a result, EFSA concluded that it is not possible to establish a tolerable intake level for ndl-PCBs (EFSA, 2005a).

Neurotoxic effects of dioxins and dl-PCBs are well known. The ndl-PCBs act via several different mechanisms and not via the AhR. Mechanistic studies indicate that they may affect components of the nervous system in several different ways. They alter intracellular signal transduction pathways by interfering with intracellular sequestration of calcium and increase the activation of protein kinase C (PKC). Induction of apoptosis and increased production of reactive oxygen species and changes in levels of neurotransmitters such as dopamine and acetylcholine have been reported. The latter is suggested to be linked to interference with PCB on thyroid hormone levels because cholinergic fibres are particularly sensitive to thyroid hormone deficiency. Furthermore, increased release of arachidonic acid has been observed. Changes in the PKC signalling pathway and calcium homeostasis as well as reduced dopamine levels have been confirmed in animal studies (EFSA, 2005a).

A provisional tolerable intake of 20 ng/kg bw/day for all 209 PCB congeners was proposed at the "2nd PCB workshop" in Brno (Czech Republic, May 2002) and has been used in France, the Netherlands, and Norway (AFSSA, 2007; Baars et al., 2001; VKM, 2008). This corresponds to a provisional tolerable daily intake of 10 ng PCB-6/kg bw/day, since half the total intake of PCBs consists of PCB-6.

[23]Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs.
[24]Analysed by the Norwegian Institute of Public Health.

The reference dose was derived from the BMDL from human studies described in the EFSA opinion from 2005. In 2008, VKM used 10 ng PCB-6/kg bw/day as a reference value in an evaluation of whether the TWI for dioxins and dl-PCBs was also protective to ndl-PCB exposure from the diet, given the relative composition of dioxins, dl-PCBs and ndl-PCBs in the food consumed in Norway. VKM concluded that with the combination of dioxins and PCBs in Norwegian food, exposure to dioxins below the TWI would also protect against toxicological effects from exposure to ndl-PCBs (VKM, 2008).

Levels and trends for ndl-PCBs in breastmilk and infant formula

From 1986 to 2005 the concentration of ndl-PCBs in breastmilk from Norway has decreased by approximately 70% (Figure 5.2).

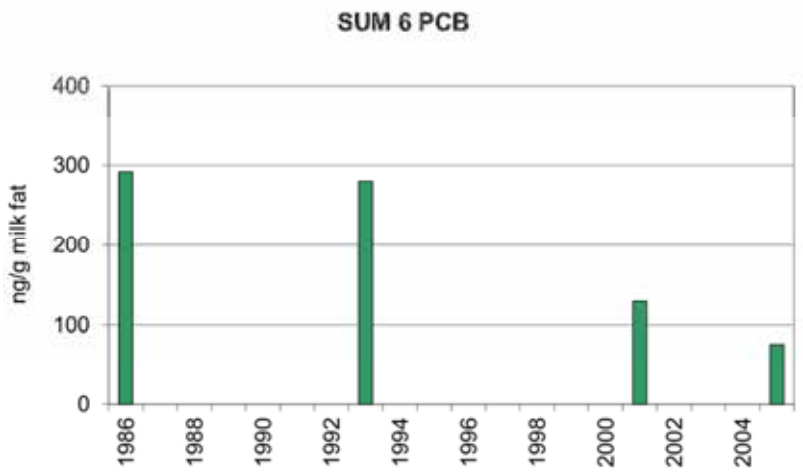

Figure 5.2: PCB-6 (ndl-PCBs) in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breastmilk (pooled data from 10-50 mothers per year). Source: "Morsmelk og miljøgifter faktaark", Norwegian Institute of Public Health based on Becher *et al*., 1995; Becher *et al*., 2002; NILU, 2013; Polder *et al*., 2009.

Individual breastmilk samples from HUMIS (n=377) collected in 2003-2005 show inter-individual variations in contamination levels between 10 to 454 ng/g lipid for sum PCB-6. Mean and median levels were 83 and 73 ng/g lipid, respectively (Polder et al., 2009). The mean, median and 95-percentile PCB-153 concentrations were 36, 32 and 64 ng/g lipids respectively (Polder, personal communication).

The maximum levels (MLs) of sum PCB-6 in infant formula is 1.0 ng/g wet weight, as laid down in the EU regulation for MLs for dioxins, dl-PCBs and ndl-PCBs in foods[25]. Provided 3.5% fat in infant formula, this corresponds to 29 ng/g fat.

Norwegian occurrence data of PCB-6 in in infant formula have not been found. The mean concentration of sum of PCB-138, -153 and -180 was 3.4 ng/g fat (range 8.1-11 ng/g fat) in six samples of infant formula analysed at the Norwegian Institute of Public Health for the Norwegian Food Safety Authority in 2008 (personal communication[26]). The mean PCB-153 concentration in these samples was 1.9 ng/g fat (range <LOQ to 4.8 ng/g fat).

[25]Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs.
[26]May Frøshaug, Norwegian Institute of Public Health.

5.2.2 Brominated flame retardants

5.2.2.1 Polybrominated biphenyls (PBBs)

PBBs are structurally similar to PCBs, but are brominated instead of chlorinated. PBBs have similar toxicological characteristics as PCBs. They have been used as flame retardants and were produced until the mid-1980s. The current levels in Europe including Norway are low and of no toxicological concern (EFSA, 2010c).

5.2.2.2 Polybrominated diphenyl ethers (PBDEs)

PBDEs are a class of brominated hydrocarbons with a basic structure consisting of two phenyl rings linked by an oxygen atom. Similarly as for PCBs, there are 209 possible congeners, with different numbers depending on the position of bromine atoms on the phenyl rings. Mixtures of PBDEs have been widely used as flame retardants in e.g. plastics, textiles, electronic casings and circuitry since the early 1970s. International agreements on regulation and use of some PBDEs have been introduced since 2004.

In general, PBDEs are persistent and bioaccumulative, with the exception of PBDE-209, which can undergo debromination reactions leading to formation of PBDE congeners containing seven to nine bromine atoms. The eight PBDE congeners; PBDE-28, -47, -99, -100, -153, -154, -183 and -209 are the ones most often found in food and consequently, in humans.

The risk from PBDE exposure was assessed by EFSA in 2011. Relevant toxicity data were only available for PBDE-47, -99, -153 and -209. Therefore a risk assessment could only be carried out for these four individual PBDE congeners (EFSA, 2011c). The main targets for PBDE toxicity are the liver, thyroid hormone homeostasis, and the reproductive and nervous system, but effects on neurodevelopment, which affect behaviour in mice, has also been identified as a critical endpoint. Due to limitations and uncertainties in the current database on PBDEs, no tolerable intakes have been established. Instead a margin of exposure (MOE) approach was used for the risk characterisation of PBDE congeners, by comparing the range of dietary intake for each of the different PBDE congeners with the estimated human intake associated with the body burden at the $BMDL_{10}$ in the experimental animal studies.

Based on interpretation of the basis for the body burden data, EFSA reasoned that a MOE larger than 2.5 might indicate that there is no health concern.

For breastfed European infants (3 months, with body weight of 6.1 kg) the MOE for PBDE-47, -99 and -153 the MOE was 12, 0.8 and 2.5 with average human milk consumption (800 ml/day). For infants with high human milk consumption (1200 ml/day), the MOEs were 8, 0.6 and 1.45, respectively. For PBDE-209, the MOE was about two orders of magnitude. EFSA concluded that the intake of PBDE-47 and -209 by breastfed infants does not constitute a health risk. For PBDE-99 and -153, the MOE is equal or smaller than a factor of 2.5 and thus exposure of breastfed infants might pose a potential health concern. It was noted that the highest mean exposure estimates across European countries were used for the MOE calculation and that the lowest estimated mean values for average and high consumption of human milk for PBDE-99and -153 are respectively about 40 and 25 times lower. In addition, EFSA noted that it takes three to four half-lives to reach steady state, i.e. 10 or more years for PBDE-99 and -153 in humans. Hence, the calculated MOEs for these PBDEs for breastfed infants based on the body burden would be an overestimation of the risk. Therefore, EFSA concluded that MOEs for PBDE-99 and -153 in human milk are unlikely to raise health concern to breastfed infants in Europe.

Levels and trends for PBDEs in breastmilk and infant formula

In contrast to dioxins and PCBs, the concentration of PBDEs in breastmilk in Norway increased until approximately 2000, after which a decline has been observed, as illustrated in Figure 5.3. Similar trends have been observed in Sweden as well as elsewhere in Europe, while the levels in the USA are in the range of 10-fold higher. (Fangstrom et al., 2008; Toms et al., 2012).

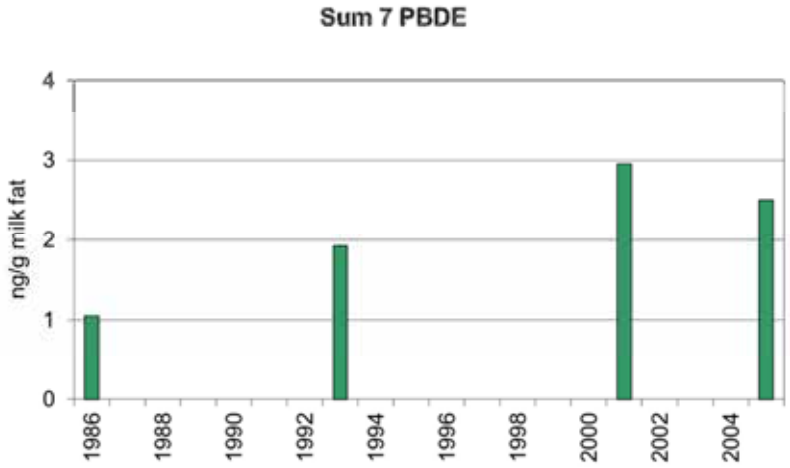

Figure 5.3: Sum of 7 PBDEs (PBDE-28, -47, -99, -100, -153, -154, -183) in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breastmilk (pooled data from 10-50 mothers per year). Source: "Morsmelk og miljøgifter faktaark", Norwegian Institute of Public Health based on Thomsen *et al*., 200; Thomsen, Stigum *et al*., 2010.

Individual breastmilk samples from HUMIS (n=393) collected in 2003-2005 provide information on inter-individual variation in contamination level for sum 7 PBDEs in women in Norway (Thomsen, Stigum et al., 2010). The results are presented in Table 5.1 and indicate a wide variation among breastmilk samples.

Table 5.1: Concentration of sum 7 PBDEs (ng/g lipid) in individual breastmilk samples collected in 2003-2005 in the HUMIS project (n=393) (Thomsen, Stigum et al., 2010).

Substance	Min	Median	Mean	75-percentile	95-percentile	Max
Sum 7 PBDEs	0.48	2.1	3.4	3.2	3.7	82

According to EFSA 2011, the average concentrations of the predominant PBDE congeners in human milk show a comparable mean contamination across various European countries. PBDE-47 had mean concentrations across countries of 0.14 to 3.0 ng/g lipid and the range of means for PBDE-99 and PBDE-153 were found to be <0.03-1.1 ng/g lipid and 0.10-2.4 ng/g lipid, respectively. There were wide concentration ranges for several PBDEs from various countries. For PBDE-209, mean concentrations between 0.21 and 2.9 ng/g lipid were reported for seven European countries (EFSA, 2011). The Norwegian data (Thomsen, Stigum et al., 2010) were part of the assessment.

The mean concentration in six samples of infant formula analysed at the Norwegian Institute of Public Health for the Norwegian Food Safety Authority in 2008 was for PBDE-47: 0.06

(range 0.03-0.13) ng/g lipid (personal communication[27]). The range of PBDE-99 was 0.04-0.16 ng/g lipid and of PBDE-153 <LOQ-0.013 ng/g lipid. The mean BDE-209 concentration was 0.73 (range 0.25-2.2) ng/g lipid.

5.2.2.3 Other brominated flame retardants

Hexabromocyclododecane (HBCDD) and tetrabrombisphenol A (TBBPA) are two other classes of brominated flame retardants which were assessed by EFSA in 2011 (EFSA, 2011b; EFSA, 2011d). Knowledge about the toxicity of these two compounds is limited and no tolerable intakes have been established. Based on present knowledge, the margins of exposure (the margin between levels causing toxic effects in animals and levels of exposure in humans, including from breastmilk) are large.

5.2.3 Chlorinated pesticides

DDT and many other chlorinated and persistent pesticides (HCH, aldrin/dieldrin, endrin, HCB, endosulphane and heptachlor) have been used extensively in the past. These have now been phased out and are generally no longer in use. DDT is an exception and is still used to fight mosquitoes carrying malaria in some non-European countries.

DDT and HCB are the most studied pesticides, and as no epidemiological data were available for other pesticides (see section 7.1) the description in the sections below is restricted to these two pesticides.

5.2.3.1 Dichlordiphenyltrichlorethane (DDT)

DDT was commercially introduced as an insecticide in the 1940s. Technical DDT contains 65-80% *p,p'*-DDT, which harbour the main insecticide activity. Major breakdown products of DDT are *p,p'*-DDE and *p,p*'-DDD. Sum of DDT refer to *p,p'*- DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p*'-DDD and *o,p'*-DDD.

DDT was banned in many European countries for most uses in the early 1970s and was banned in the EU in 1986. DDT is still used for vector control, especially in areas with endemic malaria. Extended use was recently recommended by WHO for indoor residual spraying to control malaria.

Because of the lipophilic properties and persistence in the environment, DDT and related compounds bioaccumulat and biomagnify along the food chain.

The DDT metabolite DDE has antiandrogenic activity, while p,p'-DDT (a main component) has little or no androgenic or estrogenic activity. The minor component o,p'-DDT has weak estrogenic activity. DDT and its metabolites have neurotoxic capacity and can directly affect nerve cells by interference with the membrane transport of potassium, sodium, calcium, or chloride ions. Inhibition of selective enzymatic activities, or contribution to the release and/or the persistence of chemical transmitters at nerve endings which results in repetitive discharges in neurons have also been reported.

The main target organs are the nervous system and the liver. It also affects hormonal tissues, reproduction, fetal development and the immune system. DDT is classified by IARC as

[27]May Frøshaug, Norwegian Institute of Public Health.

possibly carcinogenic to humans (group 2B). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) derived a provisional tolerable daily intake (PTDI) for DDT of 0.01 mg/kg bw (WHO, 2000).

According to EFSA (2006), a mean dietary intake for adults and children is approximately 5-30 ng/kg bw/day (EFSA, 2006a). This exposure level is more than two orders of magnitude below the PTDI of 0.01 mg/kg bw.

Levels and trends for DDT in breastmilk and infant formula

Since chlorinated pesticides are phased out and no longer in use in Norway, the level of these substances in the environment and in breastmilk has declined dramatically, as illustrated in Figure 5.4. Breastmilk from 10 to 50 mothers were combined and analysed as one sample and represent a mean concentration for each year.

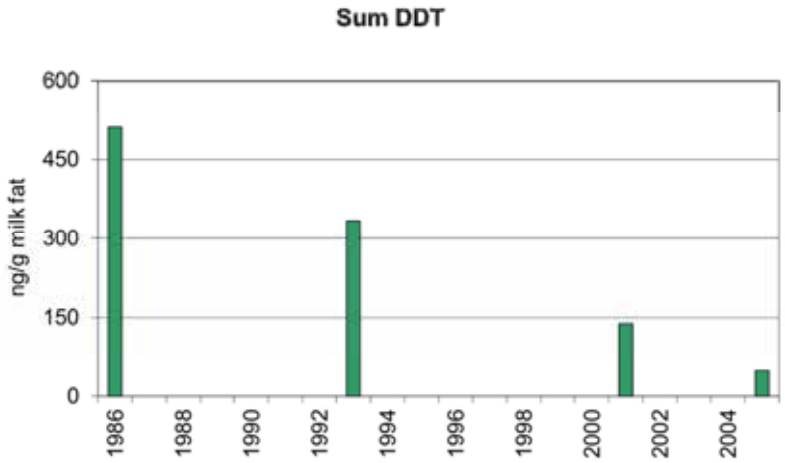

Figure 5.4: Sum DDT (*p,p'*- DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breastmilk. Source: "Morsmelk og miljøgifter faktaark", Norwegian Institute of Public Health based on Polder *et al*., 2008; Polder *et al*., 2009.

A general default EU maximum residue level (MRL) for baby food/infant formula of 0.01 mg/kg is applicable to all pesticides unless specific MRLs lower than 0.01 mg/kg were established in EU legislation[28] for this food type. No specific data on levels of DDT or derivatives in infant formula in Norway or Europe were found. According to a recent report from EFSA, no baby food from Norway had pesticide concentrations above 0.01 mg/kg (EFSA, 2013b).

5.2.3.2 Hexachlorbenzene (HCB)

HCB as pesticide (fungicide) has been phased out but it is still used as an industrial chemical to some extent. It is released as a by-product from the manufacture of industrial chemicals and several pesticide formulations, and can be released to the environment during incineration.

HCB is readily absorbed in humans and animals. It has low acute toxicity. The liver is the

[28]Commission Directive 2006/141/EC for infant formulae and follow-on formulae and in Commission Directive 2006/125/EC for processed cereal-based foods and baby foods for infants and young children.

predominant organ to be affected resulting in enzyme induction and porphyria. HCB is classified by IARC as a possible human carcinogen and since HCB exhibits weak mutagenic activity in some tests, a genotoxic mode of action cannot been excluded.

Although neurobehavioural effects in rat pups exposed to HCB has been reported, no data were located regarding possible mechanisms of HCB-induced neurotoxic effects (EFSA, 2006b).

Foods containing animal fat are the major source of HCB exposure in humans. There is no tolerable intake of HCB because it might be a genotoxic carcinogen. EFSA stated in 2006 (EFSA, 2006b), that there is a 1.6-80 x 10^5 margin between a dose causing 5% liver tumours in rats and the human exposure, and concluded that current HCB exposure was of low concern. EFSA estimated the exposure in breastfed infants to be approximately two orders of magnitude higher (per kg body weight) than the median daily intake in adults.

Levels and trends for HCB in breastmilk and infant formula

After the ban as pesticide in Europe in 1981, the level of HCB has declined approximately 95% in Norwegian breastmilk as illustrated in Figure 5.5. Breastmilk from 10 to 50 mothers were combined and analysed as one sample and represent a mean concentration for each year. The same trend has been seen in other European countries (Furst, 2006).

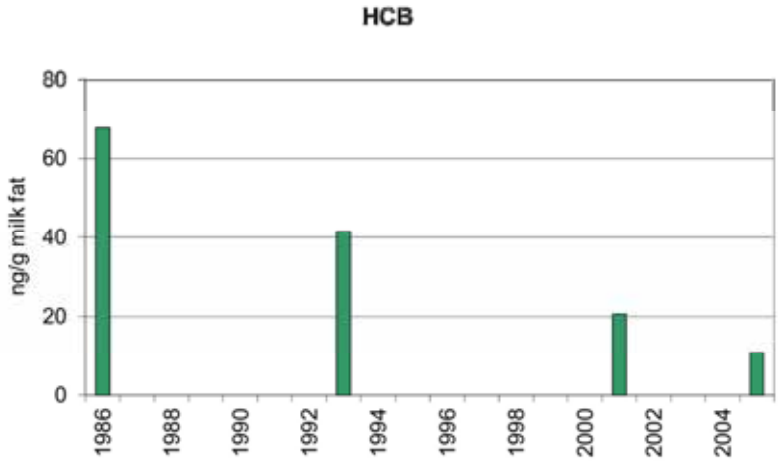

Figure 5.5: HCB in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breastmilk. Source: "Morsmelk og miljøgifter faktaark", Norwegian Institute of Public Health based on Eggesbo *et al*., 2009; Polder *et al*., 2009.

Individual breastmilk samples from HUMIS (n=423) collected in 2003-2005 provide information on inter-individual variation in contamination levels between 3.6 to 42 ng/g lipid for HCB (12-fold variation from lowest to highest concentration) in women in Norway. Mean and median levels were 12 and 11 ng/g lipid, respectively (Polder et al., 2009).

No specific data on levels of HCB in infant formula in Norway or Europe was found. According to a recent report from EFSA, no baby food recently reported from Norway had pesticide concentrations above the default EU MRL of 0.01 mg/kg (EFSA, 2013b).

5.2.4 Fluorinated compounds

Perfluoroalkylated substances (PFAS) consist of neutral and anionic surface active compounds with high thermal, chemical and biological inertness. Perfluorinated compounds are generally hydrophobic, but also lipophobic, and will therefore not accumulate in fatty tissues as is usually the case with other persistent halogenated compounds. An important subset is the organic surfactants, to which PFOS and PFOA belong. It is generally recognised that food is a major source to PFOS and PFOA exposure. A recent study on Norwegian women showed that the indoor environment may be an important contributor to human exposure (Haug et al., 2011).

Since PFOS and PFOA show highest occurrence and have been most studied, these are shortly described below.

5.2.4.1 PFOS

In sub-acute and chronic studies of PFOS, the liver is the major target organ, but developmental toxicity has also been seen. Other sensitive effects were changes in thyroid hormone- and high density lipoprotein levels in rats and Cynomolgus monkeys. PFOS induced liver tumours in rats, which appears to be due to a non-genotoxic mode of action.

EFSA (2008) established a TDI for PFOS of 150 ng/kg bw per day by applying an overall uncertainty factor (UF) of 200 to the NOAEL of 0.03 mg/kg bw per day in a study with subchronic exposure in monkeys (EFSA, 2008a). Updated exposure calculations from EFSA indicate that for PFOS, the dietary exposure estimate in the adult population is <3.5% of the TDI for average consumers and <6.7% of the TDI in high consumers, and that exposure in toddlers is 2 to 3 times higher than in adults (EFSA, 2012b).

5.2.4.2 PFOA

In studies with sub-acute and chronic exposures, PFOA primarily affected the liver. PFOA can also cause developmental and reproductive toxic effects at relatively low dose levels in experimental animals.

EFSA has established a TDI for PFOA of 1.5 µg/kg bw per day by applying an overall UF of 200 to the lowest $BMDL_{10}$ for increased liver weight in rodents (EFSA, 2008a). Updated exposure calculations from EFSA indicate that for PFOA, the dietary exposure estimates in the adult population was 0.3% of the TDI for average consumers and 0.5% of the TDI in high consumers, and that exposure in toddlers was two to 3 times higher compared to adults (EFSA, 2012b).

Levels and trends for PFOS and PFOA in breastmilk and infant formula

The concentration of PFOS and PFOA in breastmilk is low compared to blood. Still, Thomsen *et al*. (Thomsen, Haug et al., 2010) showed that the intake of PFCs through breastmilk (per kg body weight) is similar to the dietary intake for Norwegian adults.

In breastmilk in Sweden, PFOS and PFOA showed increasing trends from 1972 to 2000, with concentrations reaching a plateau in the 1990s. Then there was a significant decreasing trend from 2001 to 2008. At the end of the study, in 2008, the measured concentrations of PFOS, and PFOA in pooled human milk were 75 pg/mL and 74 pg/mL, respectively. The temporal trends for PFOS and PFOA observed in human milk are parallel to those reported in serum in the the general population in Sweden and Norway (Haug et al., 2009; Karrman et al., 2007).

The concentrations in breastmilk in Norway are in the same range as in Sweden (Haug et al., 2011).

5.2.5 The relationship between children's exposure to persistent organic pollutants and body burden

Persistent organic pollutants (POPs) accumulate in the body over time and it is generally recognised that toxicity is more related to the body burden than to recent dietary exposure. The body burden is the total amount of a substance in the body, and is commonly expressed per kilo body weight. For lipophilic POPs, such as PCBs, virtually all will be found in the lipid compartments of the body. Furthermore, the contaminant concentration in body lipids, (e.g. different fat deposits, blood lipids, membranes) are in equilibrium with each other, and all body fats can be regarded as one compartment. Thus, the PCB body burden can be calculated based on the concentration of PCBs in blood lipids, body weight, and percent fat in the body. Several POPs follow 1st order toxicokinetics, meaning that the half-life (the time needed to reduce the concentration in the body to the half in the absence of exposure) is constant and therefore independent of the concentration in the body. In an individual with constant exposure, equilibrium between the intake and excretion will be reached after 3-5 half-lives, and the body burden (or the concentration in blood) is then constant (illustrated in Figure 5.6). It therefore takes approximately 25 years to reach steady state for a substance with a half-life of 7.5 years (e.g. dioxins).

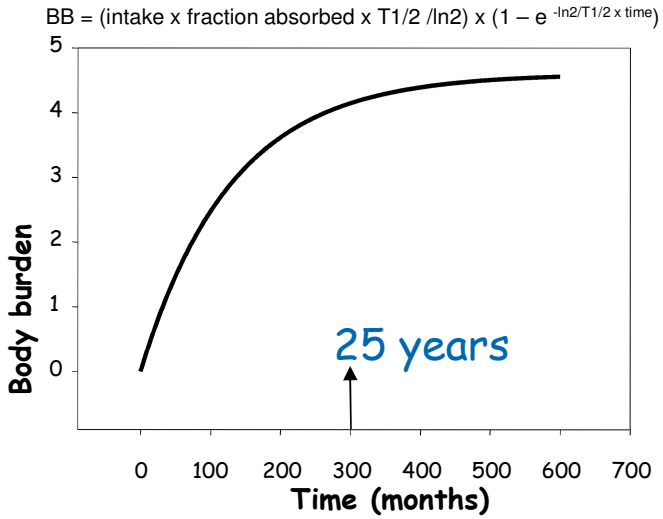

Figure 5.6: Illustration of dioxin body burden over time when exposure and body weight is constant. A half-life (T1/2) of 7.5 years has been used. Sorurce: HK Knutsen.

The illustration in Figure 5.6 is a simplification of the development of the body burden in adults. Depending on physical and chemical properties, environmental contaminants are to different extents excreted in breastmilk, and the concentration in the maternal body will be reduced during the breastfeeding period. In a study of breastmilk in 10 Norwegian women, the change in contaminant concentration in the milk during a 12 months nursing period was monitored. After a year of breastfeeding, the concentrations of different PCBs, PBDEs and PFCs were reduced by 15-94% (Thomsen, Haug et al., 2010). For several reasons, children do not have a steady state concentration in the body. First, they are born with approximately the

same blood concentration as the mother. After birth the blood concentration increases with their dietary exposure, but the increase is not as steep as adults would have had with similar contaminant intake, because the child's body weight is increasing and the amount of body fat is changing. Furthermore, the exposure duration in childhood is too short to reach a steady state.

The most important determinant of postnatal exposure to POPs in breastfed infants is the concentration in the breastmilk, breastmilk consumption and duration of breastfeeding. Although it is difficult to obtain blood samples from children at the end of the nursing period, several studies have documented a relationship between breastfeeding and children's POP concentration in blood, and thereby the body burden (see below). It is well recognised that breastfed infants have substantially higher exposure to persistent organic pollutants than formula fed infants. However, the difference in the concentration in the breastfed infant and the unborn child (postnatal versus prenatal exposure) is not well described. This difference might be of high toxicological relevance, since the infant may be equally susceptible to environmental contaminants as the unborn child as a concervative approach.

In a study from Germany, Lackman *et al*. compared concentrations of contaminants at birth, 6 weeks and 6 months in 10 breastfed and 10 formula fed infants. At 6 weeks, the infants' serum concentrations of the POPs measured were approximately 10-fold higher in the breastfed than in the formula fed. An almost doubling in PCBs and DDE was observed in the breastfed from 6 weeks to 6 months of age, as illustrated in Figure 5.7 (Lackmann et al., 2004; Lackmann et al., 2005). The study group was small, but analytical results from children at this low age are rare. Comparison with maternal exposure level was not available in this study.

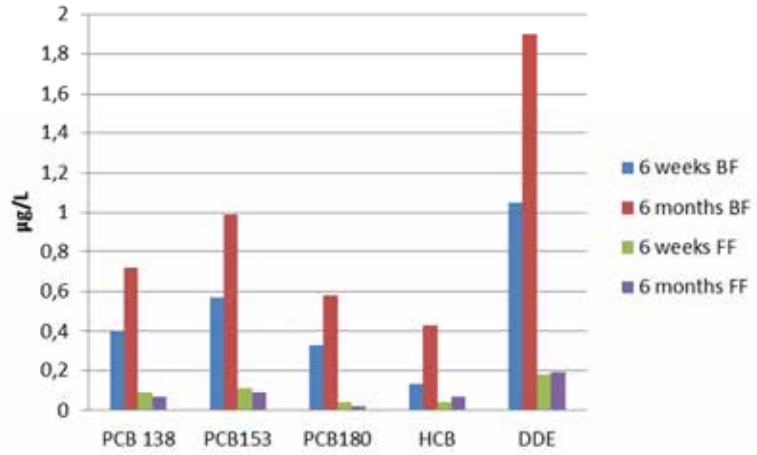

Figure 5.7: Median serum organochlorine concentration in 10 breastfed (BF) and 10 formula fed (FF) German children sampled at 6 weeks and 6 months. Based on data in Lackmann *et al*. 2005.

Data from 6-month old infants are available from an Inuit cohort, where the concentration of PCB-153 in serum from 6-month old children that were breastfed for less than 3 months (n=26) was 1.6-fold that of those never breastfed (n=14). The serum concentration in those breastfed for more than 3 months (n=50) was slightly more than 4-fold higher than in those breastfed for less than 3 months. In comparison with the maternal concentration in the overall cohort (n=128), the level was 1.5-fold that of the mean maternal concentration in those breastfed for more than 3 months (Ayotte et al., 2003).

In the Faroe Islands cohort 3, the serum PCB concentration at age 18 months was slightly higher than the average maternal level during pregnancy. The average exclusive and total breastfeeding period was 4.6 months and 9.8 months (Heilmann et al., 2010).

In children from Menorca born in 1997-98, the concentration of several different chlorinated contaminants was significantly higher (approximately 3-fold for PCB-153 and DDE) in breastfed 4-year olds (n=244, 83% breastfed, duration 0.93 to 96 weeks) than in non-breastfed. Compared with the concentration in cord blood, the concentration of PCB-153 was almost 2-fold higher at 4 years than at birth, while the concentration of DDE was only marginally higher. In formula fed children the concentration of most compounds were lower at 4 years of age than at birth (Carrizo et al., 2006). The difference between ever breastfed and formula fed children differed depending on the length of breastfeeding. The PCB concentration (PCB-153, -138, -180) at 4 years of age was approximately twice as high in children breastfed for 3 months or longer compared with a median duration of breastfeeding shorter than 3 months (Grimalt et al., 2010). For other chemicals the differences were even more marked (for instance DDE), while for a few substances, there was little difference (for instance PCB-118). In the same cohort from Menorca, levels of PBDEs were measured in cord blood and sera at 4 years of age. A lower concentration of e.g. the abundant PBDE-47 was found in formula fed than in ever breastfed infants. Since birth, the concentration had decreased markedly in the formula fed children, but was in average 20% higher in serum at 4 years than in cord blood in the breastfed group (Carrizo et al., 2007).

In a German cohort recruited in 1993-95, a 4.5-fold higher PCB concentration was reported in 4-year old children who had been breastfed more than 4 months compared with children who had been breastfed two weeks or less (Walkowiak et al., 2001). The data did not allow for comparison with levels at birth.

Several POPs were analysed in serum of 323 Swedish pregnant women born in the 1980s with a mean age of 28 years (Glynn et al., 2007). When these women were infants in the 1980s, they were breastfed with a mean duration of 4 months. Concentrations of PCB-156, PCB-180 and p, p'-DDE were significantly associated with the duration these women had been breastfed. The largest effect was observed for current adult levels of DDE, where a 34% increase was estimated for 10 months of breastfeeding. Significant associations were also shown for PCB-180 and PCB-156, although weaker.

These studies all show that body burdens are strongly influenced by duration of breastfeeding and that this influence might be of long-lasting character and can still be evident in adulthood.

The effect of breastfeeding on levels measured in blood are not directly comparable because of differences in maternal exposure, age of the children when contaminants were measured, duration of breastfeeding, and the ratio of exclusive to partial breastfeeding. With increasing child age, the influence of other factors than breastmilk, such as individual differences in growth/body size increase and individual differences in the diet, will have an increased impact on body burdens. More importantly, none of the studies described above had results that allow for an assessment of amount of POPs increase per month of breastfeeding. None of the studies allow for a separate evaluation of the effects of exclusive and partial breastfeeding.

A way of estimating body burdens due to breastfeeding is by modelling the change in body burden over time, taking excretion and dilution due to the expansion in the child's body mass into account (PBPK) as proposed by Verner *et al*. (Verner et al., 2009). Recently, Verner's model was further refined and validated on two datasets which contained repeatedly measured concentrations of PCB-153 and p, p'-DDE in infants/children, and a good prediction was obtained (Verner et al., 2012). According to this model, POP levels drop drastically after birth

in babies who are not breastfed due to the fast growth during the first months after delivery, and the thereby resulting dilution of the body burden at birth. In contrast, as long as the baby is still breastfed, a steady increase occurs. Four months of breastfeeding results in a doubling of the concentration from birth. Then, after cessation of breastfeeding at 4 months, the levels decline back to the levels observed at birth over the next 6 to 8 months. Twelve months of breastfeeding results in a doubling of the level at 4 months, and after cessation of breastfeeding at one year, the levels are not estimated to decrease markedly over the next two years. This is due to the fast growth spurt during the infant's first year of life which levels off at 1 year of age. Taken together this results in 4-fold higher PCB and DDE levels at both one and two years of age in children breastfed for 12 months compared to children breastfed for 4 months.

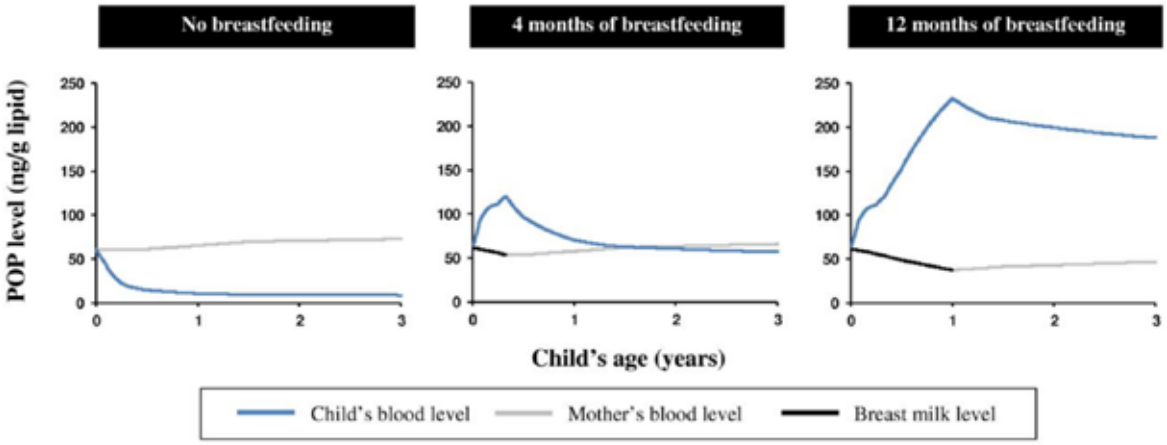

Figure 5.8: Examples of simulated toxicokinetic profiles in children breastfed for 0, 4 and 12 months. Simulations were carried out using a daily dose of 5 ng PCB-153/kg bw/day in mothers (Verner *et al.*, 2012).

Similar illustrations have not been found for breastfeeding beyond 12 months, but it is reasonable to predict that the body burden will increase as long as the child is breastfed. The example in Figure 5.8 used 5 ng PCB-153/kg bw/day as a daily intake in the mothers. The median and 95-percentile PCB-153 intake was 0.73 ng/kg bw/day and 5.8 ng/kg bw/day, respectively, in Norwegian mothers participating in the MoBa study between 2002 and 2009 (Caspersen et al., 2013).

There is no toxicokinetic model for dioxins and dl-PCBs in breastfed children. There is, however, a high correlation between exposure to ndl-PCBs and dioxins and dl-PCBs, and a rough estimate can be done based on this correlation. An exposure to dl-compounds at the TWI of 14 pg TEQ/kg bw/week is associated with a daily intake of approximately 10 ng/kg bw/day of PCB-6 with the present composition of dl-compounds and ndl-PCBs in Norwegian food (VKM, 2008). Consequently, a PCB-6 intake below this level would protect against eventual toxic effects of both ndl-PCBs and dl-compounds. Approximately 33% of PCB-6 in the diet is PCB-153, thus approximately 3.3 ng PCB-153/kg bw/day would correspond to an intake of 10 ng/kg bw/day of PCB-6 (VKM, 2008). In other words, with the present composition of dioxins and PCBs in food in Norway, a daily intake of approximately 3.3 ng PCB-153/kg bw/day will give a concomitant intake of dioxins and dl-PCBs approximately at a level which is similar to the TWI for this group of substances. The median intake of PCB-153 in Norwegian pregnant women is estimated to be much lower, while the 95-percentile is less than 2-fold higher than 3.3 ng/kg bw/day, as indicated above.

5.3 Process-generated contaminants

During processing of food, contaminants can be formed, often because of heat treatment. Acrylamide, PAH, furan and 3-monochloropropane 1,2-diol (3-MCPD) are examples of substances that may be of relevance for infant formula, and for which some data were retrieved by literature search (Appendix 6).

5.3.1 Acrylamide

Acrylamide is produced at higher temperatures when preparing foods containing both proteins and carbohydrates. Food is the major acrylamide source in non-smokers. Acrylamide is classified by IARC as "probably carcinogenic to humans" (group 2A) (IARC, 1994). It is water soluble and does not accumulate in the food chain. JECFA concluded in their risk assessment that the MOEs are low and may indicate a human health concern. Therefore, appropriate efforts to reduce acrylamide concentrations in foods should continue (JECFA, 2005).

The low MOEs were confirmed in an updated exposure assessment in Europe performed by EFSA. For toddlers (1-3 years) mean intake was estimated to range between 1.2 and 2.4 µg/kg bw per day and 95th percentile between 2.4 and 6.5 µg/kg bw per day. Major contributors to acrylamide exposure were fried potatoes, bread and biscuits (EFSA, 2011a).

Acrylamide levels in infant formula or breastmilk have not gained much attention, being a much smaller source than the foods mentioned above. However, occurrence in both infant formula and breastmilk was reported in a Swedish study on baby food (Fohgelberg et al., 2005). For all breastmilk samples except one, the acrylamide level was below the limit of quantification (0.5 µg/kg). Assuming an acrylamide level of 0.25 µg/kg in breastmilk, the mean acrylamide intake during the first 6 months for children who were exclusively breastfed was estimated to be 0.04 µg/kg bw/day. In three out of eight analysed samples of infant formula, the acrylamide content was verified and possible to quantify (0.6, 0.6, and 0.7 µg/kg, respectively).

In a Bavarian study conducted in 2005, acrylamide concentrations ranging between 0.1 and 1.3 µg/kg (median: 0.11 µg/kg; 95th percentile: 0.33 µg/kg) were discovered in 172 breastmilk samples. The infant formula investigated in the same study showed comparable results, which were on average slightly above the mean level of breastmilk (German Federal Environment Agency, 2008).

5.3.2 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are formed during incomplete combustion of organic materials and are therefore produced in industrial processes, during heating of wood or fossil fuel, and during food preparation (e.g. barbequing and smoking). PAHs generally occur in complex mixtures which may consist of hundreds of compounds. Several PAHs evaluated by EFSA in 2008 have been classified as genotoxic and carcinogenic. Of these, benzo(a)pyrene (BaP) is most extensively characterised (EFSA, 2008b). For non-smokers the major route of exposure is consumption of food, and grain-based food and seafood are the largest contributors.

According to EFSA, the MOEs in Europe indicate a low concern for consumer health at the average estimated dietary exposures. However, for high level consumers the MOEs are close to or less than 10,000, indicating a potential concern for consumer health (EFSA, 2008b).

In a study from urban and rural areas in Tuscany in Italy, PAH was determined in milk collected in 2004-2005 from 32 women. PAH concentration was higher in milk from women living in urban than in rural regions. Furthermore, the PAH level in breastmilk was highly influenced by cigarette smoking (Zanieri et al., 2007). In this study, the BaP concentration was below the detection limit in all non-smoking donors, and ranged from below the detection limit to 1.17 µg/kg milk fat in breastmilk from smoking donors. The detection rates of benz(a)anthracene, benzo(b)fluoranthene and chrysene were low, but higher in milk from smoking than from non-smoking mothers.

The maximum level for PAH in infant formula is 1.0 µg/kg for both BaP alone and for the sum of four PAHs (BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene) (EU, 2011).

In a report from Food Standards Agency (FSA) in UK (2006), 97 samples of infant formula obtained from across the UK were tested for 15 PAHs. Most PAHs were not detected in the majority of samples. The BaP concentrations were in the range of <0.01-0.2 µg/kg in infant formulas. BaP was below the limit of detection in 57 of the samples (59%). The estimated intakes from infant formula in this survey indicated a low concern for human health (FSA, 2006).

5.3.3 3-Monochloropropane-1,2-diol (3-MCPD)

3-MCPD is formed in heat-treated foods and was first detected in various foods such as hydrolysed vegetable proteins and soy sauce. It is classified as possibly carcinogenic to humans by IARC (group 2B agent), but is not acting via a genotoxic mechanism. Studies have linked 3-MCPD with infertility in rats, suppression of the immune function and possible carcinogenicity.

In 2001, JECFA considered 3-MCPD and assigned a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day, which was retained in 2006 (JECFA, 2006).

In 2007, the presence of fatty esters of 3-MCPD (3-MCPD esters) was reported for the first time in a number of foods including refined edible fats, such as margarine and oils, as well as in infant formula and breastmilk. Since 3-MCPD can be released from the esters, the presence of 3-MCPD esters should be addressed. While there are a number of toxicological animal studies on 3-MCPD, little is known about the occurrence, toxicokinetics or toxicity of 3-MCPD esters. Such data are needed to assess the possible risks to human health.

There are reports on 3-MDCP esters in both infant formula and in breastmilk, indicating higher levels in infant formula than in breastmilk (BfR, 2007; Zelinkova et al., 2006; Zelinkova et al., 2008).

5.3.4 Furan

Furan is a volatile and lipophilic compound formed in a variety of heat-treated commercial foods, including infant formula. The presence of furan is of potential health concern because it has been classified as “possibly carcinogenic to humans” (group 2B agent) by IARC. A genotoxic mechanism is likely in furan-induced carcinogenesis and in a risk assessment from 2012, VKM concluded that the current exposure to furan in all age groups, particularly among infants and children, is of health concern (VKM, 2012).

No occurrence data for furan in infant formula sold on the Norwegian market is available. VKM recently published a risk assessment of furan (VKM, 2012). The calculated furan exposures were based on the mean furan concentration (upper bound) in dry infant formula of

3.2 µg/kg (n=11) from data in an EFSA monitoring report (EFSA, 2011f) and the mean and 95-percentile consumption of infant formula reported for all participants and for the non-breastfed 6-month old infants in a Norwegian dietary survey (Spedkost, 2006-2007) (consumers only for both groups). A mean furan exposure from infant formula was 0.02 µg/kg bw/day for consumers only among all 6-month olds, while the 95-percentile exposure was 0.05 µg/kg bw/day. For the 6- and 12-month old infants, especially jarred baby food contributed more to the exposure than infant formula.

No data on concentration of furan in breastmilk has been identified.

5.4 Substances migrating from food contact materials – bisphenol A and phthalates

The infant feeding bottles and the attached teats used to supply formula or expressed breastmilk are potential sources of exposure to contaminants for infants. Likewise, substances leaking from food packaging material into food consumed by the mother can also be present in breastmilk. Food contact materials can also leak into infant formula. Two classes of hormone active substances (bisphenol A and phthalates) which are considered to be of special relevance are described shortly below. These substances are short-lived and will therefore not accumulate in the food chain or in the body.

5.4.1 Bisphenol A (BPA)

Bisphenol A (BPA) is a hormone active substance and acts by activation of the estrogen receptor. A main concern regarding exposure in early life has been migration of BPA from baby bottles. BPA is permitted for use in food contact materials in the EU under a regulation relating to plastic materials and articles intending to come into contact with foodstuffs[29]. In 2011, the European Commission adopted a directive prohibiting the use of BPA for the manufacture of polycarbonate infant feeding bottles[30]. Norway also banned the use of BPA in baby bottles from 2011.

EFSA completed its full risk assessment of BPA in 2006 and set a TDI of 0.05 mg/kg bw/day for this substance (EFSA, 2006c). EFSA also evaluated intakes of BPA through food and drink for adults, infants and children and found that they were all well below the TDI.

Following further consideration of new scientific studies, EFSA in 2012 decided to undertake a full re-evaluation of the human risks associated with exposure to BPA through the diet, also taking into consideration the contribution of non-dietary sources to the overall exposure to BPA. The new opinion (expected to be published in 2014) will review all the available data and scientific studies on dietary exposure published since EFSA's 2006 Opinion.

Since the ban of the use of BPA in polycarbonate (PC) baby bottles in 2011[31], baby bottles made of other plastics materials have appeared on the market. As part of this benefit and risk

[29]Commission regulation No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.
[30]Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles and Commission implementing regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles.
[31]Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union, L12: 1–89 and Commission implementing Regulation (EU) No. 321/2011 of 1 April 2011 amending Regulation (EU) No. 10/2011 as regards the restriction of use of bisphenol A in plastic infant feeding bottles. Off J Eur Union, L87: 1–2.

assessment of breastmilk, VKM asked various EU member states for information about the materials used in baby bottles and possible migration of chemicals from infant feeding (baby) bottles and teats after the BPA ban. Information about chemical contaminants in infant formula was also requested. The information received on the basis of this call is summarised in Appendix 8.

5.4.2 Phthalates

Based on their chemical and physical properties, phthalates have been commonly used for decades as plasticizers to soften consumer products and make them more flexible and resilient. Phthalates may be present in foods as a result of migration from food contact materials. Phthalates commonly found are di-2-ethylhexyl phthalate (DEHP), di-n-butyl phthalate (DnBP) and di-isobutyl phthalate (DiBP). Although it is reported that phthalates are no longer used in the production of food contact plastics, they may be used as carriers for pigments in printing inks and in adhesives applied to a plastic food contact material. Further, phthalates are commonly found in food contact materials made of paper and board, in particular when the material has been recycled. As a result of their widespread use and their moderate resistance to degradation, phthalates are also ubiquitous in the environment. Therefore, they may also be present in foods as a consequence of environmental contamination.

Phthalates have adverse effects on the liver, kidneys and reproductive system in particular and they can act on endocrine systems. For DEHP, a TDI of 50 µg/kg bw was established by EFSA in 2005 based on developmental and testicular toxicity (e.g. germ cell depletion and reduced testis weight) in the F1 generation of rats[32]. For DnBP, a TDI of 10 µg/kg bw was set based on reproductive effects in rats (EFSA, 2005b). A TDI has not been established for DiBP.

In a recent German study on 78 breastmilk samples, median concentrations were 3.9 ng/g for DEHP, 0.8 ng/g for DnBP, and 1.2 ng/g for DiBP. In infant formula (n=4), mean values were 19.7 ng/g for DEHP, 3.8 ng/g for DnBP, and 3.6 ng/g for DiBP. The authors concluded that the calculated intake for breastfed infants corresponded to about 2% to 7% of the tolerable daily intake. For infants nourished with formula, phthalate intake was of the same magnitude or slightly higher (DEHP) than for exclusively breastfed infants (Fromme et al., 2011).

In a study from 2012 by the Food Standards Agency in UK determining phthalates in food, among 16 samples of infant formula, one sample of infant milk formula contained 13 µg/kg DiBP and two samples, goat milk infant formula and organic infant milk formula, contained 96 and 125 µg/kg DEHP, respectively (Food Standard Agency, 2012).

Data on phthalates in breastmilk or infant formula from Norway has not been found.

[32]Statement of the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on the possibility of allocating a group-TDI for Butylbenzylphthalate (BBP), di-Butylphthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP), di-Isononylphthalate (DINP) and di-Isodecylphthalate (DIDP) (Minutes statement expressed on 28 June 2005 at its 12th Plenary meeting, corresponding to the item 10 of the agenda).

5.5 Microorganisms that may influence infant health if breastmilk is replaced by infant formula

The description below is focused on powdered infant formula since it is not sterile (in contrast to available liquid, rehydrated, ready-to eat infant formula).

Based on the strength of the evidence of a causal association between the presence of microorganisms in powdered infant formula and illness in infant, FAO/WHO placed microorganisms of concern into three categories (FAO and WHO, 2004):

Category A organisms – clear evidence and causality: Bacterial species belonging to the family *Enterobacteriaceae*, like *Salmonella* spp. and *Cronobacter* spp.

Category B organisms – causality plausible, but not yet demonstrated: Other bacterial species within the family *Enterobacteriacae*, such as *Pantoea agglomerans*, *Escherichia vulneris*, *Hafnia alvei*, *Klebsielle pneumoniae*, *Citrobacter koseri*, *Citrobacter freundii*, *Klebsiella oxytoca*, and *Enterobacter cloacae,*

Category C – causality less plausible, or not yet demonstrated: *Bacillus cereus*, *Clostridium perfringens*, *Clostridium difficile*, *Clostridium botulinum*, *Staphylococcus aureus*, and *Listeria monocytogenes*.

Among these bacterial species, microorganisms belonging to Category A are of special concern. Several outbreaks of *Salmonella* infection among infants have been attributed to contaminated, powdered infant formula, resulting in diarrhoea and, in some infants, bacteraemia and meningitis (Cahill et al., 2008). According to Cahill and co-workers, outbreaks of salmonellosis among infants due to powdered infant formula are probably underreported. Nevertheless, they highlight the need to recognise powdered infant formula as a potential source of *Salmonella* infection in infants.

In particular *Cronobacter* spp. (formerly *Enterobacter sakazakii*), which comprise six different genomospecies (Healy et al., 2010) is a rare cause of invasive infection with high death rates in neonates (Lai, 2001). Clinical symptoms related to this emerging opportunistic pathogen include necrotizing enterocolitis (NEC), meningitis and sepsis both in premature and full-term infants. Neonates aged <28 days and infants <2 months are at greatest risk (FAO and WHO, 2004). The high tolerance of *Cronobacter* spp. to osmotic stress and elevated temperature may contribute to survival of the bacteria in powdered infant formula (Healy et al., 2010). *Cronobacter* spp. is ubiquitous in nature and food. Its natural habitat may be plant material like herbs and spices (Iversen and Forsythe, 2013).

Powdered infant formula may be contaminated by *Cronobacter* spp. either due to introduction during the manufacturing process or from the use of contaminated utensils, such as blenders or spoons used in reconstitution of powdered infant formula (Noriega et al., 1990). Enteral feeding tubes and feeding bottle teats may harbour the bacterium, since *Cronobacter* has ability to grow on plastics and silicon rubber surfaces by producing and growing in biofilm (Zogaj et al., 2003). The formation of biofilm may decrease their susceptibility to anti-microbial agents and probably to soap and dish detergents.

According to recommendations from the Norwegian Food Safety Authority, only one serving infant formula should be prepared at each meal, to prevent outbreaks of *Cronobacter* spp. and *Salmonella*. The most important source of information on outbreaks in Norway is The Norwegian Surveillance System for Communicable Diseases (MSIS) and the web-based outbreak reporting system (Vesuv). As an official monitoring system for infectious diseases, MSIS collates notifications from microbiological laboratories, hospitals and physicians of

new cases of a number of infectious diseases. There have been no registered outbreaks due to infant formula in Vesuv or MSIS, however information about sporadic cases is not available.

5.6 Summary of contaminants and other undesirable compounds in breastmilk and infant formula

Lead and mercury concentration in breastmilk and infant formula are generally low, and exposure among infants is generally not above tolerable intake levels set by EFSA or JECFA (Table 5.2), although higher exposure in some infant groups (both breastfed and formula fed) cannot be excluded. There are indications that exposure to other elements (e.g. aluminum, manganese and molybdenum) might be substantially higher in infants given infant formula compared to breastfed infants.

For halogenated organic compounds, there are generally large differences in levels between breastmilk and infant formula. They are found in the highest concentrations in organisms located high up in the food chain, which include humans. The tolerable intakes are summarised in Table 5.3.

Table 5.2: Tolerable intakes for lead, mercury and cadmium.

Metals	Tolerable intake	References
Lead	No tolerable intake, but $BMDL_{01}$ =0.5 µg/kg bw/day	(EFSA, 2010b)
Methylmercury **Inorganic mercury**	1.3 µg/kg bw/week 4.0 µg/kg bw/week	(EFSA, 2012a)
Cadmium	2.5 µg/kg bw/week	(EFSA, 2009a; EFSA, 2011e)

Table 5.3: Tolerable daily intake (TDI) and other safety limits for human intake of organic contaminants used in the risk assessment.

Contaminants	Tolerable intake or other safety parameters	Reference
Dioxins and dl-PCB	14 pg TEQ/kg bw/week	(SCF, 2001)
Sum PCB-6*	10 ng PCB-6/kg bw/day	(AFSSA, 2007; VKM, 2008)
PBDE PBDE-47, -99, -153 and -209	MOE approach for each individual congener	(EFSA, 2011c)
DDT	0.01 mg/kg bw/day	(WHO, 2000)
HCB	MOE approach	(EFSA, 2006b)
PFOS	150 ng/kg bw/day	(EFSA, 2008a)
PFOA	1.5 µg/kg bw/day	(EFSA, 2008a)

*PCB-28, -52, -101, -138, -153, -180.

The levels of several of these compounds have been declining, especially for dioxins, PCBs and chlorinated pesticides. This is illustrated in Figure 5.9, where also the concentrations of these substances relative to each other are shown. In contrast, levels of the brominated flame retardants belonging to the class PBDEs were increasing until 2000, when they levelled off and have later decreased. The fluorinated surfactants PFOS and PFOA have shown a resembling time trend as the PBDEs.

Because of a long half-life, and that breastmilk is a dominating source for exposure for the baby, the infant's body concentration (and thus serum concentration) of POPs is strongly influenced by the duration of breastfeeding. The serum concentrations in children increase during the breastfeeding period, and thereafter decline. Simulation data indicate that the maximum serum PCB-153 concentration in the child can reach approximately 4-fold the concentration at birth after 12 months of breastfeeding. At 4 month of exclusive breastfeeding, simulation data indicate a doubling of the concentration at birth.

Whereas the hormone active substance BPA has recently been banned in infant feeding bottles in EU and Norway, little is known about their replacement substances. Substances from food packaging material such as phthalates may be present in both breastmilk and infant formula, as well as process-generated substances such as acrylamide, PAHs, furan and 3-MDCP. Occurrence data in breastmilk and formula are scarce, and it is not clear whether exposure is different in breastfed and formula fed infants. Tolerable intakes for such substances are listed in Table 5.4.

Table 5.4: Tolerable intakes of process-generated contaminants and substances migrating from food contact materials

Contaminants	Tolerable intake or other safety parameters	Reference
Acrylamide	No tolerable intake, MOE approach	(EFSA, 2006c)
PAH	No tolerable intake, MOE approach	(EFSA, 2006c)
3-MDCP	No tolerable intake	(JECFA, 2006)
Furan	No tolerable intake, MOE approach	(VKM, 2012)
BPA	0.05 mg/kg bw/day	(EFSA, 2006c)
DEHP	50 µg/kg bw/day	(EFSA, 2005b)
DnBP	10 µg/kg bw/day	(EFSA, 2005b)
DiBP	No tolerable intake	(EFSA, 2005b)

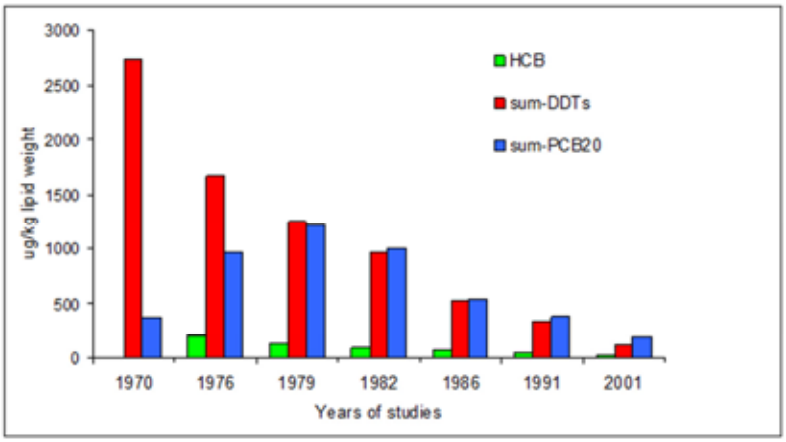

Figure 5.9: Time trends of persistent organic pollutants in Norwegian breastmilk. Source: See Polder *et al.*, 2008 and references herein.

To sum up, the main difference between the contaminants in breastmilk and in infant formula is that breastmilk generally contains higher levels of POPs, while most of the unwanted substances imposed by infant formula or feeding bottles have a shorter half-life and do not accumulate to the same degree.

Several worldwide outbreaks of *Salmonella* infections among infants have been attributed to contaminated, powdered infant formula, resulting in diarrhoea and, in some infants, bacteraemia and meningitis. In addition *Cronobacter* spp. (formerly *Enterobacter sakazakii*) is a rare cause of invasive infection with high death rates in neonates. There have been no registered outbreaks due to infant formula in Norway.

6 Positive health effects associated with consumption of breastmilk

This chapter describes epidemiological studies of positive health effects associated with breastmilk consumption. To comply with the terms of reference, the assessment of benefits is based on systematic reviews and meta-analyses published within the last 10 years.

In general, studies on health outcomes associated with any exposure need to take into account the concentration of components in breastmilk and duration of breastfeeding, since the exposure usually is highly dependent on these two variables. However, the issue of breastfeeding, i.e. whether exclusively breastfed or not, and for how long, is often not clearly defined in all papers. Many studies compare ever breastfed children to those who were never breastfed. Other studies compare infants who are breastfed for less than a given number of weeks or months, often 2-3 months, to those breastfed for longer periods. Few studies treat breastfeeding duration as a continuous variable, which would allow for dose-response analyses. Furthermore, more detailed data would have allowed for assessment of effect of partial breastfeeding with duration up to a year or more, which is the case for many children in Norway. At present, few studies exist with good data on long-term breastfeeding and health effects.

The methodological challenges are discussed in the reviews and meta-analyses used as a basis for sections under 6.2, and in addition in papers by Kramer and Brion (Brion, 2010; Kramer, 2009) in particular, and will also be discussed in chapter 9.

First and foremost, breastmilk provides the baby with a perfectly balanced supply of nutrients with high bioavailability. The nutrient concentrations in breastmilk form the national and international standard for infant formula. Some nutrients in breastmilk also have additional immunological properties. Thus, the health effects of breastmilk relates to nutritious as well as immunological properties not exhibited by infant formula.

Breastmilk influences the immune system of the child in different ways. While numerous immunity-related components such as SIgA, leukocytes, lactoferrin, oligosaccharides and interferons provide protection by preventing pathogens from mucosal adherence, other breastmilk constituents like fatty acids, enzymes, hormones and growth factors may be of importance for health in the long term. Another important function relates to the different immunomodulating components of breastmilk, which contribute to the development of the infant's immune-system (described in section 3.4). Benefits of breastmilk feeding, which may be directly explained by the various nutrients and immune components, are presented below.

6.1 Methodology, the literature assessment of positive health effects associated with breastmilk

The description of positive health effects associated with consumption of breastmilk in this report is based on conclusions from systematic reviews and meta-analyses published within the last 10 years. The main reason for this approach is that the Norwegian Directorate of Health has had a group working in parallel to VKM, covering the benefits of breastfeeding, in the revision of the present-day Norwegian recommendations on infant nutrition (healthy infants 0-12 months). The working group has focused on breastfeeding in relation to health outcomes based on an extensive literature review. Revision of the Norwegian national guidelines on infant nutrition is scheduled to be published in 2014. In addition, the basis for the revised Nordic Nutrition Recommendations (NNR) became available at the end of our

work with the present report (Hornell et al., 2013). The revised NNR is based on a full-scale systematic literature review in compliance with international guidelines. Finally, an updated systematic review from WHO regarding long-term effects of breastfeeding became available in May 2013 (WHO, 2013). The conclusions from the Nordic systematic literature review Hörnell (2013) and WHO (2013) have been added to relevant sections below.

Thus the sections reporting positive health effects associated with consumption of breastmilk are mainly based on (1) *Breast-feeding: A Commentary by the ESPGHAN Committee on Nutrition* (Agostoni et al., 2009), (2) *The influence of maternal, fetal and child nutrition on the development of chronic disease in later life* from the Scientific Advisory Committee on Nutrition in UK (SACN, 2011), (3) *Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th Nordic Nutrition Recommendations* (Hornell et al., 2013) and (4) Long-term effects of breastfeeding: A systematic review (WHO, 2013). An overview is presented in Figure 6.1, and the methods used in these systematic reviews and meta-analyses are described below.

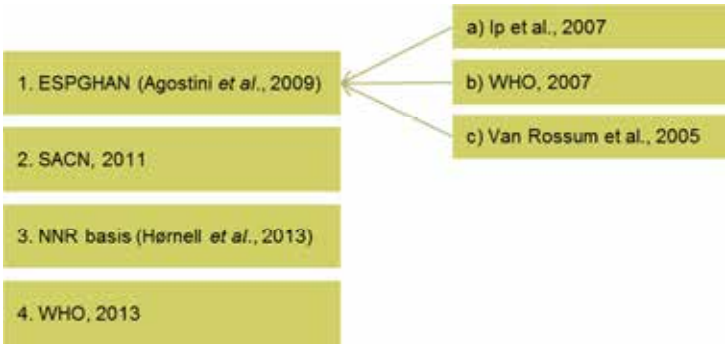

Figure 6.1: Overview of the most important reviews and meta-analyses included in the sections for benefits of breastfeeding under section 6.2. These are also the reviews and meta-analyses included in Table 6.1.

1) *Breast-feeding: A Commentary by the ESPGHAN Committee on Nutrition* (Agostoni et al., 2009) is a medical position paper grading evidence on breastmilk and health outcomes, and includes the following meta-analyses and systematic reviews:

   a) *Breastfeeding and maternal and infant health outcomes in developed countries* (Ip et al., 2007)[33].
   Literature search: Until May 2006.
   Aim: To examine the evidence concerning the benefits and harms of breastfeeding on 13 different short- and long-term infant outcomes (and six maternal health outcomes).
   Type of studies: Systematic reviews, meta-analyses, randomised controlled trials (RCTs), non-RCT comparative trials, prospective cohort, and case-control studies.
   Category of breastfeeding: Breastfeeding, exclusive or partially feeding[34].
   Length of breastfeeding: From zero up to more than one year. Most of the studies did not differentiate between exclusive and partial breastfeeding.

[33]Main paper is from 2007 (Ip, 2007), and a shorter version was presented in 2009 (Ip, 2009).
[34]The majority of the studies did not distinguish between exclusively and partially breastfed infants or explain the difference between "breastfeeding" and "feeding of expressed breastmilk". The term "breastfeeding" was selected for studies in full-term infants. All definitions of "exclusive breastfeeding" were accepted as provided by the study authors of the studies included, but Ip *et al*. qualified their findings by the details regarding those definitions.

Comparator: Formula feeding (preterm or term formula, fortified or unfortified) and different durations of breastfeeding.
Evaluated for methodological quality: Yes.
Grading of evidence: No.

Ip *et al*. include systematic literature reviews and meta-analyses published before May 2006, from developed countries. Associations between differential exposure to breastfeeding and health outcomes were examined; 13 infant and six maternal. For three infant outcomes (asthma, cognitive development and childhood diabetes) previously published systematic reviews were updated. For the rest of the outcomes, previous systematic reviews were summarised, or new systematic reviews were conducted; randomised and non-randomised comparative trials, prospective cohorts, and case-control studies were included. Adjusted estimates were extracted from non-experimental designs. The studies were graded for methodological quality. Only reviews with rigorous criteria for study selection, unbiased data collection, and appropriate methods of analyses were included. Included studies had to have either a comparator arm that evaluated formula feeding or different durations of breastfeeding. This systematic review is later summarised in 2009 (Ip et al., 2009), and the summarised version (2009) will be referred to in the following sections.

b) *Evidence on the long-term effects of breastfeeding* (WHO, 2007).
Literature search: 1966 until March 2006.
Aim: To assess the long-term consequences of breastfeeding on blood pressure, diabetes and related indicators, serum cholesterol, overweight and obesity, and intellectual performance.
Type of studies: The majority were observational studies.
Category of breastfeeding: Most of the reviewed studies compared ever breastfed subjects to never breastfed. Other studies compared breastfeeding less than a given number of months (often 2 to 3 months; including those who were never breastfed) to those breastfed for longer periods. Few studies treated breastfeeding duration as a continuous or ordinal variable with several categories, thus allowing dose-response analyses. Breastfeeding patterns (exclusive, predominant or partial) have rarely been assessed.
Length of breastfeeding: Different durations of breastfeeding, see above.
Comparator: Mostly different types of formula. In some of the oldest cohorts, diluted animal milk may have been used. Different durations of breastfeeding.
Evaluated for methodological quality: Yes.
Grading of evidence: No.

The report from WHO (2007) includes systematic reviews and meta-analyses from 1966 to March 2006. Long-term consequences of breastfeeding on blood pressure, obesity/overweight, total cholesterol, type 2 diabetes and intellectual performance were assessed. Observational and randomised studies were selected, and the following possible sources of heterogeneity were considered: Year of birth (the content of different formulas potentially affecting long-term health), length of recall of breastfeeding, source of information on breastfeeding duration, categories of breastfeeding duration (time and exclusivity), study-setting (nearly all studies from high-income countries/Caucasians). Only studies with internal comparison groups were included. Two reviewers independently evaluated study quality, using a standardised protocol, and disagreement was resolved by consensus rating. Fixed and

random-effects models were used to pool the effect estimates, and a random-effects regression was used to assess several potential sources of heterogeneity.

c) *Quantifications of health effects of breastfeeding. Review of the literature and model simulation* (van Rossum et al., 2005).
Literature search: 1980 until February 2005.
Aim: To give an overview of the literature on breastfeeding and health (including both beneficial and harmful effects) in infants (and mothers).
Type of studies: Mainly observational.
Category of breastfeeding: Every paper was evaluated regarding its quality according to a clear definition of (exclusive) breastfeeding and clear statements about the duration of (exclusive) breastfeeding. Also all publications reported the time of assessing breastfeeding (ideally not longer than 12 months after birth).
Length of breastfeeding: From zero to ≥12 months.
Comparator: Formula feeding, different durations of breastfeeding.
Evaluated for methodological quality: Every paper evaluated on its quality; if a paper did not fulfil every quality requirement the study was excluded.
Grading of evidence: Yes (according to the criteria given by WHO in 2003; (WHO, 2003a)).

Van Rossum *et al*. is a systematic literature review of published epidemiological studies conducted in the general ('western') population, including papers from 1980 to February 2005 published in English or Dutch. In addition, only study populations from Western Europe, North America, Australia and New Zealand were included in the overview. Every paper was evaluated on its quality according to the following points: Time of assessing breastfeeding data (<12 months after birth), clear definition of (exclusive) breastfeeding and clear statement about duration, blind assessment of breastfeeding data and health outcome(s), well-defined health-outcome(s), correction for relevant confounders. Inclusion presupposed that all quality requirements were fulfilled. The strength of evidence for an association as convincing, probable, possible or insufficient was based on WHO criteria (2003) for evidence. In addition the terms "conflicting evidence" and "no evidence" were added.

2) *The influence of maternal, fetal and child nutrition on the development of chronic disease in later life* (SACN, 2011).
Literature search: Systematic literature review originally from 2004, now including additional relevant evidence published before January 1st 2010.
Aim: To review the influence of child nutrition, including growth and development in utero and up to the age of 5 years, on the development of chronic disease in later life. SACN 2011 has focussed attention on cardiovascular disease, diabetes and cancer as the leading causes of mortality, but recognises that early life nutritional exposures may affect many other outcomes.
Type of studies: Systematic reviews and meta-analyses, with additional studies published after 2004 considered separately. In the absence of reviews, relevant retrospective or prospective studies and trials were identified.
Category of breastfeeding: Breastfeeding versus formula feeding, exclusive breastfeeding versus formula feeding.
Length of breastfeeding: Different lengths of breastfeeding.
Comparator: Formula feeding. Different durations of breastfeeding.
Evaluated for methodological quality: SACN 2011 did not evaluate the quality of meta-

analyses or systematic reviews and the heterogeneous nature of this evidence obviated the use of standard grading methods (often employed to judge the quality of evidence) applicable to systematic reviews of the human studies.
Grading of evidence: No.

3) *Breastfeeding, introduction of other foods and effects on health: A systematic literature review for the 5th Nordic Nutrition Recommendations* (Hornell et al., 2013).

Literature search: Until June 2011.
Aim: To review recent scientific data valid in a Nordic setting on the short- and long-term health effects of breastfeeding (duration of both any and exclusive breastfeeding) and introduction of foods other than breastmilk in order to assess the validity of the current Nordic recommendations.
Type of studies: Systemic literature reviews, meta-analyses, prospective cohort-studies (including the PROBIT Study), reports from organisations, committees and similar.
Category of breastfeeding: Different durations and exclusivity of breastfeeding. Studies defining breastfeeding as ever or never were excluded. Retrospective studies with recall feeding data older than 3 years were excluded.
Length of breastfeeding: Varying from zero to ≥1 year.
Comparator: Formula, different durations of breastfeeding¸ introduction of solid foods.
Evaluated for methodological quality: Yes.
Grading of evidence: Yes.

The paper by Hörnell *et al*., which has formed the basis for the new NNR, is a systematic literature review including papers until June 2011. Sixty studies were included and graded for methodological quality using the Quality Assessment Tools (QAT) developed in NNR5 (AMSTAR) (Shea et al., 2007).

The aim of the study was to review recent scientific data on the short-term (infections) and long-term health outcomes (growth and overweight/obesity, blood-pressure, serum cholesterol, diabetes mellitus type 1 and 2, cancer, atopic disease, asthma, IQ and neurological development, coeliac disease and inflammatory bowel disease) related to various degrees and durations of breastfeeding.

The grade of evidence was classified as convincing (grade 1), probable (grade 2), limited-suggestive (grade 3) and limited-no conclusion (grade 4) depending on the number and quality of supporting, non-supporting and contradicting studies.

4) *Long-term effects of breastfeeding. A systematic review* (WHO, 2013).

Literature search: Updated search from 2006 to September 2011. The former search with results published in 2007 (WHO, 2007) included articles from 1966 until March 2006.
Aim: To assess the long-term consequences of breastfeeding on blood pressure, type 2 diabetes, serum cholesterol, overweight and obesity and intellectual performance.
Type of studies: Observational and randomised studies. Studies restricted to outcome measures in infants were excluded from the meta-analyses.
Category of breastfeeding: Some of the reviewed studies compared ever breastfed subjects to never breastfed. Other studies compared breastfeeding less than a given number of months to those breastfed for longer periods. Few studies treated breastfeeding duration as a continuous or ordinal variable with several categories, thus allowing dose-response analyses. Breastfeeding degree (exclusive, predominant or partial) has rarely been assessed.

Length of breastfeeding: Different durations of breastfeeding, see above.
Comparator: Mostly different types of formula. In some of the oldest cohorts (first decades of the 20th century), diluted whole cow's milk or top milk with high sodium concentrations and levels of cholesterol and fatty acids that were similar to those in mature breastmilk, may have been used. Later, commercially prepared formulas changed in composition, until today, where formulas have levels of nutrients (more) similar to those in breastmilk.
Evaluated for methodological quality: Yes.
Grading of evidence: No. However, in addition to the studies forming the basis for the WHO 2007 version, 60 additional studies have been included in the 2013 review, allowing meta-analysis for all studies as well as meta-analysis for the studies judged as "high quality studies". High quality studies include those with larger sample sizes and adjustment for confounding variables relevant to each outcome.

The report from WHO (2013) is an updated systematic review with meta-analyses, based on the former version published in 2007, now including studies from 1966 until September 2011. Long-term consequences of breastfeeding on blood pressure, obesity/overweight, total cholesterol, type 2 diabetes and intellectual performance were assessed. Observational and randomised studies were selected, and the following possible sources of heterogeneity were considered: Year of birth (the content of different formulas potentially affecting long-term health), length of recall of breastfeeding, source of information on breastfeeding duration, categories of breastfeeding duration (time and exclusivity), study-setting (nearly all studies from high-income countries/Caucasians). Only studies with internal comparison groups were included. Two reviewers independently evaluated study quality, using a standardised protocol, and disagreement was resolved by consensus rating. Statistically, fixed and random-effects models were used to pool the effect estimates, and a random-effects regression was used to assess several potential sources of heterogeneity.

6.1.1 Additional reviews, meta-analysis or single studies

In addition to the reviews and meta-analyses presented above, some other reviews or single studies of high quality have been included in sections 6.2.1-6.2.6 if recent publications of relevance to the benefit assessment have appeared, or the above described reviews and meta-analyses have not commented on diseases/conditions of interest.

Included several places
(Kramer and Kakuma, 2012): There are few studies investigating advantages of exclusive breastfeeding beyond 3, 4 or 5 months of age. In the updated literature review by Kramer & Kakuma (2012) *Optimal duration of exclusive breastfeeding,* an update of the reviews from 2002 and 2004, this issue was specifically in focus.

Included in section 6.2.1 Cognitive development
(Brion et al., 2011): See above.
(Kramer et al., 2008): Follow-up of the children 6.5 years from the cluster-randomised trial described below (included in Agostini (ESPGHAN, 2009) and Hörnell (NNR, 2012)).

Included in section 6.2.2 Infections
The Generation R Study is a Dutch prospective birth cohort study taking place in Rotterdam. The children form a prenatally recruited birth cohort and will be followed until young adulthood. In total, 9778 mothers with a delivery date from April 2002 until January 2006

were enrolled in the study. Of all eligible children at birth, 61% participate in the study. A large part of the study cohort consists of ethnic minorities (see http://www.generationr.nl/researchers.html).

Included in section 6.2.3 Immune response-associated diseases
(Brandtzaeg, 2010a): Review on food allergy with focus on immunological mechanisms as well as the scientific basis for future food allergy prevention.
(Nwaru et al., 2010; Nwaru et al., 2013): A prospective birth cohort study including 994 and 3781 children respectively, with susceptibility to type 1 diabetes mellitus. The aim was to examine the relationship between age of introduction of solid foods during the first year and allergic sensitisation at 5 year.
(Greer et al., 2008): Statement regarding effects of early nutritional interventions on the development of atopic disease in infants and children.
(SACN and COT, 2011) *(Scientific Advisory Committee on Nutrition/Committee on Toxicity Joint Statement, March 2011)*: Timing of introduction of gluten into the infant diet.

Included in section 6.2.4 The metabolic syndrome
(Brion et al., 2011): A novel approach for improving causal inference in observational studies by comparing cohorts from high-income with low- or middle-income countries; where confounding structures differ. This approach was applied for assessing the confounding structure of breastfeeding by socio-economic position in the British Avon Longitudinal Study of Parents and Children (ALSPAC; N~5000) and Brazilian Pelotas 1993 cohorts (N~1000). Analyses were extended to include results from a meta-analysis of five low- or middle-income countries (N~10 000).
(Gillman et al., 2006): Within family analysis surveying 5614 siblings aged 9-14 years and their mothers; these children were a subset of participants in the Growing Up Today Study in USA. Prevalence of overweight in siblings who were breastfed longer than the mean duration of their sibship was compared with those who were breastfed for a shorter period.

Included in section 6.2.6 Sudden infant death syndrome
(Hauck et al., 2011): Meta-analysis including studies from 1966-2009, revealing 18 studies, measuring the association between breastfeeding and sudden infant death syndrome.

6.1.2 The PROBIT Study from Belarus

Although included in the reviews of ESPGHAN, SACN, Hörnell and WHO, one breastfeeding study deserves special attention, i.e. the Promotion of Breastfeeding Intervention Trial (PROBIT) conducted in the Republic of Belarus. It is the only RCT of its kind, it is very large, and it allows for investigating the impact of exclusive versus partial breastfeeding.

The PROBIT Study is a cluster-randomised trial including 17 046 mother-infant pairs at 31 maternity hospitals and polyclinics, with 96.7% and 81.5% follow-up at 1 and 6.5 years, respectively (Kramer et al., 2001; Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Mazer, 2007). The aim of the study was to assess the effect of structured lactation counselling. The intervention sites in this trial received qualified lactation and postnatal breastfeeding support based on the Baby-Friendly Hospital Initiative of the WHO and United Nations Children's fund, while the control intervention received conventional counselling on infant feeding practices. The intervention led to increased exclusive (44.4% vs 6.4%; $p<0.001$ at 3 months) and partial breastfeeding rates. It is important to be aware that non-breastfed infants were excluded from the study. Thus the study does not allow for comparison of non-breastfed versus breastfed children – the study

compares longer and more exclusive breastfeeding against shorter and less exclusive breastfeeding. Furthermore, the mothers, in general, were highly educated, and maternity leave in Belarus is three years. Thus, the children would have a different exposure pattern to microbes than children entering kinder garden at an earlier age (Martens, 2012).

All included mothers initiated breastfeeding, and although the differences between the groups as to exclusive breastfeeding were impressive, the difference in *any* breastfeeding was less dramatic, as illustrated in the Figure 6.2. Both groups represent a mixture of breastfeeding practises. A consequence of this is that the effect of breastfeeding itself on outcomes is underestimated and power is reduced.

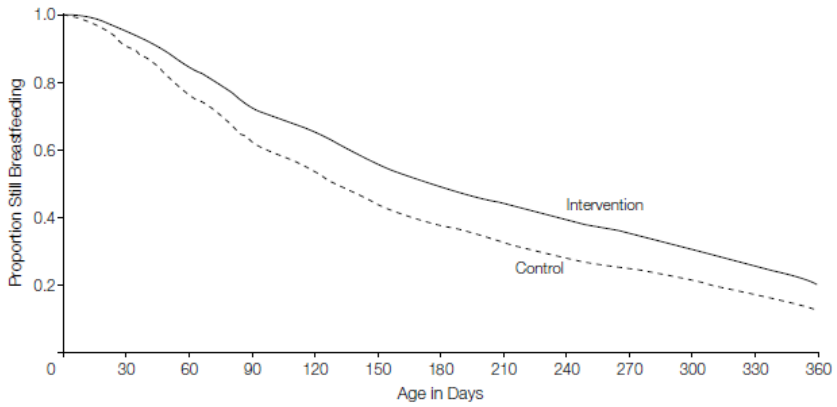

Figure 6.2: Comparison of proportion of infants still breastfeeding (to any degree) during year of follow-up. Source: Kramer *et al*., 2001.

The study was designed to assess the effects of breastfeeding promotion on breastfeeding duration and exclusivity, and the primary health outcome was gastrointestinal infections. Secondary outcomes were respiratory tract infections, atopic eczema and wheezing during the first year of life. Also results from follow-ups at 6.5 and 11 years have been published. As to statistical methodology, all results were handled as "intention to treat".

Results from the PROBIT Study have been handled in two ways, statistically speaking:

a) As originally designed, i.e. a cluster randomised controlled study
b) As an observational study, pooling all data and investigating outcomes according to exclusive vs partial breastfeeding

Results from these two ways of handling the data more or less overlap, but we choose to report them separately here.

RCT results

At 1 year of age, results from the RCT showed that children in the intervention groups had a significant reduction in the risk of one or more gastrointestinal tract infections (9.1% vs 13.2%; adjusted OR, 0.60; 95% CI, 0.40-0.91) and of atopic eczema (3.3% vs 6.3%; adjusted OR, 0.54; 95% CI, 0.31-0.95), but no significant reduction in respiratory tract infections (intervention group, 39.2%; control group, 39.4%; adjusted OR, 0.87; 95% CI, 0.59-1.28).

At 6.5 years of age, the intervention group had higher means on all of the Wechsler Abbreviated Scales of Intelligence measures, with cluster-adjusted mean differences (95%

confidence intervals) of +7.5 (+0.8 to +14.3) for verbal IQ, +2.9 (-3.3 to +9.1) for performance IQ, and +5.9 (-1.0 to +12.8) for full-scale IQ. Teachers' academic ratings were significantly higher in the intervention group for both reading and writing (Kramer et al., 2008).

Other health outcomes studied in the 6.5 years follow-up were child height, weight, adiposity and blood pressure (Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Collet et al., 2007), cited in Agostini (ESPGHAN, 2009), SACN and Hörnell (2013) and risk of allergy and asthma (Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Mazer, 2007), cited in Hörnell (2013). In these studies, no differences between the intervention and control groups were observed.

In a recent publication investigating adiposity and Insulin-like Growth Factor at age 11.5 years in the PROBIT Study, no differences were observed between the two groups (Martin et al., 2013). The authors conclude that population strategies to increase the duration and exclusivity of breastfeeding are unlikely to curb the obesity epidemic.

Results from pooling the data, treating the study as an observational trial

When pooling the data, one can compare the impact of exclusive versus non-exclusive breastfeeding and breastfeeding length across all groups participating in the study. Five publications from the PROBIT Study have treated the data this way. A number of confounding effects were controlled for, like geographic region, living urban or rural, hospital at birth, maternal education, number of siblings, birth weight and length and weight/length gain from birth to 3 months age.

At 1 year of age, the observational pooling of data confirmed the findings from the RCT that exclusive breastfeeding for 6 months are associated with a lower risk of gastrointestinal infections (Kramer et al., 2003). This publication was instrumental when WHO in 2004 revised their infant feeding recommendations and encouraged exclusive breastfeeding for the first 6 months of life. The benefit, when analysing the data this way, was limited to the actual period the child was exclusively breastfed (Kramer et al., 2003). No significant difference was observed for atopic eczema or recurrent wheezing, in contrast to what was found in the RCT data.

Investigating the impact of 3 vs 6 months of exclusive breastfeeding on health at 6.5 years, very few differences were found. In fact, the children exclusively breastfed for at least 6 months had slightly higher BMI, triceps skinfold thickness and hip circumference (Kramer et al., 2009), although this probably is due to residual confounding. The results at 6.5 years for cognitive development, including verbal, performance and full-scale IQ, showed no statistical difference between 3 and 6 months of exclusive breastfeeding.

Conclusion, the PROBIT Study

For the purpose of the present benefit risk assessment, the most important finding in the PROBIT Study was that exclusive breastfeeding for 6 months substantially reduceed the risk of gastrointestinal infections. There are uncertainties as to whether the effect lasted beyond the period of exclusive breastfeeding. At 6.5 years of age, the intervention group in the RCT study scored substantially higher in various IQ tests than the control group, but this result was not found when using the observational design. As to other health outcomes, irrespective of being measured at 1 or 6.5 years, the study found little or no difference between the groups who were breastfed exclusively for 3 compared to 6 months.

6.2 Beneficial health outcomes reported in the literature

Table 6.1 shows a list of health outcomes based on conclusions in the evidence based reviews Ip (2009), WHO (2007) and van Rossum (2005), and is modified from ESPGHAN (Agostoni et al., 2009). Our modification from ESPGHAN is an inclusion of results of health outcomes from SACN (2011), Hörnell (2013) and WHO (2013). The list of health outcomes is retrieved from the available documentation on associations between breastmilk consumption and health outcomes. The arrows represent reduced risk of disease, and may be regarded as a reflection of present scientific knowledge about breastmilk and health outcomes. It should be emphasised that the content in Table 6.1 is a summary of results from previous evidence based reviews and is not evaluations done for this report. It should also be noted that the systematic reviews and meta-analyses do not differentiate on length of breastfeeding.

Table 6.1: Comparison of recent systematic reviews and meta-analyses on children's health effects of breastfeeding in developed countries (adapted from Table 1 in ESPGHAN (Agostoni, 2009). As for WHO (2013) two numbers are given; the first represents the pooled effect for all studies included, and the second represents the high quality studies only.

Main results in infants	WHO, 2007	Ip, 2007	van Rossum, 2005	SACN, 2011*	Hörnell,2013 (NNR)*	WHO, 2013*
Intelligence test score	↑ MD 4.9 (2.97; 6.92)[a]	-	-	-	Probable ↑	↑ MD 3.45 (1.92; 4.98) [a] ↑ MD 2.19 (0.89; 3.50) [b]
Intellectual and motor development	-	-	Probable evidence ↑	-	-	-
Otitis media	-	↓	Convincing evidence ↓	-	Convincing ↓	-
Gastrointestinal infections	-	↓	Convincing evidence ↓	-	Convincing ↓	-
Respiratory infections	-	-	Possible evidence ↓	-	Convincing ↓	-
Severe lower respiratory tract infections	-	↓	-	-		-
Atopic disease	-	-	Probable evidence ↓	-	Limited, no conclusion	-
Atopic dermatitis	-	↓	Eczema, Probable evidence ↓	-	Limited, no conclusion	-
Asthma (young children)	-	↓	Probable evidence ↓	-	Limited, no conclusion	-
Wheezing	-	-	Probable evidence ↓	-	Limited, no conclusion	-
Obesity	↓ OR 0.78 (0.72; 0.84)[c]	↓	Convincing evidence ↓	↓	Convincing ↓	↓ OR 0.76 (0.71; 0.81)[c] ↓ OR 0.88 (0.83; 0.93)[d]
Coeliac disease	-	-	-	-	Probable ↓	-
Type 1 diabetes		↓	Possible evidence ↓	No evidence	Probable ↓	-

Main results in infants	WHO, 2007	Ip, 2007	van Rossum, 2005	SACN, 2011*	Hörnell,2013 (NNR)*	WHO, 2013*
Type 2 diabetes	↓ OR 0.63 (0.45; 0.89)[c]	↓	-	↓	Probable ↓	↓ OR 0.66 (0.49; 0.89)[c]
Childhood cancer	-	↓	Possible evidence ↓	↓[1]	Limited, suggestive ↓	-
SIDS	-	↓	Insufficient evidence	-	-	-
NEC	-	↓	-	-	-	-
Cardiovascular diseases	-	Not clear	No evidence	No evidence[2]	-	-
Crohn's disease	-	-	Possible evidence ↓	-	Probable ↓	-
Ulcerative colitis	-	-	Insufficient evidence	-	Probable ↓	-
High blood pressure	↓ Systolic MD-1.2mmHg (-1.72; -0.70) [a] ↓ Diastolic MD-0.49mmHg (-0.87, -0.11) [a]	-	Convincing evidence ↓	↓	Probable ↓	↓ Systolic MD-1.02mmHg (-1.45; -0.59)[a] ↓ Systolic MD-0.71mmHg (-1.24; -0.19)[b] ↓ Diastolic MD-0.37mmHg (-0.71; -0.04)[a] ↓ Diastolic MD-0.27mmHg (-0.64; 0.09)[b]
Serum cholesterol	Adulthood ↓ MD-0.18mmol/L (-0.30; -0.06)[a] Children and adolescents NS	-	-	↓	Probable ↓	No evidence

*Not included in Table 1 in ESPGHAN (Agostoni et al., 2009).
[a]Mean difference (MD) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies.
[b]Mean difference (MD) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies, restricted to studies considered high quality
[c]Odds ratio (OR) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies.
[d]Odds ratio (OR) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies, restricted to studies considered high quality.
[1]Reduced risk for acute lymphoblastic leukemia, Hodgkin's disease and neuroblastoma.
[2]SACN 2011 has looked into cardiovascular mortality whereas the others have reported morbidity.
- (=Not investigated).
MD: Mean difference.
↓Reduced risk.
↑Increased score.

As pointed out in the introduction to this chapter, many studies compare ever breastfed children to those who were never breastfed. Other studies compare infants who are breastfed for less than a given number of weeks or months, often 2-3 months, to those breastfed for longer periods. Few studies treat breastfeeding duration as a continuous variable, which would allow for dose-response analyses. Furthermore, more detailed data would have allowed for assessment of effect of partial breastfeeding with duration up to a year or more, which is the case for many children in Norway. The results in the Table 6.1 are described more detailed in the sections below.

6.2.1 Cognitive development

Several observational studies have found a positive correlation between breastmilk consumption and favourable neurodevelopment (reviewed in van Rossum 2005, Espghan 2009 and WHO 2007). However, socio-economic factors, parental intelligence and HOME score are vital confounding factors. Of interest is therefore a study from The Philippines, where breastfeeding was inversely correlated to socio-economic status and other healthy maternal behaviours. Cognitive scores at 8.5 years were higher for infants breastfed longer (1.6 points and 9.8 points higher among normal birth weight and low birth weight infants respectively, breastfed for 12-18 months versus <6 months) (reviewed in Agostini (ESPGHAN, 2009)). According to the authors, using the same novel approach as described below for *Overweight/obesity* for improving causal inference in observational studies, earlier findings that breastfeeding may have causal effects on IQ are supported (Brion et al., 2011). Long-chain polyunsaturated fatty acids, docosahexanoic acid (DHA) and arachidonic acids (AA) are important for brain development and accumulate in the brain most rapidly in the last trimester of pregnancy and the first months after birth. This is therefore a potential mechanism for an effect of breastfeeding on intellectual development. An intervention study in the United Kingdom in the early 1980s involved preterm infants randomised to receive either breastmilk or formula. At 7.5-8 years of life, children who had consumed breastmilk had an 8.3 point advantage in IQ after adjustment for differences in mother`s education and social class. Both breastmilk and formula were given by tube in order to eliminate a possible benefit of the breastfeeding process (reviewed in van Rossum (2005) and WHO (2007)).

The PROBIT Study (for further details, see section 6.1.2) cannot be used to compare effects of breastmilk versus formula, only varying durations of breastfeeding, as breastfeeding rates were high in both control and intervention groups. Despite this fact, higher means of verbal IQ as measured by the Wechsler Abbreviated Scales of Intelligence was significantly higher in the intervention group (verbal IQ +7.5, (+0.8-14.3)), (Kramer et al., 2008) reviewed in Agostini (ESPGHAN, 2009) and in Hörnell (Hornell et al., 2013).

In the systematic literature review by Hörnell *et al*. it is concluded that there is probable evidence (grade 2) that breastfeeding is beneficial for IQ and developmental scores of children, with increasing benefit with increasing duration (Hornell et al., 2013).

WHO (2013) found an increase in test scores both in the pooled analysis of all studies, as well as in the high-quality studies included. They conclude that there is a strong evidence of causal effect of breastfeeding on IQ, although the magnitude of this effect seems to be modest (WHO, 2013).

6.2.2 Reduction of infectious diseases in developed countries

A reduced risk of lower respiratory infections, especially the severe ones, as well as gastroenteritis and otitis media in breastfed versus non-breastfed children is well documented (Agostoni et al., 2009; Ip et al., 2009; van Rossum et al., 2005). Such a reduced risk is demonstrated when comparing exclusive with partial breastfeeding or formula feeding and when comparing different lengths of exclusive or partial breastfeeding with formula feeding.

In the recent systematic literature review by Hörnell *et al*. (2013) it is concluded that the evidence is convincing (grade 1) that breastfeeding protects infants in industrialised countries against overall infections, acute otitis media and gastrointestinal and respiratory tract infections. The magnitude of the effect varies depending on the specific outcome and the exclusiveness of breastfeeding (Hornell et al., 2013).

Some examples of dose-response associations with infectious outcomes and breastfeeding - both regarding exclusivity and duration from the included literature, are described below.

Gastroenteritis

Raisler *et al*., included in van Rossum *et al*. (2005), compared risk of diarrhoea related to degree of breastfeeding 0-6 months in a cohort with 7092 children from USA. Using formula fed infants as reference, the odds ratio (OR) for gastroenteritis was 0.54 (0.43-0.66) for exclusively breastfed infants at 6 months. Results for partially breastfed infants (breastfeeding<formula feeding), OR=0.95 (0.78-1.16) or (breastfeeding>formula feeding) OR=0.83 (0.69-0.99) indicated a dose-response relationship (Raisler et al., 1999).

A similar dose-response relationship between breastfeeding and risk of gastroenteritis was found in a cohort from USA study including 1743 children 0-7 months. Scariati *et al*., included in van Rossum *et al*. (2005), compared the risk of diarrhoea with regard to exclusivity and duration of breastfeeding. Compared to exclusive breastfeeding for two to 7 months, the OR for gastroenteritis was 1.8 ($p<0.05$) for formula fed infants. Partial breastfeeding between two and 7 months of age gave an intermediate OR (Scariati et al., 1997).

In the Generation R study, Duijts *et al*. found that compared with never breastfed, those exclusively breastfed for 4 months and thereafter partially breastfed, had a lower risk of gastrointestinal infections until 6 months (adjusted OR (95% CI): 0.41 (0.26, 0.64). No significant differences were found for partial breastfeeding up to 6 months compared to formula fed children (Duijts et al., 2010), see also Table 6.2.

Results from the PROBIT Study showed that infants in the intervention group (with a higher degree of both partly and exclusive breastfeeding) had a significantly reduced risk of one or more gastrointestinal infections during the first 12 months compared to the control group (adjusted OR 0.60; 95% CI 0.40-0.90) (Kramer et al., 2001), cited in Agostini (ESPGHAN, 2009) and SACN (2011).

When comparing infants exclusively breastfed for 6 months with infant exclusively breastfed for 3 months in Kramer and Kakuma (2012), a reduced risk of one or more gastrointestinal tract infections was observed (adjusted OR 0.60, 95% CI 0.40-0.91) (Kramer and Kakuma, 2012), cited in Hörnell (2013).

Severe lower respiratory tract infection

In a meta-analysis by Bachrach *et al*., reviewed in Ip *et al*. (2009), it was reported an overall reduced risk (summary relative risk 0.28, 95% CI 0.14-0.54) of hospitalisation secondary to lower respiratory tract diseases in infants less than 1 year of age who were exclusively breastfed for 4 months or more compared with those who were formula fed. The results remained consistent after adjustment for possible confounders like smoking and socio-economic status (Bachrach et al., 2003).

In the Generation R study, Duijts *et al*. also found that compared with never breastfed, those exclusively breastfed for 4 months and thereafter partially breastfed had a lower risk of lower respiratory tract infections until 6 months (OR 0.50 (0.32, 0.79). Furthermore, they found a lower risk of lower respiratory tract infections between 7 to 12 months, adjusted OR 0.46 (95% CI: 0.31, 0.69) (Duijts et al., 2010), see also Table 6.2.

Table 6.2: Duration of exclusive breastfeeding and risk of infectious diseases in the first year of life in the Generation R study (Duijts et al., 2010).

Duration of Breastfeeding	≤6 months, 0R (95% CI)			7-12 months, 0R (95% CI)		
	URTI	LRTI	GI	URTI	LRTI	GI
Never breastfed	1.00	1.00	1.00	1.00	1.00	1.00
Partially for <4 months, not breastfed thereafter	0.96 (0.76-1.21)	1.01 (0.68-1.50)	0.77 (0.52-1.15)	0.98 (0.75-1.27)	0.78 (0.55-1.09)	0.94 (0.68-1.28)
Partially for 4-6 months	0.85 (0.67-1.07)	0.89 (0.60-1.34)	0.72 (0.48-1.09)	1.03 (0.76-1.39)	0.67 (0.47-1.02)	1.01 (0.71-1.44)
Exclusively for 4 months, not breastfed thereafter	0.70 (0.41-1.20)	0.39* (0.12-1.31)	1.01 (0.44-2.38)	1.79 (0.99-3.14)	0.45 (0.17-1.19)	1.16 (0.59-2.27)
Exclusively for 4 months, partially breastfed thereafter	0.65 (0.51-0.83)*	0.50 (0.32-0.79)*	0.41 (0.26-0.64)*	0.88 (0.66-1.16)	0.46 (0.31-0.69)*	1.07 (0.77-1.49)
Exclusively breastfed for 6 months	0.37 (0.18-0.74)*	0.33 (0.08-1.40)	0.46 (0.14-1.59)	0.63 (0.30-1.33)	0.54 (0.18-1.58)	0.93 (0.42-2.06)
P	<.01	<.01	<.01	NS	<.01	NS

Reference group is never breastfed infants. Values were adjusted for maternal education, ethnicity, smoking, gestational age, birth weight, siblings, and day care attendance. Complete information about duration of exclusive breastfeeding, infectious diseases, and all confounders until the age of 6 months was available for 3504 infants (upper respiratory tract infections), 3489 infants (lower respiratory tract infections), and 3438 infants (gastrointestinal infections). For ages 7 to 12 months, complete information on breastfeeding and infectious diseases was available for 2958 infants (upper respiratory tract infections), 3027 infants (lower respiratory tract infections), and 2938 infants (gastrointestinal infections). URTI indicates upper respiratory tract infections; LRTI, lower respiratory tract infections; GI, gastrointestinal infections; NS, not significant. *P<.01.

Acute otitis media

In the meta-analysis by Ip *et al*. (2009), infants exclusively breastfed for more than 3 or 6 months were compared with exclusively bottle-fed, and the pooled OR of acute otitis media was 0.50 (95% CI 0.36-0.70). When comparing ever with never breastfed children, the pooled adjusted OR of acute otitis media was 0.77 (95% CI 0.64-0.91) (Ip et al., 2009).

In the Generation R study, Dujits *et al*. investigated the effect of breastfeeding on upper respiratory tract infections, including ear and throat infection: Compared to formula fed children, children exclusively breastfed for 4 months and thereafter partially breastfed showed an OR for getting upper respiratory tract infections of 0.65 (95% CI 0.51-0.83). Exclusive

breastfeeding for 6 months increased this effect; OR 0.37 (95% CI 0.18-0.74) (Duijts et al., 2010).

In the study of Scariati *et al*., included in van Rossum *et al*. (2005), infants were classified as exclusively breastfed; high, middle, or low mixed breast-and formula fed or exclusively formula fed from 2 to 7 months. The risk of ear infection was significantly higher among formula fed children (RR 1.7), and a dose-response protective effect of breastmilk was demonstrated (Scariati et al., 1997).

6.2.3 Reduction of immune response-associated diseases

Asthma and allergy

The incidence of allergic diseases such as subtypes of asthma, rhinitis, atopic dermatitis and food allergies has increased over the last decades. Although allergic diseases have a clear genetic basis, environmental factors, including early infant nutrition, may exert an important influence on their development. The mainstream theory for the postulated rise in the prevalence of such disorders is the extended hygiene hypothesis. This hypothesis underscores the role of environmental changes in immune-mediated disorders and explains why these conditions represent an increasing burden in developed countries. In essence, the notion is that modern hygienic measures introduced in affluent societies have deprived infants of adequate immunological stimuli (Brandtzaeg, 2010a).

The role of exclusive breastfeeding in the prevention of allergic diseases is not clear. Conflicting findings have been reported from several studies investigating whether prolonged and exclusive breastfeeding increases, decreases or has no influence on the risk of asthma and allergy (Agostoni et al., 2009; Ip et al., 2009; Kramer et al., 2001; Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Mazer, 2007; Kramer and Kakuma, 2012; Nwaru et al., 2010; van Rossum et al., 2005)[35]. Minor dys-regulations of both innate and adaptive immunity (especially low levels of IgA, see section 3.4) have been observed in children with multiple food allergies. In line with these observations, exclusive breastfeeding up to the age of at least 4 months is associated with a reduced risk of allergy both in families with and without a predisposition to allergy (Agostoni et al., 2009; Brandtzaeg, 2010a; Greer et al., 2008; van Rossum et al., 2005).

However, it is hypothesised that introduction of foreign proteins in taste samples or complementary foods together with breastmilk before 6 months of age may promote tolerance induction. Results from a recent study support this, as introduction of i.e. cereals at 5 to 5.5 months (but not earlier than 4.5 months) was shown to reduce the risk of asthma and allergic rhinitis at the age of 5 years. A total breastfeeding period less than 9.5 months was however associated with an increased risk of non-atopic asthma (Nwaru et al., 2013). On the other hand, environmental antigens, including food proteins, present at low levels in breastmilk apparently can provide the necessary allergens for oral tolerance induction.

Several clinical studies are under way to identify the optimal timing of solid food introduction to prevent allergic disease.

As mentioned in section 6.1.2 about the PROBIT Study, they found no differences between the intervention and control groups at 6.5 years as to asthma and allergy ((Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Mazer, 2007) cited in

[35]Kramer (2001) is cited in Agostini (ESPGHAN, 2009) and SACN, 2011. Kramer (2007 and 2012) is cited in Hörnell (NNR, 2012).

Hörnell (NNR, 2012). However, both the intervention- and control group in this study had a high prevalence of breastfeeding and the main difference between them was the degree of exclusive breastfeeding.

In the systematic literature review by Hörnell et *al*. it is concluded that the evidence linking breastfeeding or introduction of solid foods to asthma and wheeze is inconsistent, and the evidence is limited and no conclusions can be drawn (grade 4) (Hornell et al., 2013).

Type 1 Diabetes

Two recent meta-analyses suggest that breastfeeding for at least 3 months reduces the risk of childhood type 1 diabetes, and the effect may persist later in life (Ip et al., 2009; van Rossum et al., 2005). Two meta-analyses included in the study by Ip report an OR of 1.23 (95% CI 1.12, 1.25) and 1.43 (95% CI 1.15, 1.77) respectively for the risk of type 1 diabetes if breastfeeding duration was less than 3 months compared to more than 3 months. It is not clear whether other environmental factors, such as early introduction of gluten and cow's milk protein, influence the risk of type 1 diabetes. Cow's milk has been regarded as the main risk factor (Agostoni et al., 2009). The suggested association between breastfeeding and type 1 diabetes may be due to breastmilk *per se* or to the associated delayed introduction of gluten and/or cow's milk protein.

In the systematic literature review by Hörnell *et al*. it is concluded that the evidence for any breastfeeding having a protective effect against type 1 and type 2 diabetes is probable (grade 2). The evidence for a stronger protective effect for longer duration of breastfeeding is still limited but suggestive (grade 3) (Hornell et al., 2013).

Coeliac disease

In a review of six observational studies, it was suggested that breastfeeding could confer protection against development of coeliac disease (Agostoni et al., 2009). Except for one small study, the risk reduction was dose-dependent. However, it is still unclear whether the protective effect is permanent or delays the onset of the disease. A joint statement from SACN and the Committee on Toxicity (COT) in 2011 concluded that introduction of gluten-containing foods before 3 months might be linked to an increased risk of coeliac disease (SACN and COT, 2011). They also stated that the evidence is not strong enough to support a recommendation to introduce gluten before 6 months of age. Thus the evidence currently available is not strong enough to make specific recommendations about the appropriate timing of introduction of gluten. There might be an increased chance of infants developing coeliac disease if they are not breastfed when gluten is introduced into the diet (SACN and COT, 2011).

In the systematic literature review by Hörnell *et al*. it is judged to be probable evidence (grade 2) for breastfeeding as a protective factor for coeliac disease if gluten is introduced in small amounts while still breastfeeding. Also this report states that it is, unclear whether the protection only delays the onset of coeliac disease or if it provides permanent protection (Hornell et al., 2013).

Inflammatory bowel disease

The potential benefit of breastmilk regarding the risk of developing inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is not clear. The Dutch meta-

analysis pointed to evidence of a protective effect of breastfeeding with regard to Crohn's disease and to insufficient evidence for ulcerative colitis (van Rossum et al., 2005).

In the systematic literature review by Hörnell *et al*. it is judged that there is probable evidence (grade 2) that breastfeeding provides protection against IBD (both Crohn's and ulcerative colitis) (Hornell et al., 2013).

6.2.4 The metabolic syndrome

The concept that early nutrition may influence or reduces the risk of later cardiovascular disease in humans is supported by the findings that breastfeeding may influence overweight and obesity, cholesterol metabolism, blood pressure, cardiovascular disease and insulin resistance.

Overweight/obesity

Findings from recent meta-analyses based on studies from the developed world suggest that breastfeeding may reduce the risk of overweight and obesity (Agostoni et al., 2009; Ip et al., 2007; SACN, 2011; van Rossum et al., 2005; WHO, 2007). However, although known confounding variables are controlled for, residual confounding is still a concern. Accordingly there is a need for methodological approaches where control of possible confounders is embedded. A novel approach has been explored for improving causal inference in observational studies by comparing cohorts from high-income with low- or middle-income countries, where confounding structures differ. By this approach, breastfeeding was not strongly associated with BMI (Brion et al., 2011). Another approach is sibling studies. As siblings have similarities in their genetic make-up and grow up in the same socio-economic environment, a number of important confounders are reduced. The association between different durations of breastfeeding on the risk of obesity was investigated in more than 5600 siblings. Increased breastfeeding duration was associated with reduced risk of obesity (Gillman et al., 2006).

As mentioned in section 6.1.2 about the PROBIT Study, they found no differences between the intervention and control groups at 6.5 years (Kramer, Matush, Vanilovich, Platt, Bogdanovich, Sevkovskaya, Dzikovich, Shishko, Mazer, 2007), cited in Hörnell (2013) or 11.5 years (Martin et al., 2013) in prevalence of obesity.

In the systematic literature review by Hörnell *et al*. (not including the recent 2013 PROBIT Study on overweight) it is concluded that growth in infancy (length and height) varied only a little between those exclusively breastfed for 4 months or 6 months. Nonetheless, it is concluded that there is probable evidence (grade 2) that exclusive breastfeeding for more than 4 months is associated with slower weight gain during later infancy compared with those exclusively breastfed for less than 4 months. The evidence is convincing (grade 1) that longer duration of exclusive breastfeeding or any breastfeeding is associated with a protective effect against overweight and obesity in childhood and adolescence. With regard to the association with overweight/obesity in adulthood, due to a scarcity of strong studies, it is judged that the evidence is limited-suggestive (grade 3) for a protective effect of breastfeeding (Hornell et al., 2013).

In the updatet WHO review; a significant reduction was found both in the pooled analyses of all studies (24% reduction) as well as in the high quality studies (12% reduction). The report

concludes that breastfeeding may provide some protection against overweight and obesity, but residual confounding cannot be ruled out (WHO, 2013).

Lipid metabolism

During early childhood, breastfed children have higher levels of total cholesterol than formula fed, reflecting the high fat content in breastmilk, which is of importance for development of the infant's central nervous system. This difference seems, however, to be temporary. Total cholesterol seems to be significantly lower among adults who had been breastfed in infancy (SACN, 2011; WHO, 2007). These studies suggest that long-term changes in cholesterol metabolism are likely to occur in breastfed individuals.

In the systematic literature review by Hörnell *et al*. it is judged to be (at least) probable evidence (grade 2) for a small reduction on blood cholesterol in adulthood from breastfeeding. There is, however, less evidence for an association between breastfeeding and blood cholesterol in childhood (Hornell et al., 2013).

In the updated review by WHO (2013), the earlier observed significant lower cholesterol level in adults was no longer demonstrable (WHO, 2013).

Blood pressure

In some reviews and meta-analysis, a small but significant association between breastfeeding and reduced blood pressure, especially systolic, has been found (Agostoni et al., 2009; Ip et al., 2009; SACN, 2011; WHO, 2007). The magnitude of such an eventual association has been estimated to be similar to the effect of salt restriction (Agostoni et al., 2009).

In the systematic literature review by Hörnell *et al*. it is judged that there is (at least) probable evidence (grade 2) for this association (Hornell et al., 2013).

The updated WHO systematic review (2013) found a small effect on systolic blood pressure, but conclude that the protective effect of breastfeeding, if any, is too small to be of public health significance (WHO, 2013).

Cardiovascular disease

Contrary to the published data on isolated risk factors (lipids, type 2 diabetes and blood pressure), findings suggesting a direct association of breastmilk on cardiovascular disease are less consistent. However, the existing studies are all based on old birth cohorts, with limited, but good quality data on breastfeeding status. In addition, the infant formula types used at that time (around 1920) differs from the present formulas. There is at present no convincing evidence that breastfeeding influences cardiovascular morbidity and mortality (Agostoni et al., 2009; Ip et al., 2009; SACN, 2011; van Rossum et al., 2005).

Type 2 Diabetes

Results from studies with different study designs suggest that breastfeeding is associated with a reduced risk of type 2 diabetes in later life compared with formula feeding (Ip et al., 2009; SACN, 2011; WHO, 2007). Possible mechanisms for the protective effect may be the content of long-chain polyunsaturated fatty acids in breastmilk as well as the lower basal and postprandial concentrations of insulin and neurotensin in breastfed children (WHO, 2007).

In the systematic literature review by Hörnell *et al*. it is concluded that the evidence for any breastfeeding to have a protective effect against type 1 and 2 diabetes, is probable (grade 2). The evidence for a stronger protective effect for longer duration of breastfeeding is still limited but suggestive (grade 3) (Hornell et al., 2013).

In the updatet WHO (2013) report, a substantial protection in the pooled analyses was found. However, only two high quality studies were identified, with conflicting results (one showing an increase and the other a reduction among breastfed subjects. The authors conclude that further studies are needed on this outcome (WHO, 2013).

6.2.5 Malignant disease

In some, but not all studies, long-term breastfeeding has been associated with a reduced risk of acute lymphatic leukaemia (Ip 2009; comparing breastfeeding > or <6 months) and childhood leukaemia in general (van Rossum 2005, showing dose-response effect from 0-15 months of breastfeeding). By comparing ever versus never breastfed children, reduced risk of acute lymphoblastic leukaemia, Hodgkin's disease and neuroblastoma was found (SACN, 2011). The possible protective mechanisms of breastmilk on the development of childhood cancers include better early defence against infectious diseases, modulation and stimulation of the immune system and the positive influence of the humoral factors in the breastmilk (e.g. HAMLET) (see section 3.4).

In the systematic literature review by Hörnell *et al*. it is judged that there are limited but suggestive evidence (grade 3) for a risk reduction of breastfeeding against childhood leukemia and possibly other childhood cancers. The effect on childhood leukemia seems larger with longer breastfeeding duration (>6 months). However, as childhood cancers are relatively rare, the public health importance of these associations may be small (Hornell et al., 2013).

6.2.6 Sudden Infant Death Syndrome

Whether breastfeeding lowers the risk of sudden infant death syndrome (SIDS) has been unclear. In the meta-analysis by Ip, 2009, three good and three fair quality studies were included. "Ever being breastfed" was associated with a statistically significant reduced risk of SIDS. A new meta-analysis by Hauck *et al*. includes 18 case-control studies from the period 1966-2009 (Hauck et al., 2011). The authors conclude that "breastfeeding is protective against SIDS, and this effect is stronger when breastfeeding is exclusive". SIDS is an exclusion diagnosis and is probably multifactorial. SIDS cases may be associated with acute upper respiratory infections or diarrhoea, and breastfeeding plays a preventive role in the etiology of these diseases. Breastfed children also seem to have a different sleeping pattern with lower arousal thresholds than formula fed infants, which may provide a mechanism for protection against SIDS.

6.3 Summary of positive health effects associated with consumption of breastmilk

The description of positive health effects presented in section 6.2 is mainly based on systematic reviews and meta-analyses, including the very recent one that forms the basis for the new Nordic Nutrition Recommendations and an updated systematic review from WHO (2013). Because few of the meta-analyses or reviews used as a basis for this benefit assessment enabled differentiation of health benefits of breastmilk in a long-term dose-

dependent manner, some additional reviews and recent single studies of high quality were included to show that this field of research is gradually accumulating more knowledge. The meta-analyses, reviews and single studies pinpoint methodological challenges connected to confounding issues, with unsatisfying measurements or descriptions of length and degree of breastfeeding being the most prominent. Most of the evidence in human studies demonstrates associations that are susceptible to confounding by environmental and behavioural factors at different stages of the life course.

Cognitive development

Neurodevelopment may be influenced by infant feeding (as performance in intelligence tests seems to be better in children breastfed in infancy). WHO (2007) reports an increased mean difference for intelligence and schooling in breastfed children, while Hörnell *et al*. (2013) conclude that there is a "probable" increase. Van Rossum *et al*. (2005) conclude that the evidence is "probable" for intellectual and motor development. The PROBIT Study found significantly higher IQ among children in the intervention group compared to the control group when the children started school.

WHO (2013) concludes that there is a strong evidence of causal effect of breastfeeding on IQ, although the magnitude of this effect seems to be modest.

Immune response-associated diseases

Infections

In spite of the methodological challenges, the meta-analyses and reviews show that up to 6 months of age, there is convincing evidence for a reduced risk of infectious diseases such as gastroenteritis, otitis media and lower respiratory tract infections with increased duration and degree (exclusivity) of breastfeeding, as illustrated in Table 6.1 which shows the ratings by Ip *et al*. (2007), van Rossum *et al*. (2005) and Hörnell *et al* (NNR, 2012). The same results have been found in the PROBIT and Generation R studies.

Asthma and wheezing

As for asthma, it is often difficult to distinguish between an allergic and a non-allergic (e.g., caused by infection) condition, and wheezing in childhood may be a sign of both. In many studies this distinction has not been made. The review by Ip *et al*. (2007) indicates that breastfeeding reduced risk for asthma, while van Rossum *et al*. (2005) state that there is "probable evidence" for reduced risk and Hörnell *et al*. (2013) conclude that there is "limited evidence" and that no conclusion can be given. The statements about wheeze are similar, and one is left with uncertainty about how the results are influenced by a protective effect against infection. In the PROBIT Study, no differences in asthma prevalence were observed between the intervention and control groups when the children were 6.5 years old.

Allergies and atopic dermatitis (eczema)

Exclusive breastfeeding for at least 4 months seems to reduce the incidence of immune-mediated diseases such as asthma and other allergic disorders. It is however questioned whether it might be the total breastfeeding period rather than its exclusivity that might confer protection. In the study by Nwaru *et al*. (2013), children introduced to cereals at the age of

5-5.5 months experienced reduced risk of asthma and allergic rhinitis, whereas children breastfed shorter than 9.5 months increased the risk of non-allergic asthma. In the PROBIT Study, no differences in allergy incidence were observed between the intervention and control groups when the children were 6.5 years old.

Atopic dermatitis is another unclear disorder, but it is often associated with food allergy. Van Rossum *et al*. (2005) conclude that there is "probable evidence" for a protective effect of breastfeeding while Hörnell *et al*. (2013) sum up the evidence saying that there is "limited evidence" and no conclusion can be given.

Type 1 diabetes

Available evidence suggests that breastfeeding may reduce the risk of type 1 diabetes mellitus. WHO (2007) concludes that the OR is substantially reduced, van Rossum *et al*. (2005) indicated "possible evidence" for a protective effect from breastfeeding while Hörnell *et al*. (2013) indicate "probable evidence".

Coeliac disease

In the systematic literature review by Hörnell *et al*. (2013) it is judged to be "probable evidence" for breastfeeding as a protective factor for coeliac disease, at least for delaying its clinical presentation, while SACN/COT (2011) states that the evidence currently available is not strong enough to make specific recommendations about the appropriate timing of introduction of gluten.

Inflammatory bowel disease

Crohn's disease is included in the reviews by van Rossum *et al*. (2005) and Hörnell *et al*. (2013). The first concludes that there is "possible evidence" for a protective effect of breastmilk while the latter concludes that the evidence is "probable". As to ulcerative colitis, van Rossum *et al*. conclude that the evidence is insufficient and Hörnell *et al*. conclude that the evidence for a protective effect from breastfeeding is "probable".

Metabolic syndrome

For disease entities belonging to the metabolic syndrome, such as type 2 diabetes mellitus, high cholesterol, high blood pressure and possibly overweight, breastfeeding in infancy may reduce the risk later in life. For the many health conditions included in this concept the following were found:

Overweight/obesity

WHO (2007), Ip *et al*. (2007) and SACN (2011) indicate a reduced risk for obesity from breastmilk, and van Rossum *et al*. (2005) and Hörnell *et al*. (2013) conclude that the evidence is "convincing". On the other hand, the PROBIT randomised, controlled intervention study in Belarus did not observe differences in obesity risk at 6.5 or 11.5 years of age.

The updated WHO systematic review (2013) concludes that breastfeeding may provide some protection against overweight and obesity, but residual confounding cannot be ruled out.

Lipid metabolism

Long-term reduction in serum cholesterol is included in the review from WHO (2007) which reports a reduced mean difference in adulthood. Also in SACN (2011) indicate reduced serum cholesterol from breastmilk and Hörnell *et al*. (2013) conclude that there is a "probable" reduction. A significant reduction in serum cholesterol among adults was not found in the updated systematic review from WHO (2013).

Blood pressure

WHO (2007) and SACN (2011) conclude that the blood pressure is reduced (systolic and diastolic). Van Rossum *et al*. (2005) indicated "convincing evidence" while Hörnell *et al*. (2013) indicates "probable evidence". The WHO (2013) systematic review found a small protective effect of breastfeeding against systolic, but not diastolic blood pressure.

Cardiovascular diseases

There is no convincing evidence that breastfeeding influences cardiovascular morbidity and mortality.

Type 2 diabetes

As to diabetes type 2, Ip *et al*. (2007) and SACN (2011) indicate a protective effect from breastmilk, while Hörnell *et al*. (2013) conclude that there is a "probable" reduced risk. WHO (2013) concludes that further studies are needed.

Malignant disease

Regarding childhood malignancies, some studies suggest a reduced incidence of some cancers among breastfed individuals. Ip *et al*. (2007) and SACN (2011) indicate that there is a reduced risk, while van Rossum *et al*. (2005) and Hörnell *et al*. (2013) conclude that the evidence for a reduced risk of childhood cancer from breastfeeding is limited suggestive.

Sudden infant death syndrome

SIDS may have several causes, and airway infection may be one predisposing factor. Breastfed children also seem to have a different sleeping pattern with lower arousal thresholds than formula fed infants, which may provide a mechanism for protection against SIDS. Ip *et al*. (2007) indicate that there is a reduced risk, while van Rossum *et al*. (2005) concludes that the evidence is "insufficient". A later meta-analysis published in 2011 Hauck *et al*., concludes that breastfeeding is protective and that the effect is stronger when breastfeeding is exclusive.

Duration of breastfeeding

As the mandate of this report explicitly says that "the assessment shall particularly focus on the duration of exclusive and partly breastfeeding…" we include a separate point on this in the summary although such results have already been partly included in the text above.
The updated 2012 Cochrane review of Kramer and Kakuma, focusing solely on the issue of optimal length of exclusive breastfeeding, concluded that children exclusively breastfed for 6

months experience less morbidity from gastrointestinal infections than those exclusively breastfed for 3 months, and no deficits have been demonstrated in growth among infants from either developing or developed countries who are exclusively breastfed for 6 months or longer.

Additional single studies support the conclusions from Kramer and Kakuma, e.g. the Dutch study (Generation R), where children exclusively breastfed for 4 months had significant reduced risk of infection of the respiratory or gastrointestinal tract during the first 12 months of life. The effect of exclusive breastfeeding was even better in children exclusively breastfed for 6 months with regard to upper respiratory tract infections.

The PROBIT studies support the conclusions that prolonged and exclusive breastfeeding reduces the risk of infections in early life and provide evidence for improvement of children's cognitive development at school age. However, this RCT study did not find differences between the intervention and control groups for the outcomes asthma, allergy, overweight and blood pressure at 6.5 years nor risk of obesity at 11.5 years.

7 Negative health effects associated with persistent contaminants in breastmilk

This chapter describes epidemiological studies of negative health effects associated with contaminants in breastmilk. To comply with the terms of reference, the assessment of risks is based on findings from a full-scale systematic literature search.

It is well known that environmental contaminants may affect the risk of a number of diseases or health problems, where detrimental effects on the cognitive development of children have received special attention the last decades. However, the issue of how contaminants in breastmilk, isolated, have an impact on health and disease risk has remained unresolved – and triggered the initiation of this benefit-risk assessment.

It is important to be aware that most studies within human toxicology have been conducted with one substance and one health outcome in focus at a time. In reality, we are exposed to multiple substances simultaneously. The impact of this aspect is an emerging science – very few studies address this issue at present.

Most of the evidence in human studies demonstrates associations that are susceptible to confounding by environmental and behavioural factors at different stages of the life course, e.g. socio-economic status. Partly independent of parents' income, educational level and occupational status, is the home environment and the degree of intellectual stimulation and emotional support a child gets. The so-called "HOME score" has been developed the last decades to capture these aspects of a child's environment (Caldwell and Bradley, 1985; Tong et al., 2007). The endpoints (health outcomes) in many studies involving environmental contaminants are related to child IQ and motor and cognitive development. If the "HOME score" is not taken into account, results may be biased.

7.1 Methodology – literature search, negative health effects associated with contaminants in breastmilk

In sections under 7.1 the methods and results of a systematic literature search based on Nordic guidelines for systematic reviews[36] are described, with some minor modifications for the purpose of this work. Together with the librarian, a PICO-table and search strategy for literature search was set up which was discussed and accepted in the working group.

7.1.1 Search strategy

Test searches were conducted to find relevant terms and search words, and controlled vocabulary (MeSH and EMTREE) in some relevant papers were examined. Search strategies were developed and literature searches conducted in Medline and Embase. Both databases were used to ensure comprehensive study retrieval. The searches were conducted in January 2011 using a combination of both controlled vocabulary (MeSH and EMTREE) and text word searching. A final search was conducted in (June) 2012 to capture papers published in 2011 until June 2012. Table 7.1 shows the PICO-table that became the basis for the search strategy. The search criteria were developed based on the experience in the project group regarding presence of contaminants in breastmilk and previous and on-going epidemiological studies

[36] A guide for conducting Systematic Literature Reviews for the 5th edition of the Nordic Nutrition Recommendations, available at http://www.slv.se/en-gb/Startpage-NNR/A-guide-for-conducting-systematic-literature-reviews-for-the-5th-edition-of-the-Nordic-Nutrition-recommendations-/.

and health effects. Malignancies were not included in the search because an initial pilot search gave no harvest. Theoretically, one would need child cohorts with several hundred thousand participants to get statistical power to study eventual associations between exposure to contaminants and these rather rare outcomes. So far, such large cohorts, including relevant data, do not exist and may explain why no scientific publications cover the issue.

Table 7.1: PICO-table for search strategy in Medline and Embase[1].

Population	Intervention or exposure	Comparison	Outcome
Children receiving breastmilk or infant formula: Breast feeding Breastfeeding Human milk Mother's milk Maternal milk Infant formula Artificial milk	Pesticides DDT/DDE Persistant organic pollutants Environmental contaminants Dioxins PCB Heavy metals Mercury Lead Cadmium PFOS PFOA Brominated flame retardants	Formula fed infants No, low, medium length of breastfeeding as defined Short vs long duration of breastfeeding as defined	Child/infant growth Child/infant development Preschool child Language development Child intelligence Cognitive development Cognition Cognitive disorders Intellectual impairment Neurodevelopment Immune system Allergy Sensitization Hypersensitivity Behaviour Sexual maturation Thyroid Infections Autism/autistic disorder Childhood mental disorder Adolescent mental disorder

[1]Exact controlled vocabulary used in Medline and Embase (MeSH and EMTREE) is not listed as these differ.

The environmental contaminants were divided into three main groups:

- Pesticides (DDT[37] and HCB[25])
- Other halogenated organic pollutants (PCBs[25], dioxins[25], brominated flame retardants[25], perflourinated compounds[25])
- Heavy metals (lead, mercury and cadmium)

The substances mentioned in parentheses were the ones focused on in this report. The two main reasons for choosing these compounds were
a) most of the compounds are included in the Stockholm Convention

b) these are the bioaccumulating environmental toxicants most extensively studied till now

The search period covered publications from 1990 onwards unless substantial contributions to the field had been published before this date. An extensive review by Wigle was used to check if relevant articles published before 1990 were missed in the literature search (Wigle et al., 2008). Articles in English, German and a Scandinavian language were included in the search. The method allows for inclusion of studies found in references.

[37]Compounds included in the Stockholm Convention (see http://chm.pops.int/Home/tabid/2121/mctl/ViewDetails/EventModID/871/EventID/230/xmid/6921/Default.aspx)

Animal studies were not included in the search even if they give extensive information on dosages and mechanisms of toxic action and are thus a basis in many of the works cited in chapter 5. They may also hint at where to look for negative health effects in humans, or they may confirm observations done in humans. It is recognised that animal and epidemiological studies complement each other in risk assessments (Boyes et al., 2007; Brunekreef, 2008). However, with the time frame given to conduct the assessment, we concluded that well performed epidemiology studies are sufficient and provide adequate methodology for determining the human risks and the effects of persistent environmental toxicants.

7.1.2 Publication selection

Two reviewers working separately assessed the titles and abstracts of all papers identified in the search process for relevance, see Figure 7.1. Grey literature such as dissertations, conference proceedings and reports were excluded. Papers were excluded if they did not include information on breastfeeding in addition to a health outcome. Reviews were also excluded.

In the next step, two reviewers working alone or together, assessed full text papers. A second selection was performed (see Figure 7.1), and papers were excluded if they:

- did not allow for a clear separation between prenatal and postnatal exposure
- included preterm infants who were not separated in the statistical analyses
- did not seem relevant for Nordic background levels. This would include exceptionally high exposures as a result of heavy industrial contamination
- in other ways were irrelevant

Examined full text papers that were excluded according to the criteria listed above are shown in Appendix 1.

7.1.3 Data extraction, relevance and quality assessment

To assess the relevance and quality of included studies a three-category (A-B-C) rating system based on the NNR5 AMSTAR Quality Assessment Tool (QAT) was applied (Shea et al., 2007), modified for our purposes, see Appendix 2. The rating system included questions for evaluating all aspects of a study, e.g. study design, population characteristics, assessment of breastfeeding, assessment of outcome, relevance for the present purpose, confounding factors, methods, results etc. Only studies categorised as A or B are included in the results. The studies included in the results are listed in Appendix 3. Studies categorised as C are listed in Appendix 4 where also the reason for category C is described. Two independent reviewers performed data extraction for all included papers for halogenated compounds and pesticides. For capacity reasons, only one person extracted data from the heavy metal papers. Extracted data were compared and any inconsistencies noted and corrected as necessary. Extracted data were entered into Summary Tables and Quality Assessment Tool Tables. The Summary Tables (Appendix 5, Table 1-24) and Quality Assessment Tool Tables show how each article has been evaluated.

Figure 7.1 shows a flow chart for the literature search and selection process

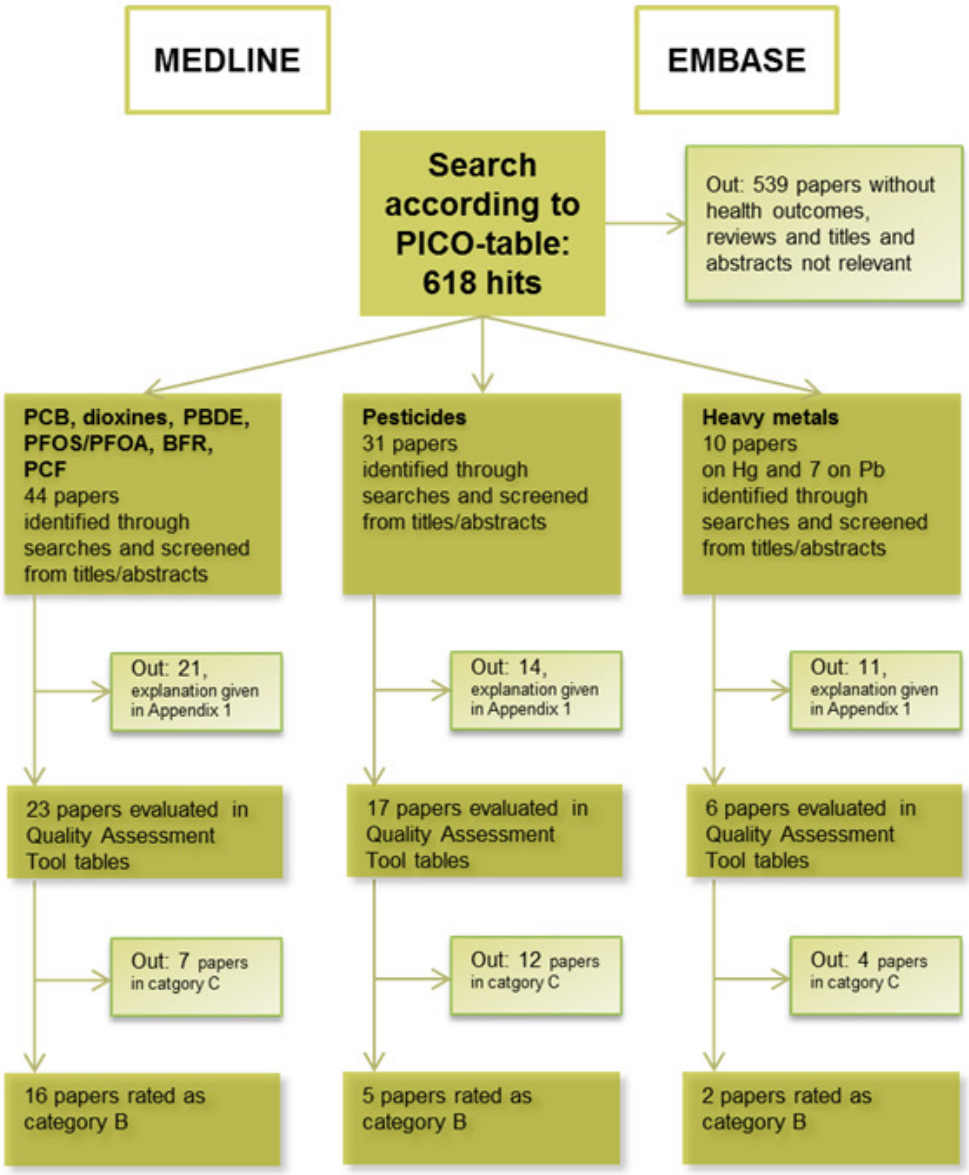

Figure 7.1: Flow chart for literature search and data extraction in chapter 7. The figure does not include papers which were added from the last literature search in June 2012.

After the last literature search in June 2012, six additional papers were identified and examined in full text. Five of these met the inclusion criteria, and are included in the results. Some of the included papers investigate combinations of contaminant groups, and all together 24 papers are included in the results. None of the papers investigate Norwegian infants.

The quality of exposure information varied substantially between publications. Four main ways of measuring or estimating exposure are shown in Table 7.2.

Table 7.2: Ways of reporting exposure to contaminants, pre- and postnatally.

Time point	Measurement	Quality assessment
During pregnancy	Contaminants in mother's blood, urine.	Studies with additional postnatal exposure measurements or postnatal exposure estimates (e.g. PBPK) were included. Qualified for A or B if outcomes were adequately described and duration of breastfeeding was appropriately taken into consideration in statistical analyses.
At birth	Contaminants in cord blood, mother's hair or blood.	Studies with additional postnatal exposure measurements or postnatal exposure estimates were included. Qualified for A or B if outcomes were adequately described and duration of breastfeeding was appropriately taken into consideration in statistical analyses.
During lactation	Contaminants in breastmilk.	Studies included if duration of breastfeeding was given. Qualified for A or B if outcomes were adequately described and duration of breastfeeding was appropriately taken into consideration in statistical analyses.
Postnatal or in childhood	Contaminants in child's blood or hair in a relevant period of exposure for the outcome.	Studies included if duration of breastfeeding was given together with a measure of prenatal exposure. Qualified for A or B if outcomes were adequately described, duration of breastfeeding was appropriately taken into consideration and associations between prenatal exposure and outcomes were adjusted for in statistical analyses.

7.2 Negative health effects – overview of results

Overview of results – contaminants

Some of the contaminants included in the literature search did not appear in the papers fulfilling our inclusion criteria. We found no papers with lead, cadmium, PFOS, PFOA and brominated flame retardants studying breastmilk levels and the health outcomes in question.

Overview of number and types of cohorts

The literature search revealed that a limited number of birth cohorts laid the foundation for most of the studies published as of (June 2013). These are cohorts with both lactation information and contaminant measurements. Table 7.3 gives an overview of the included cohorts. They are all prospective observational studies. In order to facilitate comparison of contaminant exposure between the cohorts, median PCB-153 concentrations in maternal serum and/or cord serum as well as cord serum median DDE is indicated for cohorts from which such information was found. Some of the concentrations were obtained by conversion from breastmilk or maternal serum samples to cord serum by use of conversion factors (explained in Govarts *et al*., 2012). In order to facilitate comparison with levels in Norway, results from the HUMIS study is shown in the last row.

Table 7.3: Overview of the cohorts included in the assessment, including (where available) selected indicator contaminant levels and compared with Norwegian contaminant levels.

Cohort, enrolment years	Number of children in the cohort	Exposures assessed of relevant contaminants	Maternal pregnancy serum, median PCB-153 (ng/g lipid)[1] (n=analysed samples)[1]	Cord serum, median PCB-153 (ng/L)[2]	Cord serum, median DDE (ng/L)[2]
The USA cohorts					
The North Carolina cohort, born 1978-1982	858	Breastmilk PCBs (Rogan and Gladen, 1991)	80 (n=872)		
The Michigan cohort, born 1980-81	313, of which 236 were exposed to mercury-contaminated fish	Maternal and cord blood PCBs, breastmilk PCBs. PCBs, PBBs, DDT and HCB in 4-year old children (Jacobson et al., 1990)	120 (n=196)		
The Faroe Islands birth cohorts					
Faroe 1, born 1986-87	1022	Mercury and PCBs at birth and at age 7, 14 and 23 years. Hair Hg in mother at birth and in child at 12 months (Grandjean et al., 1995)		a (n~1022)	
Faroe 2, born 1994-95	182	Mercury in cord, PCBs in breastmilk and serum at age 54 months (Grandjean et al., 2003)	450 (n=173)	484 (n=167)	1208 (n=167)
Faroe 3, born 1999-2001	656	Pregnancy and milk PCB, child PCB at 18 months and 5 & 7 years. Hg in maternal hair, cord, and in child blood at 5 and 7 years (Heilmann et al., 2010)		346 (n=549)	730 (n=549)
The Dutch cohort born 1990-92	418	Maternal and cord blood PCBs. Breastmilk dioxins and PCBs 2 weeks postpartum (Huisman et al., 1995; Koopman-Esseboom et al., 1996)	100 (n=415)	150 (n=523)	
The German cohort (Düsseldorf) born 1993-1995	171	Cord blood and breastmilk PCBs. Serum PCBs at 42 months (Walkowiak et al., 2001)	140 (n=126)		
The Canadian/Northern Quebeck cohorts born 1995-1998	204	PCBs, PCP and HCB in maternal plasma at delivery, cord plasma and in infant plasma at 7 months (Dallaire et al., 2009)	100 (n=159)		
born 1995-2002	333	PCB-153, DDE, Pb, Hg in cord blood and infant blood at 6th months (Verner et al., 2010)	134[3] (n=164)		
The Spanish cohorts (INMA)					
The Menorca region born 1997-1999	482	DDE and DDT in cord blood and child serum at 4 years (Sunyer et al., 2006)		134* (n=1227)	596* (n=1515)
The Sabadell, Valencia and Gipuzkoa regions born 2003-2008	2150	Pregnancy PCBs, DDE and HCB in maternal serum and postnatal exposure estimated month by month through PBPK modelling		40[3]** (n=1175)	132[3]** (n=1175)

Cohort, enrolment years	Number of children in the cohort	Exposures assessed of relevant contaminants	Maternal pregnancy serum, median PCB-153 (ng/g lipid)[1] (n=analysed samples)[1]	Cord serum, median PCB-153 (ng/L)[2]	Cord serum, median DDE (ng/L)[2]
		(Gascon et al., 2013)		46 (n=856)	131 (n=857)
The Slovakian cohort born 2002-2004	1134	Maternal, and 6- and 16 month infant blood (serum) (Jusko et al., 2012)		143[3] (n=1104)	
Norway, HUMIS for comparison levels born 2002-2006	409	The concentration in the next two columns are estimated levels based on breastmilk analyses		39 (n=409)	50 (n=409)

[1)]Contaminant concentration from Longnecker *et al*. unless other reference is given (Longnecker et al., 2003). All values are maternal plasma or serum levels apart from the Düsseldorf study which are converted breastmilk levels.
[2)]Contaminant concentration from Govarts *et al*. unless other reference is given (Govarts et al., 2012). Some of the concentrations are obtained by conversion from breastmilk or maternal serum samples.
[3)]Contaminant concentration from the cohort reference paper.
*Cord serum. The n indicates that several INMA cohorts are included.
**Maternal blood. The n indicates that several INMA cohorts are included.
[a)]In Grandjean *et al*. 2012 they analysed the geometric mean of cord blood total PCB sum(-118, -138, -153, and -180)x2 (n="almost all cohort members"). Not lipid adjusted, the value was 1860 ng/L (Grandjean, Weihe et al., 2012).

In the Dutch cohort, 418 children have been studied as a whole or in a sub-group of Rotterdam children. In Spain, four regions are included in the INMA cohort.

Overview of results, quality assessment

In spite of excellent design, adequate descriptions of breastfeeding quality (e.g. exclusive or partial) and length and correction for relevant confounders in many of the included studies, none achieved the category A. The main reasons were inadequacies in their description of statistics, e.g. not describing statistical power in sufficient detail, or no correction for home environment e.g. HOME score. Description of statistical power is set as a criterion for category A in the NNR5 AMSTAR system and seems strict for the included studies, but is maintained in this report in order to be in compliance with the NNR-method. The reasons appear in more detail in the Quality Assessment Tool (QAT) Tables[38]. However, within the B-category, there were also relatively large differences in quality, so we decided to indicate this by giving the best studies a plus, i.e. B+. It should be kept in mind that the QAT used in this report was developed solely on the basis of a study's relevance for this risk assessment and is not an evaluation of the papers *per se*. Category C studies were excluded from further analyses first and foremost because they could not separate the impact of pre- and postnatal exposure.

Some papers were categorised as B for one contaminant group (e.g. PCBs) but as category C for another (e.g. mercury).

[38]Available at VKM website: http://www.vkm.no/eway/default.aspx?pid=277&trg=Content_6498&Main_6177=6498:0:31,2369&Content_6498=6187:1781229::0:6271:3:::0:0.

Overview of results, health outcomes

The most common outcomes studied in relation to contaminant exposure in these cohorts were neurodevelopment, respiratory diseases, immunology and allergies, growth and thyroid status. Details are described in the sub-sections below. Table 7.4 gives an overview of the included studies, sorted by outcome.

Table 7.4: Overview of studies included in the results of risks related to persistent contaminants in breastmilk (sorted by health outcome).

Outcome type	Reference	Country	N*	Prenatal contaminant sampling	Postnatal contaminant sampling	Quality
Neurodevelopment	Gascon *et al.*, 2013	Spain, mainland	2150, 1175 at 14 months follow-up	PCBs, DDE, HCB in maternal pregnancy serum	Child's exposure to PCBs, DDE and HCB estimated month by month by PBPK-modelling	B+
Neurodevelopment	Forns *et al.*, 2012	Spain, Menorca	482, 470 at 4 years follow-up	PCBs, DDE, HCB in cord blood	PCBs, DDE, HCB in child blood at 4 years	B
Neurodevelopment	Verner *et al.*, 2010	Canada	333, 168 at 11 months follow-up	PCBs in maternal blood, PCB-153, DDE, Pb and Hg in sub-group cord blood	Child's exposure to PCBs, DDE, Pb and Hg estimated by PBPK-modelling	B
Neurodevelopment	Jensen *et al.,* 2005	Faroe Islands, cohort 1	1022, 910 at 7 years follow-up	Hg in maternal hair, cord Hg	Hg in child hair at 12 months	B
Neurodevelopment	Winneke *et al.,* 2005	Germany	171, 70 at 6 years follow-up	PCBs in cord blood	PCBs in breastmilk 2 weeks postpartum and serum PCBs at 42 months	B
Neurodevelopment	Vreugdenhil *et al.,* 2002 (1)	The Netherlands	207, 158 at 7 years follow-up	PCBs in plasma and cord blood	PCBs and dioxins in breastmilk within 2 weeks postpartum	B+
Neurodevelopment	Vreugdenhil *et al.,* 2002 (2)	The Netherlands	418, 372 at 6 years follow-up	PCBs in plasma and cord blood	PCBs and dioxins in breastmilk within 2 weeks postpartum	B+
Neurodevelopment	Walkowiak *et al.,* 2001	Germany	171, 91 at 42 months follow-up	PCBs in cord blood	PCBs in breastmilk 2 weeks postpartum and serum PCBs at 42 months	B
Neurodevelopment	Patandin *et al.,* 1999	The Netherlands	418, 395 at 42 months follow-up	PCBs in plasma and cord blood	PCBs and dioxins in breastmilk 6 weeks postpartum. Child blood at 42 months	B+
Neurodevelopment	Lanting *et al.,* 1998	The Netherlands	418, 394 at 42 months follow-up	PCBs in serum and cord blood	PCBs and dioxins in breastmilk 6 weeks postpartum. Child blood at 42 months	B+
Neurodevelopment	Koopman-Essebom *et al.,* 1996	The Netherlands	207, 182 at 7 months follow-up	PCBs in plasma and cord blood	PCBs and dioxins in breastmilk within 2 weeks postpartum	B+
Neurodevelopment	Huisman *et al.,* 1995	The Netherlands	418, 373 at 18 months follow-up	PCBs in serum and cord blood	PCBs and dioxins in breastmilk within 2 weeks postpartum	B
Neurodevelopment	Gladen & Rogan, 1991	USA, The North Carolina cohort	858, 712 at 3, 4 and 5 years follow-up	PCBs and DDE in serum, cord blood and/or placenta	PCBs and DDE in breastmilk postpartum and at 3 months	B

Outcome type	Reference	Country	N*	Prenatal contaminant sampling	Postnatal contaminant sampling	Quality
Neurodevelopment	Rogan & Gladen, 1991	USA, The North Carolina cohort	858, 670 at 12 and 24 months follow-up	PCBs and DDE in plasma and placenta and cord blood	PCBs and DDE in breastmilk postpartum and at 3 months	B
Neurodevelopment	Grandjean *et al.,* 1995	Faroe Islands, cohort 1	1022, 583 at 1 year follow-up	Hg in maternal hair at delivery	Hg in child hair at 12 months	B
Neurodevelopment and growth	Jacobson *et al.,* 1990	USA, The Michigan cohort	313, 231 (growth)/ 265 (activity) at 4 year follow-up	PCBs in cord blood	PCBs in breastmilk and child serum at 4 years. Additionally, lead and a number of pesticides were analysed in the 4-year olds	B
Immunology and allergy	Jusko *et al.*, 2012	Slovakia	1134, 100 at 6 and 6 months follow-up	PCBs, dioxins in pregnancy	PCBs, dioxins in child serum at 6 and 16 months.	B
Immunology and allergy	Grandjean *et al.,* 2010	Faroe Islands, cohort 3	656, 464 at 7 year follow-up	PCBs in serum, maternal hair and cord blood Hg	PCBs in breastmilk postpartum. PCBs and Hg in child blood at 5 and 7 years	B
Immunology and allergy	Heilmann *et al.,* 2010	Faroe Islands, cohort 3	656, 587 at 5 and 7 year follow-up	PCBs in child blood	PCBs in postpartum milk	B
Immunology and allergy	Sunyer *et al.,* 2006	Spain	482, 462 at 6 year follow-up	DDE and DDT in cord blood	DDE and DDT in child serum at 4 years	B
Immunology and allergy	Weisglas-Kuperus *et al.,* 2000	The Netherlands	207, 175 at 42 months follow-up	PCBs in plasma and cord blood	PCBs and dioxins in breastmilk within 2 weeks postpartum	B+
Weight and height	Grandjean *et al.,* 2003	Faroe Islands, cohort 2	182, 171 at 18 months and 154 at 42 months, 121 at 54 months follow-up	Hg in cord blood	PCBs in postpartum breastmilk	B
Weight, height and puberty signs	Gladen *et al.,* 2000	USA, The North Carolina cohort	858, 594 at follow-up at puberty	PCBs in plasma, cord blood, placenta	PCBs, DDE in breastmilk postpartum and at 3 months	B
Thyroid hormones	Dallaire *et al.,* 2009	Canada (Inuits)	204, 95 umbical cords, 130 infants at 7 months	PCBs, PCP and HCB in mothers at delivery, cord blood	PCBs, PCP and HCB in child blood at 7 months	B

*N=number of children at inclusion in cohort and at follow-up.

Some of the studies include more than one contaminant group, e.g. halogenated organic pollutants in combination with pesticides or heavy metals. These papers are included and discussed in the sections relevant for the contaminant groups included in the cohort. This implies that some studies are mentioned twice, e.g. if a study has analysed both PCBs and pesticides (DDT), it may appear both in the halogenated organic pollutant sections and in the pesticide sections. There is, however, only one Summary Table for each paper.

7.2.1 Halogenated organic pollutants (PCBs, dioxins, brominated flame retardants, perfluorinated compounds)

As indicated in the flow chart (Figure 7.1), the search strategy identified 44 papers as relevant for halogenated organic pollutants and their full texts were retrieved. Examination of full texts resulted in the exclusion of 21 papers which did not meet the inclusion criteria; i.e. did not allow for assessment of postnatal exposure or did not include breastfeeding information or did not describe a specific health outcome or did not seem relevant for Nordic background levels. A final total of 23 publications were identified for PCBs, dioxins, PBDEs, PFOS and PFOA and assessed in Summary Tables and Quality Assessment Tool Tables. Seven papers were categorised as C, and therefore not further interpreted. Five additional papers were identified as relevant and included after the last literature search. Finally, 21 papers with halogenated organic pollutants were categorised as B. No papers fulfilled the criteria for being categorised as A. An overview of the publications on halogenated compounds included in the results in this opinion is provided in Table 7.4. For a detailed presentation of the papers, see the Summary Tables (Appendix 5).

7.2.1.1 Halogenated organic pollutants – neurodevelopmental outcomes

Fourteen papers reported results of breastfeeding exposure to halogenated organic compounds in relation to neurodevelopmental milestones. Two of these were based on the North Carolina cohort (Gladen and Rogan, 1991; Rogan and Gladen, 1991), one from the Lake Michigan cohort (Jacobson et al., 1990), six from the Dutch cohort (Huisman et al., 1995; Lanting et al., 1998; Patandin, Lanting et al., 1999; Vreugdenhil, Slijper et al., 2002; Vreugdenhil, Lanting et al., 2002), two from the German cohort (Walkowiak et al., 2001; Winneke et al., 2005), one from the Canadian cohort (Verner et al., 2010) and two from the INMA cohort in Spain (Forns et al., 2012; Gascon et al., 2013). A brief overview of measurements and results are given in Table 7.5 below. If reported, results from investigation of *prenatal* exposure and health outcome have been included in the table.

Table 7.5: Evidence table for outcomes - halogenated organic pollutants included for assessment on neurodevelopment associations.

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
North Carolina cohort	Rogan & Gladen, 1991	PCBs, DDT/ DDE	Mostly or partly breastfed	Short: 0-9 weeks (0-4 mostly breastfed), medium: 10-19 weeks (0-4 mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks	Psychomotor scores (Bayley Scales) at 18 and 24 months	No consistent association between exposure to PCBs or DDE through breastmilk and outcomes
						Psychomotor delay up to 2 years of age associated with the highest 5% of transplacental exposure to PCBs
	Gladen & Rogan, 1991	PCBs, DDT/ DDE	Mostly or partly breastfed	As above	McCarthy Scales at 3, 4 and 5 years. Information about school grades	No consistent association with lactational exposure to PCBs and DDE was observed at 3, 4 and 5 years on neuropsychological development
						No association with prenatal exposure either

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
Michigan cohort	Jacobsen *et al.,* 1990	PCBs, (PBB, DDT)**	Not given	Length of breastfeeding mean 29.6 weeks, SD*** 29.0	Growth and activity in children; McCarthy Scales at 4 years	Activity level decreased with increasing postnatal PCB exposure. The association was negligible unless the infant was breastfed for more than one year
Dutch Cohort	Huisman *et al.,* 1995	PCBs, dioxins	Fully breastfed	Zero, short (6-16 weeks) or long >16 weeks	Neurological examination at 18 months, focus on motor functions.	No association between lactational exposure to PCBs and dioxins and neurological conditions
						Prenatal PCB exposure had a weak negative association to the neurological condition at 18 months of age
	Koopman-Esseboom *et al.,* 1996	PCBs, dioxins	Fully breastfed	Zero, short (at least 6 weeks) or long	Mental and psychomotor scores (Bayley Scales) at 3, 7 and 18 months of age	At 7 months the psychomotor scores for the highest exposed breastfed children were comparable to bottle-fed children. At 18 months mental and psychomotor development affected neither by PCB/dioxin exposure nor by feeding type.
						Prenatal PCB exposure was associated with small reduction in psychomotor score at 3 months
	Lanting *et al.,* 1998	PCBs, dioxins	Fully breastfed	Zero, short (6-16 weeks) or long >16 weeks	Neurological examination at 42 months, focus on motor functions	Neither prenatal PCB nor postnatal exposure to PCBs/dioxins related to neurological condition at 42 months
	Patandin *et al.,* 1999	PCBs, dioxins	Fully breastfed	Zero, short (6-16 weeks) or long >16 weeks	Cognitive abilities (Kaufman Assessment Battery) at 42 months	No associations between lactational exposure to PCBs/dioxins nor to current PCB level in child and cognitive abilities at 42 months of age
						In utero exposure to PCBs associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure especially at risk
	Vreugdenhil *et al.,* 2002 (1)	PCBs, dioxins	Fully breastfed	Zero, short (6-16 weeks) or long >16 weeks	Play behaviour at school age	No association between postnatal exposure and play or problem behaviour at school age
						Some associations from prenatal exposure

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
	Vreugdenhil *et al.,* 2002 (2)	PCBs, dioxins	Fully breastfed	Zero, short (6-16 weeks) or long >16 weeks	Cognitive and motor abilities; the Dutch version of McCarthy Scales at school age	Postnatal exposure to PCBs/dioxins through lactation not significantly related to GCI, memory, and motor scores, and effects of postnatal exposure were not significantly modified by parental and home environmental characteristics
						Subtle neurotoxic effects of prenatal exposure to PCBs and dioxins persist into school age
German cohort	Walkowiak *et al.,* 2001	PCBs	Not given	Breastfeeding length (<2 weeks; >2 weeks-4 months; >4 months)	Mental and psychomotor scores (Bayley Scales, motor and mental) at 7, 18 and 30 months, and at 42 months of age, intelligence was measured with the German version of Kaufman Assessment Battery	Negative associations between early milk PCBs and mental/motor development at all ages, significant from 30 months onwards. Intelligence at 42 months negatively assoc. with calculated breastmilk PCB exposure and PCB-level in serum from 42-month olds
						Prenatal PCB exposure was associated with impaired child development. A favourable home environment had the opposite effect and supported mental and motor development until 42 months of age
	Winneke *et al*., 2005	PCBs	Not given		Intelligence at 72 months follow-up was measured with the German version of Kaufman Assessment Battery	The negative associations with PCBs in milk (prenatal exposure) and in 42-month-serum (postnatal exposure) was no longer significant at 72 months, although associations still remained negative
Canadian cohort	Verner *et al*., 2010	PCBs	Exclusive breastfeeding included in the PBPK	Length of exclusive breastfeeding recorded. Mean 155.9 days (range 0-466)	Infant behaviour at 11 months of age. Behaviour Rating Scales, BRS, of Bayley Scales, BSID-II.	Postnatal exposure associated with activity level, measured by non-elicited activity. The strongest association was found for simulated PCB levels during the 4th month of life
						Inattention was related to prenatal exposure
INMA cohort	Gascon *et al*., 2013	PCBs, DDE, HCB	Recorded	Recorded. Mean duration exclusive breastfeeding 3.8 months and partly breastfeeding 6.1 months	Child neuro-psychological assessment. Bayley Scales, BSID-I test giving MDI and PDI test scores at around 14 months of age	Postnatal PCB-153 exposure was not associated with any of the outcomes
						Prenatal PCB-153 exposure was associated with PDI, but not MDI (as reported separately, from this cohort), also when postnatal PCB-153 exposure was included in the model

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
	Forns *et al*., 2012	PCBs, (DDE, HCB)**	Recorded, but not given	Recorded, but not given	Neuro-psychological development. McCarthy Scales adapted to Spanish population, MSCA and MSCA subtests	The levels of postnatal PCBs at 4 years of age were not associated with neuropsychological development
						No statistically significant effects of sum of prenatal PCBs on MSCA scores, but significant detrimental effects of prenatal PCB-153 on the majority of MSCA scores, while no effects reported for the other PCB congeners

*Degree of breastfeeding is defined in a number of different ways in the papers.
**Contaminants in parentheses are analysed, but not included in the reported health outcomes in the paper.
***SD=standard deviation.

In the North Carolina cohort (1978-82) (n=858), the follow-up period was 5 years and the outcomes studied were child development (psychomotor scores, Bayley Scales of Infant Development (BSID)) at 18 and 24 months of age (n=670 at 24 month follow-up) (Rogan & Gladen, 1991), and neuropsychological development (McCarthy Scales of Children`s Abilities (MSCA) at 3, 4 and 5 years of age). Information about school grades was also included (n=712 at 5 year follow-up) (Gladen and Rogan, 1991). Both PCB and DDT/DDE exposures were studied. Degree of breastfeeding is described as mostly or partly breastfed and breastfeeding length as short, medium, long or very long, see Table 7.5. Rogan & Gladen (1991) concluded that there was no evidence of an association between motoric maturation and postnatal exposure to PCBs through breastmilk, while they found small, but stronger with age, association to psychomotor development and prenatal exposure after a threshold of 3.4 ppm PCBs (Rogan and Gladen, 1991). Gladen & Rogan (1991) found some significant and consistent patterns, however, due to inconsistent dose-response patterns, they concluded that no association between neither pre- nor postnatal PCB and DDE exposure could be observed at 3, 4 and 5 years of age on neuropsychological development as assessed by McCarthy Scales and school grades.

In the Michigan cohort (1990-92) (n=313), the follow-up period was 4 years and the outcome studied was activity in children assessed by McCarthy Scales of Children`s Abilities (n=231 (growth) and n=265 (activity) at follow-up) (Jacobson et al., 1990). The exposure was PCBs, PBB and some chlorinated pesticides. Degree of breastfeeding was not described. Mean breastfeeding length was reported as 29.6 weeks. The 15 children whose mothers had higher than average breastmilk PCB levels and who nursed for more than a year, scored lower in activity measurements when they were 4 years old than children of mothers who breastfed shorter than a year. In the rest of the group, n=297, no associations between breastmilk PCB levels and activity scores were observed.

There are three relevant cohorts from Europe for the neurodevelopment endpoint; the Dutch cohort (Vreugdenhil, Lanting et al., 2002; Vreugdenhil, Slijper et al., 2002; Patandin, Lanting et al., 1999; Lanting et al., 1998; Koopman-Esseboom et al., 1996; Huisman et al., 1995), the German cohort (Walkowiak et al., 2001; Winneke et al., 2005) and the INMA cohort from Spain (Gascon *et al*., 2013 (three regions at mainland) and Forns *et al*., 2012 (Menorca)).

In the Dutch cohort (1990-1992) (n=418, (211 in Groningen and 207 in Rotterdam)), the follow-up periods were 3, 7, 18, 42 and 78 months and the outcome studied were

neurodevelopment with focus on cognitive and motor abilities (3,7, 18 and 42 months), and play behaviour (78 months) (Koopman-Esseboom et al., 1996; Lanting et al., 1998; Patandin, Dagnelie et al., 1999; Vreugdenhil, Slijper et al., 2002; Vreugdenhil, Lanting et al., 2002). Different approaches and methods to study the health outcomes were used in these papers, all studying exposures from PCBs and dioxins. Degree of breastfeeding is described as fully breastfed and breastfeeding length as zero, short or long, see Table 7.5.

Koopman-Essebom *et al.*, (1996) demonstrated a slight negative association between prenatal exposure to PCBs and dioxins and psychomotor functions at 3 months of age (n=182 at 7 month follow-up) in the Rotterdam arm of the Dutch cohort (Koopman-Esseboom et al., 1996). PCB and dioxin exposure through breastfeeding had adverse effect on psychomotor outcome at 7 months of age. There was however a beneficial effect of breastfeeding overall, so that breastfed infants with the highest exposure had test results similar to the formula fed. At 18 months of age, neither PCBs nor dioxin or feeding type affected the psychomotor outcome. Furthermore, no such influence was seen at 42 months of age (n=394 at follow-up) (Lanting et al., 1998). Huisman *et al*. (1995) found that prenatal PCB exposure had a weak negative association with psychomotor function (neurological optimality score (NOS), quality of movements in terms of fluency; normal, mildly abnormal, abnormal NOS, fluency cluster score, different measurement of outcome compared with Koopman-Essebom *et al.* (1996) at 18 months of age (n=373 at follow-up), but found no effect of exposure via breastfeeding (Huisman et al., 1995). Patandin *et al*. (1999) found that *in utero* exposure to "background PCBs levels" was associated with poorer cognitive functions in preschool children at 42 month of age (n=395 at follow-up), and that children of mothers at the upper end of PCB and dioxin exposure were particularly at risk (Patandin, Dagnelie et al., 1999). No associations between lactational exposure to PCBs and dioxins nor level in the blood at 42 months and cognitive abilities were found at this age (Koopman-Esseboom et al., 1996). Vreugdenhil *et al*., 2002 (1 and 2) who studied cognitive and motor abilities (n=372 at follow-up), and play behaviour (n=158 at follow-up) of Dutch children at school age, found that neurotoxic effects of prenatal exposure to PCBs and dioxins persisted into school age, resulting in a subtle cognitive and motor development delay. More optimal intellectual stimulation provided to the child may counteract the effects of prenatal exposure to PCBs and dioxins. They found no associations between the studied outcomes and postnatal exposure through breastfeeding (Vreugdenhil, Slijper et al., 2002; Vreugdenhil, Lanting et al., 2002).

In the German Düsseldorf cohort (1993-1995) (n=171), the initial follow-up period was up till 42 months (n=91 at follow-up). The outcome studied was psychomotor and cognitive development assessed at 7, 18, 30 and 42 months of age, and the exposure measured was PCBs (Walkowiak et al., 2001). Degree of breastfeeding is not given, but breastfeeding duration is reported in weeks or months, see Table 7.5. Of the Bayley Scales of Infant Development, the mental and motor scales were used at 7, 18 and 30 months of age, while intelligence was measured at 42 months of age with the German version of the Kaufman Assessment Battery for Children (KABC). Scores from the Sequential Processing and Simultaneous Processing subscales were combined to yield the Mental-Processing-Composite-Index that was standardised to a mean of 100 and SD 15. No significant or borderline association with calculated postnatal PCB exposure was found for any of the Bayley Scales. However, a significant negative association was found between postnatal exposure and Kaufman Assessment Battery at 42 months of age. Increasing PCB levels in early milk combined with duration of breastfeeding, as well as measured PCB level in serum from 42-month old children, predicted decreased Kaufman Assessment Battery scores at 42 months. The authors concluded that prenatal PCB exposure at current European background

levels inhibits, and a favourable home environment supports, mental and motor development until 42 months of age. PCBs also had an effect postnatally.

In a later follow-up at 72 months (6 years) the children (n=70) were again assessed by the Kaufman Assessment Battery for Children and the HOME score was again assessed. At 72 months the negative associations with PCBs in milk (prenatal exposure) and in 42-months-serum (postnatal exposure) and intelligence, were no longer significant, although associations still remained negative. The positive effect of the home environment became even more pronounced. The authors concluded that early PCB exposure at levels in their cohort possibly induces transient delay in cognitive development rather than irreversible deficit (Winneke et al., 2005).

In the Canadian cohort, conducted among Inuits by the Hudson Bay coast in Artic Quebec (1995-2002) (n=333), the follow-up period was 11 months (n=168 at follow-up). Infant behaviour was assessed at 11 months of age, and the exposure measured was PCB-153 in cord or maternal blood obtained at delivery or within a few weeks postpartum (n=168). Hg, Pb, PCBs, and other chlorinated compounds were analysed in a subset of cord blood and infant blood at 6 months of age (n=83-85). Infant blood PCB-153 profiles were estimated month by month using a previously validated (at 6 months) PBPK modelling framework developed by Verner *et al*. (2009), (Verner et al., 2010). Mean length of exclusive breastfeeding was 155.9 days (range 0-466). Exclusive breastfeeding only was included in the PBPK model. The outcomes were assessed using Behaviour Rating Scales (BRS) of Bayley Scales of Infant Development, BSID-II at 11 months of age. Video coding of inattention and activity was measured during administration of the mental developmental subscale of BSID-II. The estimated pre- and postnatal PCB exposure measures predicted significant increases in inattention and activity at 11 months of age. Inattention was mostly related to prenatal exposure, while activity level, measured by non-elicited activity, was best predicted by postnatal exposure, with the strongest association obtained for simulated PCB levels during the fourth month of life. Thus, windows of susceptibility were revealed by simulated infant toxicokinetic profiles for the first year of life during which PCBs may impair infant attention and activity.

In the Spanish INMA cohort (Menorca) (1997-1999) (n=482), the follow-up period was 4 years (n=472 at follow-up). The outcome studied was general neuropsychological development at 4 years of age, and the exposure measured was PCBs -28, -101, -118, -138, -153,- 180, DDE and HCB (355 mother-child pairs with complete information of outcome assessment and contaminant levels in cord blood, and child blood at 4 years of age from 285 children) (Forns et al., 2012). Length and degree of breastfeeding were recorded but not given. McCarthy Scales of Children's Abilities was adapted to the Spanish population. The general cognitive scale and the five subtests were reorganised into sub-area scores (executive functions, working memory, quantitative and motor) and were examined. In addition, new measures were included by reorganising the McCarthy Scales sub-tests into new sub-area scores (executive functions, working memory, visual and verbal span, verbal memory, gross and fine motor skills, and cognitive functions of posterior cortex) according to those tasks that are highly associated with specific neuropsychological functions. Postnatal exposure to PCBs was not associated with neuropsychological development in the preschool period. Prenatal PCB exposure, particularly PCB-153, was adversely associated with general neuropsychological development at 4 years of age, including negative effect on executive function, verbal functions and visiospatial abilities, but not on motor development.

In the Spanish INMA cohort (Gipuzkoa, Catalonia and Valencia) (2003-2008) (n=2150), the follow-up period was 14 months, (range 11-21 months) (n=1175 at follow-up). Child

neuropsychological development was assessed at 14 months (mean), and the exposure measured was PCB-153, DDE and HCB in maternal serum (at pregnancy week 7-26, median 12.9 weeks), while the child`s exposure was estimated month by month by a physiologically based pharmacokinetic modelling (PBPK) developed by Verner *et al.*[39] (Gascon et al., 2013). Length and degree of breastfeeding were recorded, and the mean duration of exclusive breastfeeding was 3.8 months and partly breastfeeding 6.1 months. Breastmilk consumption during partial breastfeeding was calculated as a constant decrease in intake. The outcomes were measured by Bayley Scales of Infant Development, BSID-I test (giving mental development index (MDI) and psychomotor development index (PDI) test scores) in children aged around 14 months of age (range 11-21 months). Mean estimated PCB-153, DDE and HCB levels increased in child's blood after birth due to breastfeeding. Prenatal PCB-153 exposure was associated with PDI, but not with MDI (has been reported earlier from this cohort), also when postnatal exposure was included in the model. Postnatal PCB exposure was not associated with any of the outcomes. Thus, even though breastfeeding increased the levels of POPs in children`s blood during postnatal life, no associations were found between different periods of postnatal exposure to these POPs and mental or psychomotor scores. Thus, the authors suggested that deleterious effects of PCB-153 on early brain development, particularly on psychomotor development, are mainly attributable to prenatal exposure to low levels of POPs.

7.2.1.2 Halogenated organic pollutants – immunological or allergic outcomes

Four papers reported results of breastmilk exposure to halogenated organic compounds in relation to immunological or allergic outcomes. Two of these were based on the Faroe Islands cohort 3 (Grandjean et al., 2010; Heilmann et al., 2010), one was from the Dutch cohort (Weisglas-Kuperus et al., 2000), and one from the Slovakian cohort (Jusko et al., 2012). A brief overview of measurements and results are given in Table 7.6 below. If reported, results from *prenatal* exposure and health outcome have been included in the table.

In the Faroe Islands cohort 3 (1999-2001) (n=656), Grandjean *et al.* (2010) studied allergy, asthma and sensitisation during childhood with prenatal and lactational exposure to marine pollutants (PCBs and Hg). The degree of breastfeeding is described as exclusive breastfeeding or any breastfeeding and breastfeeding duration is reported in months, see Table 7.6.

The immunological outcomes were total IgE, grass-specific IgE and occurrence of allergic disease in 7-year old children (n=464 at follow-up). The results indicate that PCBs in breastmilk may cause an IgE increase in children and thus interact with the beneficial effects of breastfeeding, but a history of asthma or atopic dermatitis was not associated with the duration of breastfeeding (Grandjean et al., 2010).

[39]Verner *et al*., 2009.

Table 7.6: Evidence table for outcomes - halogenated organic pollutants included for assessment of immunological or allergic parameters.

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
Faroe Islands cohort 3	Grandjean *et al.,* 2010	PCBs, (Hg)**	Exclusive breastfeeding vs not exclusive	Exclusive breastfeeding 4.6 +/- 2 months. Any breastfeeding 9.8 +/- 6.6 months	Allergy, asthma and IgE sensitisation during childhood	Dependant on outcome measures, postnatal PCB exposure both increased and decreased the risk of allergic disease
						Prenatal exposures to PCBs were inversely associated with a history of atopic dermatitis, but showed a week positive association with asthma
	Heilmann *et al.,* 2010	PCBs	Exclusive breastfeeding vs not exclusive	Exclusive breastfeeding 4.6 +/- 2 months. Any breastfeeding 9.8 +/- 6.6 months	Vaccine response to diphtheria and tetanus toxoids	Breastmilk PCB exposure associated with immunotoxic outcomes on serum concentration of antibodies against diphtheria and tetanus vaccinations
						No significant association observed with prenatal PCB exposure
Dutch cohort	Weisglas-Kuperus *et al.,* 2000	PCBs, dioxins	Fully breastfed	Zero, short (0-6 weeks) or long >16 weeks	Prevalence of infectious and allergic diseases at school age	PCB plasma levels in 42-month old children were associated with higher prevalence of chicken pox and recurrent middle-ear infections and a lower prevalence of allergic reactions. A higher dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm
						Prenatal exposures associated with higher levels of immunological parameters
Slovakian cohort	Jusko *et al*., 2012	PCBs	Fully breastfed	0-<3 months, 3-6 months, >6 months	Thymus volume	6 month PCB concentration in infant plasma was inversely associated with thymus volume. The association became positive at 16 months of age
						Prenatal PCB concentration associated with smaller thymus volume at birth. No association beyond the neonatal period

*Degree of breastfeeding is defined in a number of different ways in the papers.
**Contaminants in parentheses are analysed, but not included in the reported results in the paper.

Heilman *et al*. (2010) studied serum concentrations of antibodies against vaccine toxoids in the Faroe Islands cohort 3 at age 5 and 7 years (n=587 at follow-up). The serum PCB concentration was measured at 18 months in addition to at ages 5 and 7. Postnatal PCB exposure was associated with reduced serum concentration of specific antibodies after diphtheria vaccination and to a lesser extent after tetanus vaccination (Heilmann et al., 2010). The strongest associations suggested a decrease in the antibody levels by about 20% for each

doubling in PCB exposure. At the age of 5 years, the odds of an antidiphtheria antibody concentration below a clinically protective level of 0.1 IU/L increased by about 30% for a doubling in PCBs in milk and 18-month serum. Maternal pregnancy serum PCB concentration showed only a small and not statistically significant association with antibody concentrations and with serum PCB concentrations determined at ages 5 and 7 years (cross-sectional analyses).

In the Dutch cohort (1990-1992) (n=418, (211 in Groningen and 207 in Rotterdam)), Weisglas-Kuperus *et al.* (2000) found that the PCB concentration in serum from 42-month old children (n=175 at follow-up) was associated with a higher occurrence of chicken pox and recurrent middle ear infection, and a lower prevalence of allergic reactions to food, pollen, dust and household pets (Weisglas-Kuperus et al., 2000). The authors hypothesised that common infections acquired early in life may prevent development of allergy, so PCB exposure therefore might be associated with a lower prevalence of allergic diseases. Degree of breastfeeding is described as fully breastfed and breastfeeding length as zero, short or long, see Table 7.6. Breastfeeding duration was found to counteract the increased odds ratio for middle ear infection. The authors concluded that the study did not provide data to discourage breastfeeding at the background PCB levels in the Netherlands at that time.

In the Slovakian cohort (2002-2004) (n=1134), Jusko *et al*. (2012) found that higher maternal PCB concentration was associated with reduced thymus volume at birth, but the maternal concentration was not predictive of 6- and 16 month thymus volume. The plasma PCB concentration in infants was measured at 6 months, and was associated with a decrease in 6-month thymus volume. This association was weaker when exclusive breastfeeding was not adjusted for. An indication of a positive association between 16 month infant PCB concentration and thymus volume was also seen. Since thymus volume peaks at 6 to 8 months of age and then shrinks, the authors speculate that this could be related to delayed thymus maturation. The potential adverse effect of in utero PCB exposure on thymic development may extend beyond the neonatal period, and postnatal PCB exposure was influential. The physiological implications of the findings are however not known (Jusko et al., 2012).

7.2.1.3 Halogenated organic pollutants – child growth and weight

Three papers reported results of breastfeeding exposure to halogenated organic compounds in relation to child growth. Two were from the USA (the North Carolina cohort and the Michigan cohort) (Gladen et al., 2000; Jacobson et al., 1990) and one was from the Faroe Islands cohort 2 (Grandjean et al., 2003). A brief overview of measurements and results are given in Table 7.7 below. If reported, results from *prenatal* exposure and health outcome have been included in the Table.

In the North Carolina cohort (1978-82) (n=858) Gladen *et al*. (2000) did not observe associations between postnatal exposure to PCBs and DDE and pubertal growth and development (n=594 at follow-up) (Gladen et al., 2000). Degree of breastfeeding in the North Carolina cohort is described as mostly or partly breastfed and breastfeeding length as short, medium, long or very long, see Table 7.7.

In the Michigan cohort (1980-81) (n=316), Jacobsen *et al*. observed no association between postnatal PCB exposure and growth in children at 4 years (n=231 at follow-up) (Jacobson et al., 1990). Degree of breastfeeding is not described in the Michigan cohort. Mean breastfeeding length is reported as 29.6 weeks, see Table 7.7.

Table 7.7: Evidence table for outcomes - halogenated organic pollutants included for assessment of growth.

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
North Carolina cohort	Gladen *et al.,* 2000	Sum PCBs, DDE	Mostly or partly breastfed	Short: 0-9 weeks (0-4 w mostly breastfed), medium: 10-19 weeks (0-4 w mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks	Weight and height at 14 years, age at menses, breast stage 3-5, pubes 3-5	No association between postnatal PCB exposure and growth or puberty signs (but tendency to later maturation in bottle-fed girls)
						Prenatal exposure to PCBs positively associated with weight in girls
Michigan cohort	Jacobson *et al.,* 1990	PCBs, (PBB, DDT)	Not given	Mean breastfeeding 29.6 weeks, SD*** 29.0	Childs weight, height, head circumference at 4 years	No association between postnatal PCB exposure and growth
						Prenatal exposure associated with growth reduction at 4 years
Faroe Islands cohort 2	Grandjean *et al.,* 2003	PCBs, (Hg)**	Exclusive breastfeeding vs not exclusive	Total breastfeeding 7.8 +/- 5.7 months. Exclusive breastfeeding 3.5 +/- 2.0 months. 18.2% exclusively breastfed for 6 months	Height and weight at 18 and 42 months	At 18 months, children who had been exclusively breastfed for at least 6 months weighed 0.59 kg less and were 1.50 cm shorter than those not breastfed
						A weaker, but similar negative association was observed between cord blood PCB concentration and weight and height at 6 months

*Degree of breastfeeding is defined in a number of different ways in the papers.
**Contaminants in parentheses are analysed, but not included in the reported results in the paper.
***SD=standard deviation.

In the Faroe cohort 2 (1994-95) (n=182), Grandjean *et al*. (2003) investigated the relationship between pre- and postnatal exposure to PCBs and mercury and growth. Degree of breastfeeding is described as exclusive breastfeeding, and breastfeeding duration is reported in months, see Table 7.7. They found that children who were exclusively breastfed for the first 6 months weighed less and were shorter than those not breastfed (Grandjean et al., 2003). This was most clearly observed after 18 months (n=171), but was also observed at 42 months (n=154), albeit not statistically significant. The impact of PCB versus mercury exposure could not be separated.

7.2.1.4 Halogenated organic pollutants – thyroid parameters

One paper from Canada, studying Inuits (Dallaire et al., 2009), reported results of breastfeeding exposure to halogenated organic compounds in relation to effects on thyroid parameters. Measurements and results are given in Table 7.8 below. Results from *prenatal* exposure and health outcome have been included in the table.

Table 7.8: Evidence table for outcomes - halogenated organic pollutants included for assessment on thyroid parameters.

	Reference	Exposure	Bf degree	Bf length	Health outcome	Results
Canadian cohort	Dallaire *et al.,* 2009	PCBs, HCB, PCP	Not given	0-7 months	Thyroid hormone status in mothers and infants	No association was observed between postnatal exposure to PCBs, HCB or PCP and thyroid hormones at 7 months of age
						Prenatal exposure was negatively associated with TBG levels and T4 concentration in the newborn

In the Canadian cohort (1995-98) (n=204 at inclusion), Dallaire *et al*. (2009) studied the relationship between thyroid hormone status and postnatal exposure to PCBs, PCP and HCB in 7-month old children of Canadian Inuit women (n=130 at follow-up); no association was found (Dallaire et al., 2009). Degree of breastfeeding is not given in the Canadian cohort and breastfeeding duration is reported as 0-7 months, see Table 7.8.

7.2.2 Heavy metals – Mercury

As indicated in the flow chart (Figure 7.1), the search strategy identified 17 papers as relevant for heavy metals and their full texts were retrieved. Examination of full texts resulted in the exclusion of eleven papers which did not meet the inclusion criteria; i.e. did not allow for assessment of postnatal exposure or did not include breastfeeding information or did not describe a specific health outcome or did not seem relevant for Nordic background levels. A final total of six publications were identified for mercury and lead, and assessed in Summary Tables and Quality Assessment Tool Tables. Four papers were categorised as C and therefore not further interpreted. Finally, two papers were categorised as B. No papers fulfilled the criteria for being categorised as A. An overview of the publications on mercury included in the results in this opinion is provided in Table 7.4. For a detailed presentation of the studies, see the Summary Tables (Appendix 5).

7.2.2.1 Mercury – neurodevelopmental outcomes

Two papers from Faroe Islands cohort 1(1986-87) (n=1022) investigated mercury from breastmilk in relation to developmental milestones, first at the age of 1 (n=583) and later when the child was 7 years old (n=910) (Grandjean et al., 1995; Jensen et al., 2005). A brief overview of measurements and results are given in Table 7.9 below. Results from examination of *prenatal* exposure and health outcome have been included in the table.

Mercury exposure from breastmilk was assessed by relating mercury in the child's hair at age 1 to length of breastfeeding. Degree of breastfeeding is described as exclusive breastfeeding, and breastfeeding duration is reported in months, see Table 7.9. Neither at 1 (Grandjean et al., 1995) nor at 7 years (Jensen et al., 2005) of age was the mercury exposure from breastmilk associated with a reduction in the developmental outcomes measured. On the contrary, at age 1, higher hair mercury was associated with earlier development (earlier sitting, creeping and rising), which the authors attributed to the positive impact of breastfeeding *per se*.

However, in a recent publication from the investigators, a re-evaluation of the first data brought forth that *prenatal* mercury exposure is the primary explanatory variable for neurobehavioural deficits at the age of 7 years (Grandjean, Weihe et al., 2012).

Table 7.9: Evidence table for outcomes – heavy metals included for assessment on neurodevelopment associations.

	Reference	Exposure	Bf degree	Bf length	Health outcome	Results
Faroe Islands cohort 1	Grandjean *et al.,* 1995	Hg	Mostly or partly breastfed	Short: 0-9 weeks (0-4 w mostly breastfed), medium: 10-19 weeks (0-4 w mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks	Month of child sitting, creeping, rising	Higher Hg in hair at 12 months associated with earlier development
						Milestone development was not associated with prenatal Hg exposure as measured by hair-Hg at parturition
	Jensen *et al.,* 2005	Hg	Mostly or partly breastfed	As above	A number of neuropsycho-logycal tests at age 7	Postnatal exposure to Hg was not associated with reduction in neuropsychological performance at 7 years
						Prenatal association not reported. Adjustment for prenatal exposure to Hg, as measured by cord blood Hg, did not change the results

7.2.3 Pesticides

As indicated in the flow chart (Figure 7.1), the search strategy identified 31 papers as relevant for pesticides and their full texts were retrieved. Examination of full texts resulted in the exclusion of 14 papers which did not meet the inclusion criteria; i.e. did not allow for assessment of postnatal exposure or did not include breastfeeding information or did not describe a specific health outcome or did not seem relevant for Nordic background levels. A final total of 18 publications were identified for DDT and DDE, and assessed in Summary Tables and Quality Assessment Tool Tables. Thirteen papers were categorised as C and therefore not further interpreted. One additional paper was identified after the last literature search. Finally, six papers were categorised as B. No papers fulfilled the criteria for being categorised as A. An overview of the publications on pesticides included in the results in this opinion is provided in Table 7.4. For a detailed presentation of the studies, see the Summary Tables (Appendix 5).

7.2.3.1 Pesticides – neurodevelopmental outcomes

Three papers investigated the association between breastfeeding exposure to pesticides and neurodevelopmental outcomes. Two of these papers were from the North Carolina cohort (1978-82) (n=858), (Rogan and Gladen, 1991; Gladen and Rogan, 1991) and one was from the INMA mainland sub-cohort (2003-2008) (n=2150) (Gascon et al., 2013). A brief overview of measurements and results are given in Table 7.10 below. If reported, results from investigation of *prenatal* exposure and health outcome have been included in the table.

Table 7.10: Evidence table for outcomes – pesticide studies included for assessment of neurodevelopment outcomes.

	Reference	Exposure	Bf degree*	Bf length	Health outcome	Results
North Carolina cohort	Gladen& Rogan 1991	DDE, PCBs	Mostly or partly breastfed	Short: 0-9 weeks (0-4 mostly breastfed), medium: 10-19 weeks (0-4 mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks	McCarthy Scales at 3, 4 and 5 years. Information about school grades	No consistent association between prenatal or lactational exposure to DDE or PCBs and neuropsychological development was observed at 3, 4 and 5 years
	Rogan& Gladen 1991	DDE, PCBs	Mostly or partly breastfed	As above	Psychomotor scores (Bayley Scales) at 18 and 24 months	No consistent association between exposure to DDE or PCBs through breastmilk and outcomes
						At 18 and 24 months there was lower psychomotoric scores among children in the fifth percentile of prenatal exposure
INMA cohort	Gascon *et al*., 2013	PCBs, DDE, HCB	Recorded	Recorded. Mean duration exclusive breastfeeding 3.8 months and partly breastfeeding 6.1 months	Child neuro-psychological assessment. Bayley Scales, BSID-I test giving MDI and PDI test scores at around 14 months of age	Neither pre- nor postnatal DDE and HCB exposure was associated with any of the outcomes

*Degree of breastfeeding is defined in a number of different ways in the papers.

In the North Carolina cohort, Rogan & Gladen (1991) assessed DDE and PCB exposure and child development at 18 and 24 months. 670 participants completed the 24 months follow-up (Rogan and Gladen, 1991). The proportion of solely formula fed was 10.8%. The degree of breastfeeding was divided into mostly and partly, and breastfeeding duration reported in four categories (*short:* mostly breastfed 0-4 weeks and weaned by 9 weeks 10.5%, *medium:* either mostly breastfed from 0-4 weeks and weaned after 9 weeks or mostly breastfed for 5 to19 weeks and weaned by 19 weeks, 19.7%, *long:* mostly breastfed for 5 to19 weeks and weaned after 19 weeks or mostly breastfed for 20 weeks or longer and weaned by 49 weeks 35.8% and *very long*: mostly breastfed for 20 weeks or longer weaned after 49 weeks 23.2%). Postnatal exposure was assessed as breastfeeding duration times concentration levels of the contaminants (sum PCBs and pp-DDE). The outcome variables were Bayley Scales PDI and MDI (see Appendix 7 for explanation) at 18 months and 24 months. At 18 and 24 months, adjusted scores on the psychomotoric scales were 4-9 points lower among children in the top fifth percentile of prenatal exposure. The association between prenatal exposure and psychomotoric development became stronger with age and was statistically significant at 24 months. Postnatal associations were not demonstrated.

Gladen and Rogan (1991) also assessed the association between DDE and PCBs and later development, measured as McCarthy Scales score at 3, 4, and 5 years. Prenatal exposure was measured in maternal blood, cord blood and placenta. The postnatal exposure was measured in breastmilk and the duration of the breastfeeding was assessed in detail. The categories given are bottle-feeding, short, medium, long, and very long period of breastfeeding (see above). DDE exposure showed borderline significance between McCarthy Scales score and

DDE, although not in a dose-response manner. Higher postnatal DDE exposure was associated with poorer English grades, of borderline significance, but not to mathematics. No association between postnatal PCB and school performance was observed. The inconsistent patterns resulted in the authors stating that no conclusive associations could be found between exposures and the McCarthy Scales or school performance (Gladen and Rogan, 1991).

In the Spanish INMA cohort (Gipuzkoa, Catalonia and Valencia) (2003-2008) (n=2150), the follow-up period was 14 months, (range 11-21 months) (n=1175 at follow-up). Child neuropsychological development was assessed at 14 months (mean), and the exposure measured was PCB-153, DDE and HCB in maternal serum (at pregnancy week 7-26, median 12.9 weeks), while the child`s exposure was estimated month by month by a physiologically based pharmacokinetic modelling (PBPK) developed by Verner *et al.* (2009) (Gascon et al., 2013). Length and degree of breastfeeding were recorded, and the mean duration of exclusive breastfeeding was 3.8 months and partly breastfeeding 6.1 months. Breastmilk consumption during partial breastfeeding was calculated as a constant decrease in intake (n=382). The outcomes were measured by Bayley Scales of Infant Development, BSID-I test (giving MDI and PDI test scores) in children aged around 14 months of age (range 11-21 months). Pre- and postnatal DDE and HCB exposure was not associated with any of the outcomes.

7.2.3.2 Pesticides – immunological or allergic outcomes

One paper from the INMA cohort (Menorca) (1997-1999) (n=482) investigated the association between breastfeeding exposure to pesticides and immunological or allergic parameters (Sunyer et al., 2006). Measurements and results are given in Table 7.11 below. Results from investigation of *prenatal* exposure and health outcome have been included in the table.

Table 7.11: Evidence table for outcomes – pesticide studies included for assessment of immunological or allergic parameters.

	Reference	Exposure	Bf degree	Bf length	Health outcome	Results
INMA cohort, Menorca	Sunyer *et al.,* 2006	DDE, DDT	Exclusive breastfeeding	Zero, 0-20 weeks, >20 weeks	Asthma and wheezing at 6 years	Postnatal exposure to DDE/DDT as assessed by child levels at 4 years: No negative association with asthma/wheezing. No modification by breastfeeding. No modification by atopy. Breastfeeding had a protective effect on asthma (relation to duration of breastfeeding not given)
						Diagnosed asthma and wheezing at 6 years were associated with DDE at birth, indicating an impact of prenatal effects

Sunyer *et al*. (2006) from the Menorca cohort investigated the exposure indicators DDE and DDT and association to asthma and wheezing, parentally reported by interviews. Degree of breastfeeding was reported as exclusive, and breastfeeding duration in weeks. The results showed that prenatal exposure to DDE was associated with increased risk of asthma, while

DDT showed no relationship (Sunyer et al., 2006). Postnatal exposure as assessed by child levels at age 4 showed no association with asthma/wheezing. There was no effect modification by breastfeeding. Breastfeeding protected against diagnosed asthma (OR=0.33, 95% CI=0.08-0.87) and wheezing (OR=0.53, 95% CI=0.34-0.82) in children with low and high DDE levels at birth. The protective effect of breastfeeding was strongest and significant for short term breastfeeding (breastfeeding>0 to 20 weeks). The authors concluded that prenatal exposure may have a negative impact on immunological parameters which is not seen postnatally.

7.2.3.3 Pesticides – child growth and weight

One paper from the North Carolina cohort (1978-1982) (n=858) investigated the association between breastfeeding exposure to pesticides and growth and puberty signs (Gladen et al., 2000). Degree of breastfeeding is described as mostly or partly breastfed and breastfeeding length as short, medium, long or very long. A brief overview of measurements and results are given in Table 7.12 below. Results from investigation of *prenatal* exposure and health outcome have been included in the table.

Table 7.12: Evidence table for outcomes – pesticide study included for assessment of relation to growth.

	Reference	Exposure	Bf degree	Bf length	Health outcome	Results
North Carolina cohort	Gladen *et al.,* 2000	DDE, Sum PCBs	Mostly or partly breastfed	Short: 0-9 weeks (0-4 w mostly breastfed), medium: 10-19 weeks (0-4 w mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks	Weight and height at 14 years, age at menses, breast stage 3-5, pubes 3-5	No association between postnatal exposure to DDE and growth or puberty signs (but tendency to later maturation in bottle-fed girls)
						Prenatal exposure to DDE was associated with tendency to higher weight in boys at 14 years, but not girls

Gladen *et al*. (2000) explored prenatal and lactational exposure to PCBs and DDE and pubertal growth and development. Postnatal measurements in breastmilk were performed in fully breastfed and partially breastfed infants. Outcome variables were weight and height at 14 years (n=594 at final follow-up), age at onset of puberty, and breast and pubes development. The conclusions were that prenatal exposure to DDE was associated with boys' length and height while in the girls a borderline positive association between prenatal exposure to PCB and weight was observed. However, no postnatal associations were observed, except a tendency for later maturation in bottle-fed girls (Gladen et al., 2000).

7.2.3.4 Pesticides – thyroid parameters

One paper from Canada, studying Inuits (Dallaire et al., 2009), reported results of breastfeeding exposure to halogenated organic compounds in relation to effects on thyroid parameters. Measurements and results are given in Table 7.13 below and include findings of associated with prenatal exposure.

Table 7.13: Evidence table for outcomes - halogenated organic pollutants included for assessment on thyroid parameters.

	Reference	Exposure	Bf degree	Bf length	Health outcome	Results
Canadian cohort	Dallaire *et al.,* 2009	PCBs, HCB, PCP	Not given	0-7 months	Thyroid hormone status in mothers and infants	No association was observed between postnatal exposure to PCBs, HCB or PCP and thyroid hormones at 7 months of age
						Prenatal exposure negatively associated with TBG levels and T4 concentration in the newborn

In the Canadian cohort (1995-98) (n=204 at inclusion), Dallaire *et al*. (2009) studied the relationship between thyroid hormone status and postnatal exposure to PCBs, PCP and HCB in 7-month old children of Canadian Inuit women (n=130 at follow-up). No association was found (Dallaire et al., 2009). Degree of breastfeeding is not given in the Canadian cohort while breastfeeding duration was reported as 0-7 months, see Table 7.13.

7.2.4 Positive effects of breastfeeding in the papers reporting negative health effects

It has to be mentioned that several of the studies and cohorts described above found positive effects of breastmilk *per se* in spite of this not being the main focus in the study.

In the Faroe Island cohort 1, an overall beneficial effect of breastfeeding on neurodevelopment was observed, and in Grandjean *et al*. (1995) the following is stated: **"**even if exposure to mercury from milk is increasing during the breastfeeding period and resulting in considerable increase in mercury concentrations in infant hair (18 months of age), duration of breastfeeding did not act as a predictor for neurodevelopmental delays (Grandjean et al., 1995). On the contrary, an extended period of breastfeeding was associated with earlier attainment of developmental milestones, indicating that beneficial effects of breastfeeding compensated for possible neurotoxic effects of mercury on milestone development". The authors concluded that when evaluating the possible health implications of neurotoxicants in human milk, the developmental benefits associated with breastfeeding must be taken into consideration. In the Faroese cohort 3, Grandjean *et al*. (2010) showed that an apparent association between breastfeeding duration and increased serum IgE in children was weakened and no longer significant after adjustment for the concomitant PCB exposure (Grandjean et al., 2010).

In the Dutch cohort, a beneficial effect of breastfeeding was observed for several of the outcomes studied. Huisman *et al*. (1995) reported that the fluency cluster score was not related to PCBs and dioxins in cord blood, maternal blood or breastmilk (Huisman et al., 1995). However, breastfed children had a higher fluency cluster score compared to formula fed. The study by Koopman-Essebom *et al*. (1996) in the same cohort showed a negative association between exposure to dioxins and dl-PCBs via breastmilk and the mental developmental index of the Bayley Scales of Infant Development at 7 months of age (Koopman-Esseboom et al., 1996). However, there was a positive effect of breastmilk *per se*, meaning that the beneficial effect of breastfeeding was negatively confounded by dioxins and dl-PCBs in breastmilk. This effect was not observed at 3 months of age, where prenatal exposure was most influencal. No association with breastfeeding duration or dioxin and dl-PCB exposure was seen at 18 months.

In another study on the Dutch cohort, Weisglas-Kuperus *et al.* (2000) reported that PCB concentration in serum from 42-month old children was associated with a higher occurrence of recurrent middle ear infection, and that breastfeeding duration counteracted the increased odds ratio for middle ear infection (Weisglas-Kuperus et al., 2000).

The impact of breastmilk *per se* was not reported in all studies (e.g. the German cohort), although most studies included breastfeeding duration as a parameter in their models.

Together, the data above indicate that possible negative effects of contaminants in breastmilk may be camuflaged by beneficial effects of breastmilk itself. This needs to be taken into consideration both when interpreting possible negative effects of contaminants and positive effects of (or lack of effects of) breastfeeding on different endpoints.

7.2.5 Studies without data on breastfeeding

The present benefit-risk assessment has had as an inclusion criterion that a paper had to have information on breastfeeding length and preferably degree of breastfeeding (e.g. exclusive versus partial breastfeeding) to be included. It is appropriate to ask if we might have missed out on important publications and knowledge by applying such strict inclusion criteria. Knowing that breastmilk is a major source to contaminants, another approach to evaluate risk from exposure to contaminants present in breastmilk would be to use the child's blood concentration of a contaminant, even if there is no information on the history of breastfeeding.

This approach may be useful when evaluating the risk of adverse health outcomes from exposure to contaminants in general, but has not been considered appropriate in this benefit- and risk assessment of breastmilk. Examples of this approach are the many publications from e.g. Slovakian cohorts, with levels of measured POPs in blood/serum of a highly exposed mother-child pairs (Jusko et al., 2010; Trnovec et al., 2008; Trnovec et al., 2010).

As another example, perfluorinated compounds assessed in pregnant women have been associated with increased risk of overweight and obesity in the offspring 20 years later (Halldorsson et al., 2012), and also to reduced sperm quality in males (Vested et al., 2013). Another study used the levels of flame retardants in breastmilk as a proxy for fetal exposure and found increased risk of cryptocordism in the boys with higher levels in the mother's milk (Main et al., 2007).

However, no breastfeeding data were included in these studies and they were therefore excluded when we applied our exclusion criteria. In a study on prenatal (measured in maternal serum) and postnatal (measured in child serum at 5 years) exposure to perfluorinated compounds and serum tetanus and diphtheria vaccine antibody concentrations in 5 and 7-year old children in the Faroe Islands (Faroe 3), it was found that at doubled postnatal PFC exposure (PFOS, PFOA, PFHxS) the antibody concentration at age 7 was approximately halved (Grandjean, Andersen et al., 2012). The difference remained after adjusting for prenatal exposure. According to the authors, the association with PFCs is much stronger than that observed for PCBs (described in 7.2.1.2). Breastmilk is an important source of PFC exposure in children and given the long half-life, it can still be an important determinant in the child's serum at 5 years. However, data on breastfeeding was not included as a parameter in the statistical analyses in this study, and it was thus not captured by the literature search.

7.2.6 Other pollutants

As mentioned in section 7.1, the focus of this assessment has been on a limited amount of persistent environmental contaminants, i.e. the pesticides DDT and HCB, the pollutant groups PCBs, dioxins, brominated flame retardants and perfluorinated compounds and last but not least, heavy metals. These bio-accumulating compounds have so far been known to have the largest potential to cause harm in early life. More pragmatically, these are the compounds most extensively studied till now in relation to detrimental health effects in children. However, no studies including brominated flame retardants, perfluorinated compound or lead and cadmium were found which fulfilled our inclusion criteria.

A number of emerging chemicals have attracted public attention the last decade, like phthalates and phenols (BPA). Many of these are not persistent, i.e. they do not accumulate in the body and are, accordingly, even more difficult to study than the persistent contaminants. It is scientifically challenging to study the separate impact of a contaminant on risk of a disease, especially if the effect is connected to a disease with low prevalence. Assessments of contaminant burdens thus often become assessments of levels in body fluids (like blood or urine), more than assessments of direct harm. Furthermore, the levels found are often so low that known harmful effects are not known.

7.3 Summary of potential negative health effects related to persistent contaminants in breastmilk

The following persistent contaminants substances were included in the literature search: halogenated organic pollutants (PCBs, dioxins, BFRs), pesticides (DDT/DDE and HCB) and heavy metals (mercury).

A full-scale literature search was conducted and studies were selected according to standard procedures for systematic reviews. An important inclusion criterion was that a study had to have well documented breastfeeding data, thus the absence of breastfeeding information was an exclusion criterion. All included studies were described in Summary Tables and evaluated in Quality Assessment Tool Tables into category A, B or C. The quality assessment resulted in no studies in category A, the main reasons being inadequacies in their description of statistics, e.g. not describing statistical power in sufficient detail, or no correction for “HOME score” or other measures of home environment. Twenty four studies qualified for category B. The category C-studies are not included for further consideration, and an explanation for category C is given in Appendix 4.

All studies are based on eleven cohorts in Europe and the North Americas, totally including approximately 7900 children at enrolment.

The health outcomes were connected to neurodevelopment (including motoric and cognitive development), infections, immunology and allergy, child growth and thyroid hormone status. A brief summary of main findings are given in the tables below.

The focus of the risk assessment was on eventual findings of negative health effects. However, many of the 24 studies included in chapter 7 observed better scoring or reduced risk in children who were breastfed, in spite of high contaminant exposure. Such results were found in e.g. the North Carolina cohort, the Faroese cohort 1, the Dutch cohort and the German cohort and are duly discussed in the respective papers (see section 7.2.4).

Summary and evaluation of the results for neurodevelopment

The methodology used to study outcomes varied between studies, as did the age of the children when subject to the various tests. Overall, the Bayley Scales of Infant Development was used before the age of 2.5 years and the McCarthy Scales of Children's Abilities was used for the older children. Several cohorts used country-adapted versions of these tests. The tests are described in more detail in Appendix 7. The HOME score is considered essential as a correction factor when assessing the impact of breastfeeding on neurodevelopment, and its use in the various studies has been included in a separate column in Table 7.14.

Table 7.14 gives an overview of time points, tests used and results in the studies of child neurodevelopment.

Table 7.14: Overview of time points, tests used and results in the studies of child neurodevelopment.

Cohort	Contaminant	Childs age, in months or years when tested: 0-5 mo	1 year	2 years	3 years	4 years	5 years	6 years	7 years	HOME score	Results, postnatal exposures	Reference
North Carolina	PCBS, DDE									No	No association	Rogan& Gladen 1991
	PCBS, DDE									No	No association	Gladen& Rogan 1991
Michigan	PCBs									Yes	No association	Jacobsen 1990
Dutch	PCBs, dioxins									Yes	Assoc at 7 mo, but not at 18 mo	Koopman Essebom 1996
	PCBs, dioxins									No	No association	Huismann 1995
	PCBs, dioxins									No	No association	Patandin 1999
	PCBs, dioxins									No	No association	Lanting 1998
	PCBs, dioxins									Yes	No association	Vreugdenhil 2002 (1)
	PCBs, dioxins									Yes	No association	Vreugdenhil 2002 (2)
German	PCBs									Yes	Association at 4y, but not at 6 y	Walkowiak 2001, Winneke 2005
Canada	PCBs									Yes	Association with activity level at 11 mo	Verner 2010
Spain, Menorca	PCBs									No	No association	Forns 2013
Spain, mainland	PCBs, DDE, HCB									No	No association	Gascon 2013
Faroe 1	Hg									No	No association	Grandjean 1995
	Hg									No	No association	Jensen 2005

The Bayley Scales of Infant Development
The McCarthy Scales of Children's Abilities
DM=Development milestones (focus on motor functions), a test described in Hempel MS, 1993
The Kaufman Assessment Battery for Children
MISC=Miscellaneous test used in the Faroese cohort

Halogenated organic pollutants

The results for halogenated organic pollutants and the endpoint neurodevelopment are based on seven cohorts (North Carolina, Michigan, Dutch, German, Canadian and Spanish) with

4725 mother-child pairs at inclusion, the smallest having 70 children at follow-up (the German cohort) and the largest having 1175 children at follow-up (three regions in Spain (the INMA cohort).

The studies had varying information about degree and duration of breastfeeding. Three cohorts did not give information on degree of breastfeeding. All papers, however, included information about breastfeeding length in intervals, e.g. short, medium or long duration, each interval defined by number of breastfeeding weeks. PBPK modelling was used in two studies.

No *consistent persistent association* between neuropsychological development (cognitive or motor abilities) and postnatal exposure to PCBs and dioxins was found in 13 out of 14 studies (see Table 7.14). In three studies, various results were reported.

One Dutch paper found that postnatal exposure to PCBs via breastmilk was associated with poorer developmental outcomes at 7 months of age, but the effects did not persist to 18 or 42 months of age. In general, the breastfed infants scored significantly higher than the formula fed infants at 7 months, but when corrected for PCB and dioxin exposure, the score was reduced and became comparable to the formula fed infants.

Results from the German cohort showed a negative overall association with prenatal PCB exposure and mental/motor development, becoming significant from 30 months onwards. Both pre- and postnatal exposure to PCBs was negatively associated with child intelligence at 4 years of age, but this was no longer significant at 6 years of age. They also found a very strong positive effect of the home environment as assessed by the HOME score.

In both the Dutch and German studies, postnatal exposure assessments mostly relied on metrics of overall exposure, such as multiplying the level of PCBs in breastmilk by the duration of breastfeeding and/or PCB concentration measured in children postnatally, which may not reveal associations with neuropsychological development where specific postnatal windows of susceptibility exist.

When using the advanced PBPK model to estimate infant blood concentration profile during the first year of life in the Canadian cohort of Inuits, the authors found an association between blood PCB-153 around 4 months of age and the infant's ability to control activity (Bayley Scales of Infant Development) at 11 months of age. So far, no follow-up of these children has been published, testing if the model can predict associations at later ages.

Table 7.3 gives an overview of PCB-153 and DDE levels in the various cohorts, presented in a comparable way. To the degree that serum PCB-153 represents most PCBs and dioxins, the PCB and dioxin exposures in the Dutch and North Carolina cohorts were more than twice the levels of today's exposure in Norway, whereas the German, Michigan, Canadian (Inuit) and the INMA (Menorca) cohorts had about 3 times higher PCB-153 levels than the present corresponding exposure in Norway. The INMA mainland sub-cohorts had PCB levels comparable to Norwegian levels.

Mercury

The results for the associations between neurodevelopment and mercury are based on one cohort, the Faroe Islands cohort 1, with 1022 mother and child pairs at inclusion, 583 children at follow-up 1 year and 910 at follow-up 7 years. In this study, the degree of breastfeeding is described as exclusive breastfeeding, and breastfeeding duration is reported in months.

The Faroe population has historically had an exceptionally high mercury intake from their traditional diet. In the decades after the first publications came out (approx. 1990), many

Faroese women have reduced their consumption of pilot whale, the main source of mercury. In the wake of this, exposure to mercury (and PCBs/dioxins) has declined substantially, as illustrated by PCB-153 in Table 7.3.

None of the two included papers showed negative associations between postnatal exposure to mercury and neurodevelopment (cognitive or motor abilities).

Pesticides

The results for pesticide exposure and neurodevelopment endpoints are based on two cohorts with 3008 mother and child pairs at inclusion. The North Carolina cohort had 712 participants at the 12 month follow-up and 670 at the 24 month investigation. The Spanish INMA study had 1175 participants at the follow-up at 24 months.

The degree of breastfeeding was divided into mostly and partly, and breastfeeding duration reported in months in the North Carolina cohort. PBPK modelling was used in the INMA cohorts.

None of the four publications showed associations between postnatal exposure to pesticides and neurodevelopment (cognitive or motor abilities).

In the North Carolina study no association was found between postnatal exposure to DDE from breastmilk and child development as measured by the McCarthy Scales or school performance. The same lack of association was reported in the two very recent Spanish publications, one from Menorca and one from mainland Spain, the latter using PBPK modelling.

The exposure levels of DDE in the North Carolina cohort were approximately twice of present-day Norwegian levels (see Table 7.3). In the Spanish sub-cohorts, the Menorca participants had approximately 10 times higher DDE levels than present-day Norwegian while the mainland participants had 3 times higher levels.

Summary and evaluation of the results for immunological or allergic outcomes

The outcomes varied between the five included studies, as did the age of the children when subject to the various tests, see Table 7.15.

Four out of five publications found associations between postnatal exposure to PCBs and dioxins and DDE/DDT and immunological and allergic outcomes.

Table 7.15: Overview of time points, tests used and results in studies of immunological or allergic outcomes.

Cohort	Contaminant	Childs age in months or years when tested: 6-16 mo	3-4 years	5 years	6 years	7 years	Results, postnatal exposures	Reference
Faroe 3	PCBs						Inverse association with vaccine antibody titers	Heilmann 2010
	$PCBs_s$, Hg						Positive association PCB_s and IgE	Grandjean 2010
Dutch	PCBs and dioxins						Increased prevalence of infectious and allergic diseases, but breastfeeding protected	Weisglas-Kuperus 2000
INMA, Menorca	DDE, DDT						No association	Sunyer 2006
Slovakia	PCBs						Small decrease in thymus voulum at 6 mo, but not at 16 mo	Jusko 2012

Vaccine antibody concentrations
IgE-changes, allergic disease occurrence
Middle ear infections, allergic reactions, chickenpox, antibody levels for mumps, measles and rubella
Asthma, wheezing
Thymus volume

Halogenated organic pollutants

The results for the halogenated organic pollutants and the immunological endpoints are based on three cohorts (Dutch, Faroe Islands cohort 3 and Slovakian cohorts) with 2208 mother-child pairs at inclusion, the smallest having 100 children at follow-up (the Slovakian cohort) and the largest (Faroe Island cohort 3) having 587 children at follow-up.

The studies had varying information about degree and duration of breastfeeding. Degree of breastfeeding is described as exclusive, fully, partial or any breastfeeding. All papers, however, included information about breastfeeding in months or in intervals, e.g. short, medium or long duration, each interval defined by number of breastfeeding weeks.

As illustrated in Table 7.15, interpretations of these papers are complex, both because different time points and endpoints have been investigated in the different cohorts, but also because results within the studies are conflicting.

In the Dutch cohort, postnatal PCB and dioxin exposure was associated with increased risk of middle ear infection, but this was counteracted by longer duration of breastfeeding. To complicate the interpretation, they also observed a lower prevalence of allergic disease with higher PCB and dioxin exposure. There was no association between postnatal PCB exposure and antibody titer to mumps, measles and rubella vaccination, however, a reduction associated with prenatal exposure was seen.

The Faroe Islands cohort 3 found that higher breastmilk PCB exposure was associated with reduced serum concentration of antibodies against diphtheria and tetanus vaccinations at 7 years of age. Furthermore, higher PCB exposure was associated with higher serum IgE at 7 years, which might be indicative of allergic disease, but no association with allergic disease was observed.

In the Slovakian cohort there was a small, but statistically significant decrease in thymus volume at 6 months, but not later, associated with breastmilk PCB exposure. The results indicate an association between pre- and postnatal PCB exposure and altered thymus

development, but even though the thymus is important for T-cell differentiation and maturation, eventual physiological implications of this observation are unknown.

To the degree that serum PCB-153 represents most PCBs and dioxins, the PCB and dioxin exposures in the Faroe Islands cohort 3 was almost 10 times higher than present-day Norwegian levels and the Dutch cohort had more than twice the levels of today's exposure in Norway (see Table 7.3 with comparison of PCB-153 concentrations). The Slovakian PCB-153 levels were approximately 3.5 times higher than present-day Norwegian levels.

Pesticides

The results for the immunological and allergic outcomes and pesticides are based on one cohort (the Menorca region in the Spanish INMA cohort) with 482 mother and child pairs at inclusion and 462 at 6 year follow-up.

Degree of breastfeeding was reported as exclusive breastfeeding and breastfeeding duration was reported in weeks.

Breastfed infants in the Menorca study had a reduced risk of asthma at the age of 6 years. The authors concluded that prenatal exposure may have a negative impact on immunological parameters which is not seen postnatally.

The exposure level to DDE in the Menorca cohort was more than 10 times the present-day Norwegian exposure levels (see Table 7.3).

Summary and evaluation of the results for child growth and weight

The classical anthropometric measurements height and weight were used to study associations between growth and contaminant exposure in two of the included studies, while a third study measured development of puberty. Table 7.16 shows that the children were measured at very different time points.

Table 7.16 gives an overview of time points, tests used and results in studies of growth and puberty signs.

Table 7.16: Overview of time points, tests used and results in studies of growth and puberty signs.

		Childs age in months or years when tested				
Cohort	**Contaminant**	**18-42 mo**	**4 years**	**Puberty**	**Results, postnatal exposures**	**Reference**
North Carolina	PCBs, DDE				No association	Gladen 2000
Michigan	PCBs				No association	Jacobsen 1990
Faroe 2	PCBs				Lower weight and height at 18 mo, same tendency at 42 mo	Grandjean 2003

Weight, height and stage of pubertal development
Weight and height (+ McCarthy Scales and activity rating)
Weight and height at 18 and 42 months

Two out of the three publications included for growth showed no associations between postnatal exposure to PCBs and dioxins and DDE and growth, while one found an association at 18 months.

Hallogenated organic pollutants

The results for the halogenated organic pollutants and the growth endpoints are based on three cohorts (Michigan, North Carolina and Faroe Islands cohort 2) with 1353 mother and child pairs at inclusion, the smallest having 171 children at follow-up and the largest having 595 children at follow-up.

The studies had varying information about degree and duration of breastfeeding. Degree of breastfeeding is described as exclusive, mostly, partial or not given. All papers, however, included information about breastfeeding in weeks or in intervals, e.g. short, medium or long duration, each interval defined by number of breastfeeding weeks.

The Michigan study found no association between weight and height at 4 years and breastmilk PCB exposure. The North Carolina study investigated height, weight and puberty development and found that trans-placental PCB exposure was associated with increased weight in a sub-set of girls. The association was attributed to prenatal exposure.

The data from the Faroe Island cohort 2 might indicate a growth-reducing effect of contaminant exposure and growth in 18 months children. However, reduced growth in breastfed versus formula fed children is well known, and cannot only be ascribed to contaminant exposure (Nommsen-Rivers and Dewey, 2009). Thus, in order to explore eventual effects of contaminant exposure on growth, comparison of children with approximately similar duration of breastfeeding but with different contaminant exposure would have been a better approach.

The PCB and dioxin exposures in the Faroe Islands cohort 2 was almost 10 times higher than present-day Norwegian levels, and the PCB-153 levels in the North Carolina and Michigan cohorts were 2 to 3 times higher than the present corresponding exposure in Norway (see Table 7.3 with comparison of PCB-153 and DDE concentrations).

Pesticides

The results for pesticides and the growth endpoints are based on one cohort (the North Carolina cohort) with 858 mother and child pairs at inclusion, reduced to 594 children at the final follow-up.

Degree of breastfeeding is described as mostly or partly breastfed and breastfeeding length as short, medium, long or very long.

The authors found no association between postnatal DDE exposure and weight and height at puberty. The exposure levels for DDE in the North Carolina cohort were about twice as high as in present-day Norway.

Summary and evaluation of the results for the thyroid parameters

There was only one study investigating associations between PCBs, HCB and thyroid parameters as outcome, and no association was found, see Table 7.17.

Table 7.17: Overview of time points, tests used and results in studies of thyroid parameters.

Cohort	Contaminant	Childs age in months when tested	Results, postnatal exposures	Reference
		7 mo		
Canada	PCB_s, HCB		No association	Dallaire 2009
	Thyroid hormone status (TSH, T4, T3 and TBG)			

Halogenated organic pollutants and pesticides

The results for halogenated organic pollutants and the endpoint thyroid parameters are based on one cohort with 204 mother and child pairs at inclusion and 130 children at 7 months follow-up.

In this Canadian cohort the degree of breastfeeding is not given, and the breastfeeding duration is reported to be 0-7 months. No association was observed between postnatal exposure to PCBs or HCB and thyroid hormones at 7 months of age. Prenatal exposure was negatively associated with TBG levels and T4 concentration in the new born.

The Canadian Inuits had 2.5 times higher levels of PCB-153 in breastmilk compared to present-day Norwegian levels.

8 Exposure to contaminants

Dietary exposure assessment of contaminants in breastmilk combines information of breastmilk consumption data with data on the concentration of contaminants in breastmilk. A variety of factors may affect both consumption data and concentration data and thus the estimation of contaminant exposure. The infant`s consumption of breastmilk will vary with age and weight of the infant. The concentration of contaminants in the breastmilk will depend on maternal exposure and body burden (including parameters such as age, body mass index, parity and the nutritional and health condition of the mother) as well as fat content of the milk, time of sampling the breastmilk during the meal, the day and the breastfeeding period.

Available data on levels of breastmilk contaminants used in this exposure assessment are presented in chapter 5. Exposure estimates have been performed for PCB-153, total TEQ, DDE and HCB. The reason for the choice of these compounds is that these were the only persistent contaminants where we have good Norwegian data on their levels in breastmilk and which were also included in chapter 7 investigating negative health effects of contaminants in breastmilk. Since breastmilk is not recognised as a substantial heavy metal exposure source, and since there are no available data from Norway, exposure estimates have not been performed for mercury.

Exposure calculations are presented in sections 8.4-8.7. Importantly, the exposures to persistent organic pollutants should be seen in light of the change in body concentration in children during the nursing period (see section 5.2.5). PCB-153 is the PCB congener most abundant in food and is often used as an indicator of total PCB exposure. The levels correlate strongly with total PCB exposure from food. PCB-153 can be seen as a marker for total dioxin and PCB exposure, since it shows high correlation with total TEQ in breastmilk and because it constitutes a large part of PCB-6. The PCB-153 exposure data are based on milk from 377 mothers and provides a better measure of variability among Norwegian women than the exposures to dioxins and dl-PCBs, which are based on only 27 samples. DDE and HCB were calculated as representatives of different classes of substances for which there were epidemiological data on associations between exposure and health outcomes.

It is well known that exposures to several persistent organic pollutants are lower from infant formula than from breastmilk, as also shown in section 5.2.5. Thus, no specific exposure calculation for infant formula has been performed.

In order to estimate contaminant exposure among Norwegian children, a mean consumption of breastmilk, the mean fat concentration in breastmilk and the weight development in children has been used, as described in sections 8.1-8.3.

8.1 Breastmilk consumption

Breastmilk consumption data (breastmilk volumes) used in the contaminant exposure assessments in sections 8.4-8.7 are based on the WHO report *Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life* (Butte N. et al., 2002). Breastmilk volumes during the first 6 months in exclusively breastfed infants are shown in Table 2.2. The volume for the rest of the breastfeeding period is set to 500 ml/day independent of age of the child since studies showed that the breastmilk volume hovered around 530 ml/day from 6-12 months of age, see Table 2.4. The same volume has been used for children 13-24 months (500 ml/day). This volume is probably an overestimate for children

this age since breastmilk consumption most likely decreases as the intake of solid foods and other drinks than breastmilk increases.

Breastmilk volumes are further described in section 2.3.

According to the Norwegian studies Spedkost and Småkost (2007), 46% of the mothers breastfeed exclusively for 4 months, while only 9% breastfeed exclusively for 6 months. Mean duration of breastfeeding is 10.3 months.

8.2 Fat concentration in breastmilk

The total fat concentration in breastmilk depends on a variety of parameters described in section 3.3.3. In the Norwegian Human Milk Study (HUMIS), mean and median fat concentration in breastmilk samples was 3.5 g/100g (n=295) (Polder et al., 2009). This is also in accordance with the EFSA opinion on PBDEs in food (EFSA, 2011c) and in the lower range of concentrations reported in studies described in section 3.3.3 (range 20-70 g/L). The fat concentration from HUMIS is used in the exposure assessments of PCB-153, total TEQ, DDT and HCB.

8.3 Infant body weight development

In the exposure assessment of PCB-153, total TEQ, DDT and HCB, body weights for children 1-24 months are based on tables (Table 39 boys and Table 50 girls) in the WHO report *Child Growth Standards* (WHO Multicentre Growth Reference Study Group, 2013). There were only small body weight differences between the genders (300-700 g in children 1-24 months), and in the exposure estimates, the median value for each month (mean for boys and girls) are used, see Table 8.1.

Table 8.1: Body weight for children 0-24 months. Source: WHO Multicentre Growth Reference Study Group, 2013.

Age, months	1	2	3	4	5	6	7	8	9	10	11	12
Mean body weight, kg	4.4	5.4	6.1	6.7	7.2	7.6	8.0	8.3	8.6	8.9	9.1	9.3
Age, months	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Mean body weight, kg	9.6	9.8	10.0	10.2	10.4	10.6	10.8	11.0	11.2	11.5	11.7	11.9

8.4 PCB-153

PCB-153 is often used as an indicator of total PCB or PCB-6 because the correlation between PCB-153 and PCB-6 is usually high. In Norway, the correlation between PCB-153 and total TEQ is also high. PCB-153 has been presented in several epidemiological studies (section 7.2.1) and is therefore selected as a representative PCB for exposure calculation in Norwegian infants.

In HUMIS, PCB-153 was analysed in 377 breastmilk samples from women with Norwegian background[40] (Polder et al., 2009), and Polder, personal communication. The mean, median and 95-percentile PCB-153 concentrations were 35.6, 32.3 and 63.6 ng/g lipids respectively. Reduction in breastmilk concentration of PCB-153 during the breastfeeding period has not been taken into consideration in the exposure estimation, which was based on a mean consumption of breastmilk, the mean body weight in children, and the mean fat concentration in breastmilk (sections 8.1-8.3) combined with the above-mentioned concentrations. The highest daily PCB-153 exposure per kg body weight was seen the first months of life and decreased with age and increasing body weight (Tables 8.2 and 8.3, Figure 8.1 and 8.2). The 95-percentile exposure was approximately 1.8-fold higher than the mean.

Table 8.2: PCB-153 exposure estimates from breastmilk in exclusively breastfed infants 0-6 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of PCB-153, ng/kg bw/day (n=377).

Age, months	1	2	3	4	5	6
Mean	197.9	168.7	153.4	145.1	137.8	140.0
Median	179.6	153.0	139.2	131.6	125.0	127.0
95-percentile	353.6	301.3	274.1	259.2	246.1	250.1

[1]See Table 2.2.

Table 8.3: PCB-153 exposure estimates from breastmilk in partially breastfed children 5-24 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of PCB-153, ng/kg bw/day (n=377).

Age, months	5	6	7	8	9	10	11	12	13	14
Mean	122.9	100.3	77.9	75.1	72.4	70.0	68.5	67.0	64.9	63.6
Median	111.5	91.0	70.7	68.1	65.7	63.5	62.1	60.8	58.9	57.7
95-percentile	219.5	179.3	139.1	134.1	129.4	125.1	122.3	119.7	115.9	113.6
Age, months	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Mean	62.3	61.1	59.9	58.8	57.7	56.6	55.6	54.2	53.3	52.4
Median	56.5	55.4	54.4	53.3	52.3	51.4	50.5	49.2	48.3	47.5
95-percentile	111.3	109.1	107.0	105.0	103.1	101.2	99.4	96.8	95.1	93.5

[1]See Table 2.2 and Table 2.4. Volume 500 ml/day is used for children 12-24 months.

[40]The women were ethnic Norwegian.

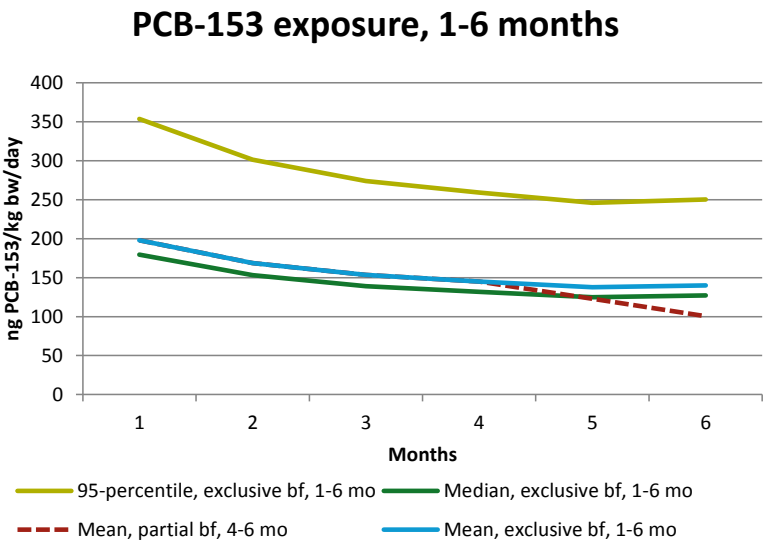

Figure 8.1: PCB-153 exposure (ng/kg bw/day) from breastmilk in infants 1-6 months, based on mean, median and 95-percentile PCB-153 concentrations in breastmilk (n=337) (bf=breasfeeding).

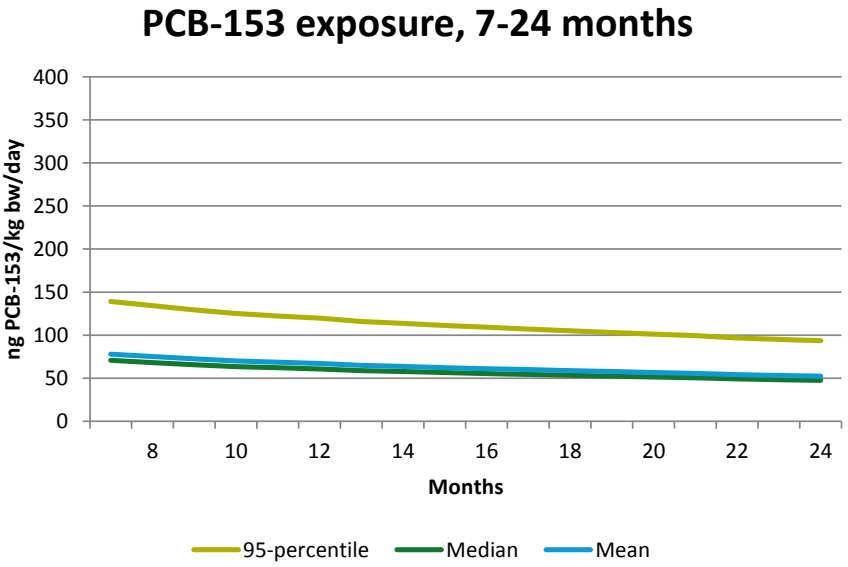

Figure 8.2: PCB-153 exposure (ng/kg bw/day) from breastmilk in partially breastfed children 7-24 months, based on mean, median and 95-percentile PBB 153 concentrations in breastmilk (n=377).

8.5 Total TEQ

In HUMIS, total TEQ (included dioxins, furans and dl-PCB) was determined in 27 individual samples from women with a Norwegian background[41] (Stigum et al., 2005). The mean, minimum and 95-percentile total TEQ concentrations were 11, 4.6 and 18 pg TEQ/g lipids respectively. Reduction in breastmilk concentration of total TEQ during the breastfeeding

[41]The women were ethnic Norwegian.

period has not been taken into consideration in the exposure assessment, which was based on a mean consumption of breastmilk, the mean body weight in children, and the mean fat concentration in breastmilk (section 8.1-8.3) combined with the above-mentioned mean and maximum concentrations. The exposure was highest in the younger age group and decreased with increasing age and body weight (Tables 8.4 and 8.5, Figure 8.3 and 8.4). The maximum exposure was approximately 1.6-fold higher than the mean.

Table 8.4: Total TEQ exposure (pg/kg bw/day) from breastmilk in exclusively breastfed infants 0-6 months, based on mean[1] and maximum concentrations in breastmilk (n=27).

Age, months	1	2	3	4	5	6
Mean	61.2	52.1	47.4	44.8	42.6	43.3
Maximum	100.1	85.3	77.6	73.3	69.7	70.8

[1]See Table 2.2.

Table 8.5: Total TEQ exposure (pg/kg bw/day) from breastmilk in partially breastfed infants 5-24 months, based on mean[1] and maximum concentrations in breastmilk (n=27).

Age, months	5	6	7	8	9	10	11	12	13	14
Mean	38.0	31.0	24.1	23.2	22.4	21.6	21.2	20.7	20.1	19.6
Maximum	62.1	50.7	39.4	38.0	36.6	35.4	34.6	33.9	32.8	32.1
Age. Months	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Mean	19.3	18.9	18.5	18.2	17.8	17.5	17.2	16.7	16.5	16.2
Maximum	31.5	30.9	30.3	29.7	29.2	28.6	28.1	27.4	26.9	26.5

[1]See Table 2.2 and Table 2.4. Volume 500 ml/day is used for children 12-24 months.

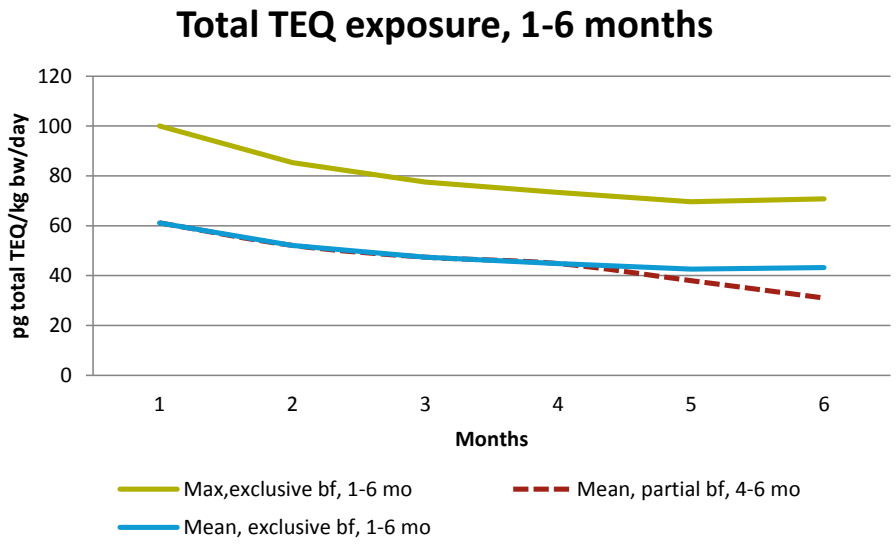

Figure 8.3: Total TEQ exposure (pg/kg bw/day) from breastmilk in infants 1-6 months, based on mean and maximum concentrations in breastmilk (n=27) (bf=breasfeeding).

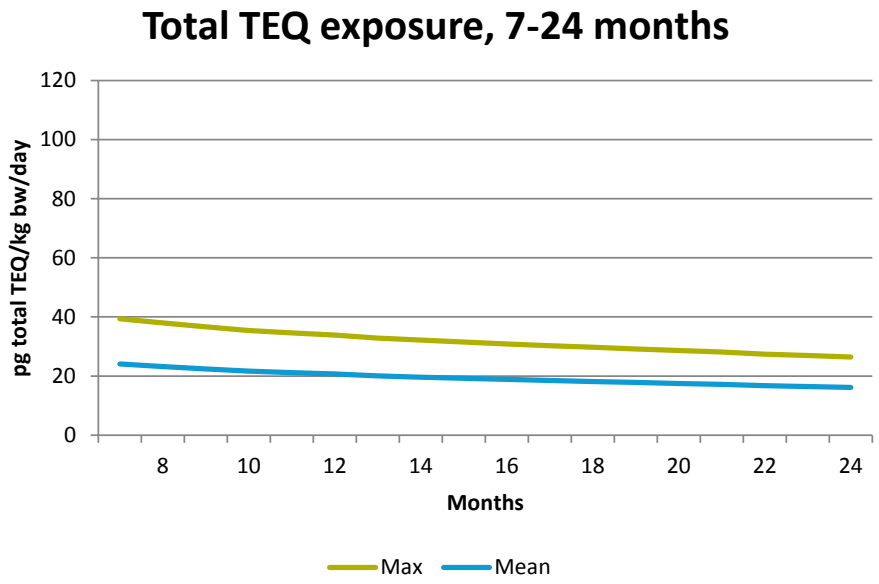

Figure 8.4: Total TEQ exposure (pg/kg bw/day) from breastmilk in partially breastfed children 7-24 months, based on mean and maximum concentrations in breastmilk (n=27).

8.6 DDE

1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p.p'*-DDE) is a major breakdown product of DDT, and was determined in 377 breastmilk samples from women with a Norwegian background[42] in the HUMIS study (Polder et al., 2009). The mean, median and 95-percentile DDE concentrations were 52.5, 40.5 and 115.9 ng/g lipids respectively. Reduction in breastmilk concentration of DDE during the breastfeeding period has not been taken into consideration in the exposure assessment, which is based on a mean consumption of breastmilk, the mean body weight in children, and the mean fat concentration in breastmilk (sections 8.1-8.3) combined with the above-mentioned concentrations. The highest daily DDE exposure per kg body weight was seen the first months of life and decreased with age and increasing body weight. (Tables 8.6 and 8.7, Figure 8.5 and 8.6). The 95-percentile exposure was approximately 2.2-fold higher than the mean.

Table 8.6: DDE exposure from breastmilk in exclusively breastfed infants 0-6 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of DDE, ng/kg bw/day (n=377).

Age, months	1	2	3	4	5	6
Mean	291.9	248.7	226.2	213.9	203.2	206.5
Median	225.2	191.9	174.5	165.0	156.7	159.3
95-percentile	644.4	549.1	499.4	472.3	448.5	455.8

[1]See Table 2.2.

[42]The women were ethnic Norwegian.

Table 8.7: DDE exposure from breastmilk in partially breastfed children 5-24 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of DDE, ng/kg bw/day (n=377).

Age, months	5	6	7	8	9	10	11	12	13	14
Mean	181.2	148.0	114.8	110.7	106.8	103.2	101.0	98.8	95.7	93.8
Median	139.8	114.2	88.6	85.4	82.4	79.6	77.9	76.2	73.8	72.3
95-percentile	400.0	326.7	253.5	244.4	235.8	227.9	222.9	218.1	211.3	207.0
Age, Months	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Mean	91.9	90.1	88.3	86.7	85.1	83.5	82.0	79.9	78.5	77.2
Median	70.9	69.5	68.2	66.9	65.6	64.4	63.3	61.6	60.6	59.6
95-percentile	202.8	198.9	195.0	191.3	187.8	184.4	181.1	176.4	173.4	170.4

[1]See Table 2.2 and Table 2.4. Volume 500 ml/day is used for children 12-24 months.

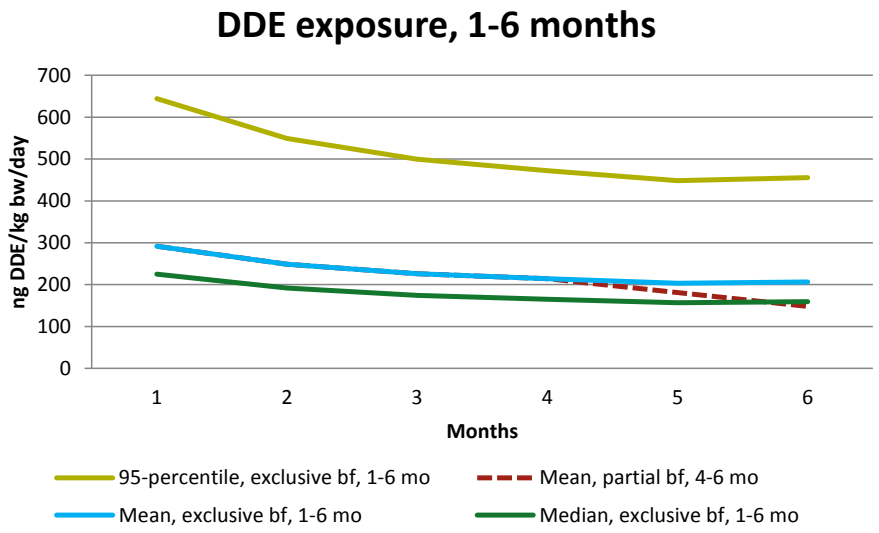

Figure 8.5: DDE exposure (ng/kg bw/day) from breastmilk in infants 1-6 months, based on mean, median and 95-percentile concentrations in breastmilk (n=377) (bf=breasfeeding).

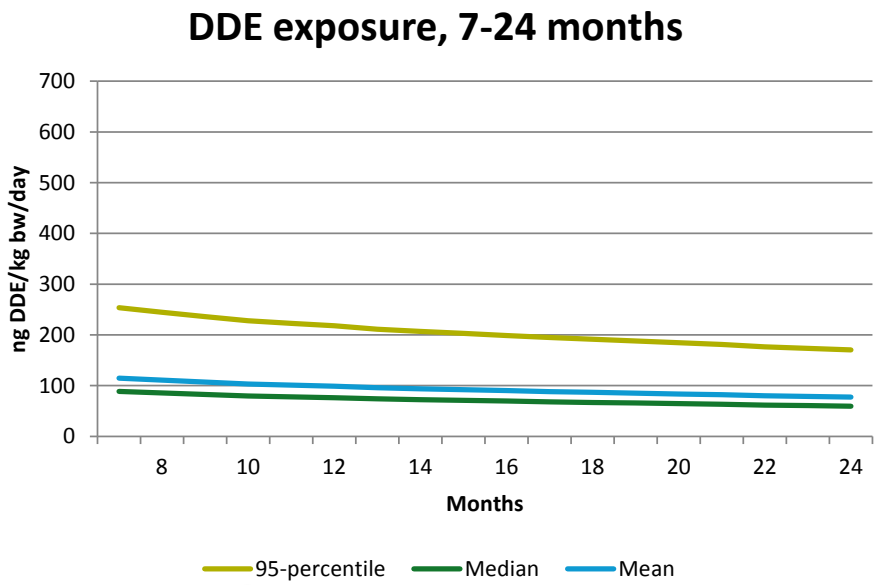

Figure 8.6: DDE exposure (ng/kg bw/day) from breastmilk in partially breastfed children 7-24 months, based on mean, median and 95-percentile concentrations in breastmilk (n=377).

8.7 HCB

In HUMIS, HCB was determined in 377 breastmilk samples from women with a Norwegian background[43] (Polder et al., 2009). The mean, median and 95-percentile HCB concentrations were 11.5, 11.1 and 18.3 ng/g lipids respectively. Reduction in breastmilk concentration of HCB during the breastfeeding period has not been taken into consideration in the exposure assessment, which was based on a mean consumption of breastmilk, the mean body weight in children, and the mean fat concentration in breastmilk (sections 8.1-8.3) combined with the above-mentioned concentrations. As for PCB-153 and DDE, the HCB exposure was highest in the first month of life and decreased with increasing age and body weight (Tables 8.8 and 8.9, Figure 8.7 and 8.8). The 95-percentile exposure was approximately 1.6-fold higher than the mean.

Table 8.8: HCB exposure from breastmilk in exclusively breastfed infants 0-6 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of HCB, ng/kg bw/day (n=377).

Age, months	1	2	3	4	5	6
Mean	63.9	54.5	49.6	46.9	44.5	45.2
Median	61.7	52.6	47.8	45.2	43.0	43.7
95-percentile	101.8	86.7	78.9	74.6	70.8	72.0

[1]See Table 2.2.

[43]The women were ethnic Norwegian.

Table 8.9: HCB exposure from breastmilk in children 5-24 months, based on mean consumption[1] of breastmilk and mean, median and 95-percentile concentrations of HCB, ng/kg bw/day (n=377).

Age, months	5	6	7	8	9	10	11	12	13	14
Mean	39.7	32.4	25.2	24.3	23.4	22.6	22.1	21.6	21.0	20.5
Median	38.3	31.3	24.3	23.4	22.6	21.8	21.4	20.9	20.2	19.8
95-percentile	63.2	51.6	40.0	38.6	37.2	36.0	35.2	34.4	33.4	32.7
Age, months	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Mean	20.1	19.7	19.4	19.0	18.6	18.3	18.0	17.5	17.2	16.9
Median	19.4	19.0	18.7	18.3	18.0	17.7	17.3	16.9	16.6	16.3
95-percentile	32.0	31.4	30.8	30.2	29.7	29.1	28.6	27.9	27.4	26.9

[1]See Table 2.2 and Table 2.4. Volume 500 ml/day is used for children 12-24 months.

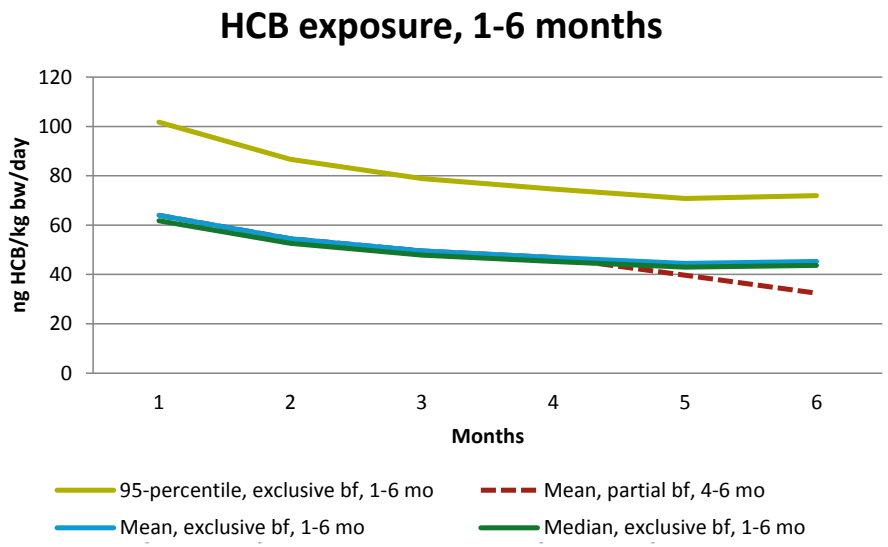

Figure 8.7: HCB exposure (ng/kg bw/day) from breastmilk in infants 1-6 months, based on mean, median and 95-percentile concentrations in breastmilk (n=377) (bf=breasfeeding).

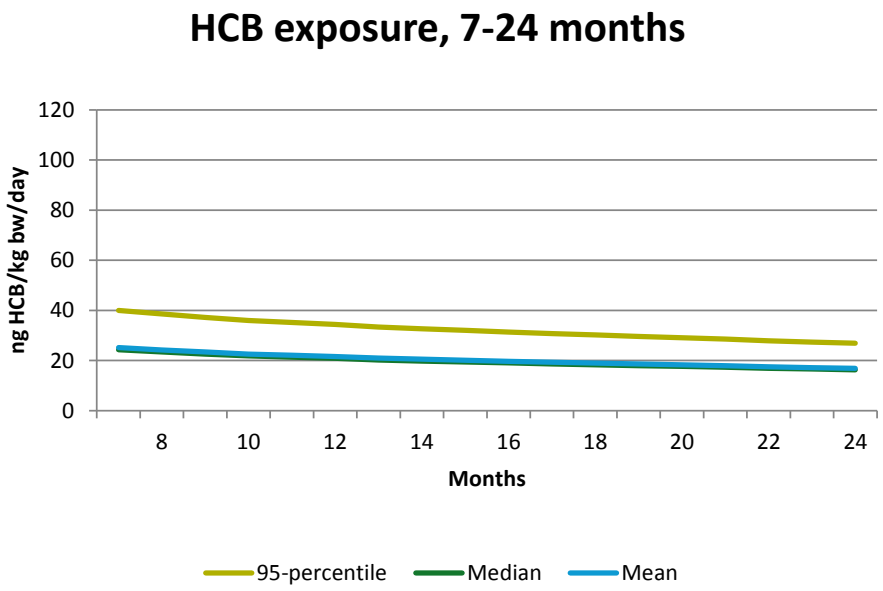

Figure 8.8: HCB exposure (ng/kg bw/day) from breastmilk in partially breastfed children 7-24 months, based on mean, median and 95-percentile concentrations in breastmilk (n=377).

8.8 Summary of exposure

Exposure estimates to PCB-153, dioxins and dl-PCBs, DDE and HCB are based on mean and high (95-percentile, maximal for dioxins and dl-PCBs) concentrations in breastmilk from women with Norwegian background combined with mean consumption of breastmilk, mean body weight in children, and the mean fat concentration in breastmilk. Highest exposure per kg bw was seen the first month of life, and it thereafter declined. High exposures were in the range of 1.6 to 2-fold the mean exposures for all the contaminants.

9 Methodological issues

In this chapter there will be a brief discussion of the methodological challenges we encountered when evaluating the literature in chapters 6 and 7. The challenges were somewhat different for the literature used in the chapter describing the positive health effects associated with breastmilk (chapter 6) compared to the chapter describing the negative health effects associated with persistent contaminants in breastmilk (chapter 7). In the benefit assessment, we used reviews and meta-analyses as a starting point. In the risk assessment, we used a systematic literature review approach and retrieved original research papers.

9.1 Study design

It is generally recognised that randomised controlled trials (RCT), if adequately designed and conducted, provide the best evidence for a causal association between exposure, such as breastfeeding, and a health and developmental outcome. However, for ethical reasons, no country would currently permit an RCT design to investigate pros and cons of breastfeeding versus formula feeding.

Therefore, all but one (the PROBIT Study) of the studies used as a basis for chapters 6 and 7 are observational studies. Observational studies have their own challenges irrespective of topic and how well or poorly they are designed. An observational study draws inferences about the possible relationship between exposure and health outcome, but is not able to say that the relationship is a cause-effect one. To be able to handle this limitation, Austin Bradford Hill in the 1960s developed what is now known as the *Hill's Criteria of Causation.* The criteria outline the minimal conditions needed to establish a causal relationship between two items and include consistency, strength of association, dose-response, time order, specificity, consistency on replication, predictive performance, biological plausibility and coherence (HILL, 1965). *Hill's Criteria* form the basis of modern epidemiological research, which attempts to establish scientifically valid causal connections between potential disease agents and the many diseases that afflict humankind.

9.2 Dose and body burden

In general, studies on health outcomes associated with any exposure need to take into account dose and duration, since the effects of exposure usually are highly dependent on these two variables. The total exposure of environmental contaminants to the infant is directly proportional to the total dose of breastmilk. However, in studies of breastfeeding, the issue of dose, e.g. whether exclusively breastfed or not and for how long, is often not clearly defined.

Many studies compare ever breastfed children to those who were never breastfed, without reporting if the children were exclusively or partly breastfed. Other studies compare infants who are breastfed for less than a given number of weeks or months, often 2-3 months, to those breastfed for longer periods. Few studies treat breastfeeding duration as a continuous variable, which would allow a dose-response analysis.

In chapter 6 (describing the positive health effects), based on reviews of the literature, four of the six reports did not focus specifically on breastfeeding duration. Thus, the impact of dose (breastfeeding length and degree) cannot be disentangled in these systematic reviews without having to read and interpret the separate papers included. This was not part of the mandate.

The two very recently published systematic literature reviews (Hörnell *et al*., 2013 and WHO, 2013) have a much larger focus on dose and thus we have chosen to give these most weight in our final analyses. In addition, we give some weight to the PROBIT Study, the only RCT so far conducted in term-born infants. Although it is a breastfeeding counselling study, investigating the impact of increased breastfeeding promotion, and does not have a classical control group, it does allow for a certain degree of dose-response information. Furthermore, when reorganising their data to an observational design, results do allow for separating the exclusively and the partly breastfed children.

In chapter 7, the 24 original research papers used are characterised by having adequate dose data as this was an inclusion criterion.

However, the methodological approaches for assessing postnatal exposure to contaminants in most of these studies only give an overall estimate of postnatal exposure because they rely on samples of breastmilk and/or infant blood collected at a single time point together with information on breastfeeding duration. Infant toxicokinetics, which is the result of complex interactions between several concurrent events including breastfeeding and growth, may influence the daily blood concentration of contaminants, but are most often not taken into consideration. Thus associations with contaminants that impact health during narrow time windows of exposure may not be elucidated and a failure to detect developmental impairment may occur (Verner et al., 2010; Verner et al., 2012).

Critical windows of exposure is an emerging issue in research related to infant development and environmental contaminants. The subject is discussed in section 10.3.1.

9.3 Prenatal versus postnatal exposure

Another methodological challenge encountered has been to separate if the health outcomes are related to pre- or postnatal exposures. In many papers it is virtually impossible to separate health effects caused by *in utero* exposure from postnatal exposure. This issue is discussed in section 10.3.1.

9.4 Uncertainty in the contaminant exposure estimations

9.4.1 Volume

Different methods of measurement have been used to quantify human milk consumption. Weighing of mother or child before and after feeding is the most common method. The uncertainties are expected to be relatively small and tend to even out when the number of observations increase.

In the present assessment, the estimates of breastmilk volume is based on WHO values (Butte N. et al., 2002) (section 2.3). The estimated monthly volume in infants 0-6 months is based on results from 28 different studies from developed countries (Butte *et al*. 2002). However, the number of studies and participants varies between different months, and the infants followed over a long time period were not the same all the time.

The monthly breastmilk volume in children 7-24 months has been chosen to have a default value of 500 g/day in the present report. This is probably an overestimation of intake, but was the best value attainable. Values for partially breastfed infants were chosen due to introduction of complementary food. This corresponds with the WHO report from 2002 (Butte N. et al., 2002), but the variation between the different months varies between 417 to

569 g/day in months 7-12. Variations in consumption of breastmilk are likely to be larger between children also having complementary food than between exclusively breastfed children.

9.4.2 Fat percentage

Persistent organic pollutants are fat-soluble, and their concentrations in breastmilk are therefore correlated to the fat percentage of the milk. The mean and median fat percentage of 3.5 in HUMIS is chosen as the default fat percentage value in this opinion. There are several factors that influence the breastmilk fat content as previously described in section 3.3.3. The variations are reported to be between 3 and 7% fat (per 100 g breastmilk), and some studies indicate that the variations might even be larger (Michaelsen, Larsen et al., 1994; Picciano, 2001). The variations are both inter-and intra-individual. The fat percentage, and thereby the exposure estimates can therefore both be an over- or an underestimate of the true value.

9.4.3 Body weight

Exposure estimates of contaminants are expressed per kg body weight. In the exposure assessments in this opinion, mean body weights reported in "Child Growth Standards" (WHO Multicentre Growth Reference Study Group, 2013), which includes Norwegian children, are used. By choosing the mean body weight, the body weight estimate and thereby the exposure estimates can both be an over- or an underestimate of the true value.

9.5 Exposure profile

Among the papers included in chapter 7 (describing negative health effects from contaminants in breastmilk), there are variations in how many substances are reported within a paper, and within a group of substances, e.g. PCBs, where variable numbers of PCB congeners are assessed. The issue can be illustrated by four main approaches when looking at it from this perspective:

a) one substance, one outcome – classical toxicology
b) several substances, one outcome
c) co-variation between substances
d) real life exposure: multiple exposures

Most studies within human toxicology have been conducted with one substance or group of substances and one health outcome in focus at a time. In reality, man is exposed to multiple substances simultaneously. The impact of real life exposure is an emerging science – very few studies address the issue at present. The co-variation between some contaminants can be considerable, e.g. mercury and dioxins/PCB have overlapping food sources, and concentrations in breastmilk may thus be strongly correlated. It may be difficult to isolate the biological impact of a specific contaminant, or to examine the independent effect in statistical analysis. Only few studies have been published where this issue has been discussed in detail. The co-variation between some contaminants and nutrients in breastmilk may also be considerable for the same reason as mentioned above; i.e. they have the same food sources. There are very few, if any, publications in this field, but when aiming to understand (positive or) lack of negative health effects of contaminants, this is a dimension to consider. However, both the prenatal and postnatal exposure include all contaminants present in either mother's

blood or breastmilk and thus possible impairment of specific health outcomes in reality integrate all exposures (see also section 10.3.4).

9.6 Confounders

Socio-economic and lifestyle factors often show co-variation with breastfeeding initiation and duration; the influences from these factors may be difficult to isolate. Factors that are independently associated with the decision to continue breastfeeding are e.g. maternal smoking, maternal age, single parent status, atopy risk and number of siblings (Haggkvist et al., 2010; Dulon et al., 2001). Nowadays it is difficult to get a paper published without adequate statistical correction for obvious confounding factors, and this was not a challenge in the risk articles apart from one parameter: lack of the HOME score assessment.

The HOME score measures how stimulating the child's home environment is and may be independent of socio-economic status. A specific confounder challenge for interpretation of studies on neurodevelopment is the lack of HOME score measurements. Where it had been included, several of the papers came to the conclusion that a stimulating home environment outbalanced an eventual negative effect of contaminants in breastmilk.

9.7 Bias

Selection bias is of particular concern since babies who receive exclusive and prolonged breastfeeding might be those growing well and remaining healthy. Furthermore, if the infant or the mother gets ill it may be weaned. Weaned infants cannot be switched back to breastmilk. Thus, there might be a selection of healthy children among those breastfed (Kramer, 2009). On the other hand, health information about the potential protective effect of breastfeeding on asthma and allergy might in particular motivate mothers with a family history of asthma or allergy to breastfeed exclusive and prolonged. Likewise, mothers whose infants experience recurrent respiratory infections might decide to prolong breastfeeding in order to provide the infant some sort of "protection". This kind of selection bias may attenuate any effect of impact of breastfeeding on these conditions.

The strong assumption of beneficial effects from breastfeeding poses a risk of report bias, e.g. that only studies confirming these positive effects are reported and cited.

9.8 Outcomes

A challenge when interpreting the benefit results was the lack of explicit sorting of results by length of breastfeeding, whether exclusive or partial. Furthermore, it has been challenging to separate the beneficial health outcomes apparent while breastfeeding, e.g. infections, from the long-term benefits measured after weaning, e.g. obesity.

As to the risk assessment, the major challenge is the heterogeneity within each main group of outcomes, e.g. immunologically or allergic related diseases, where all five papers included in reality measured widely different parameters. The outcomes investigated within the category neurodevelopment cover a number of different tests: The McCarthy Scales of Children's Abilities in four studies, the Bayley Scales of Infant Development in five studies, the Kaufman Assessment Battery for Children in two studies, but also more unspecified tests: play behaviour, or neurological examination or motoric development. Furthermore, the timepoint of investigation varied from some weeks to teenage years.

9.9 Summary of methodological issues

A number of methodological challenges are encountered when working with observational epidemiological studies. The ideal study design, an RCT, can for ethical reasons not be used to study breastfeeding and health outcomes. The particular methodological challenges appearing in this assessment were related to lack of adequate description of breastfeeding length and extent (exclusive or partial), thus making accurate doses and body burdens somewhat uncertain. Other challenges were related to whether the studies truly could separate pre- and postnatal exposure to contaminants. Many risk studies had investigated only one contaminant while in reality the infant is exposed to a mixture of many contaminants at the same time. All studies had corrected for traditional confounders like socioeconomic status, but several lacked information on HOME score, a parameter which is particularly important to include when studying the health effects of breastfeeding. We also noted that outcome measurements varied substantially between studies even within the same field of research, e.g. immune response-associated diseases, thus making comparisons of outcomes challenging. In chapter 10 we have tried to assess the impact of the methodological challenges in the final benefit risk assessment.

10 A comprehensive assessment of benefits and risks

The Norwegian health authorities' responsibility does not comprise the safety of human milk. In the wake of intermittent public debates about contaminants in breastmilk, there has been no official body to handle the unease. VKM thus took the initiative to perform a benefit and risk assessment of breastmilk with focus on the infant's health.

There is an extensive body of documentation showing that breastmilk is beneficial for the child. Apart from covering the nutritional needs of the infant, it is especially well documented that the immunological properties of human milk protect against infections and boost the child's own immunological development. Following this, a breastfed child seems to have lowered risk of a number of health conditions, both during the breastfeeding period itself and after weaning. However, there has been a continuous debate as to what the optimal duration of breastfeeding is. In 2011, the Norwegian Directorate of Health set up a working group whose mandate is to develop national guidelines on infant feeding, i.e. nutrition for healthy infants aged 0-12 months. The working group aims to finish its work in 2014. An assessment of contaminants in breastmilk is not part of the mandate of the Directorate of Health working group.

Breastmilk contains contaminants that have entered the mother's body through the lungs, skin and gastrointestinal tract. Breastmilk concentrations of contaminants reflect the contaminant history of the mother. In 1951, DDT became the first environmental pollutant to be found in human milk. The last four decades, additional chemicals have been detected in breastmilk, including persistent organic pollutants such as dioxins, PCBs, brominated flame retardants and a number of pesticides other than DDT. The majority of the most toxic early environmental contaminants have been banned, and their levels in breastmilk are decreasing (see Background chapter and the Stockholm convention), but low levels of a number of more recent contaminants have been introduced. It follows from this that previously, mothers were exposed to higher concentrations of a smaller number of contaminants, while the present-day situation may be exposure to a wider range of contaminants but at lower concentrations.

The discussion about optimal duration of breastfeeding therefore has a contaminant dimension. While there is no discussion about the benefits of breastfeeding initiation, there is a need to clarify whether environmental contaminants should have their say in the discussion about length and degree of breastfeeding.

Norwegian women have a high prevalence of breastfeeding and a particular high rate of partial breastfeeding at 12 months. Mean breastfeeding duration is 10.3 months, and at 12 month of age, 46% of the children are still breastfed. However, the prevalence of *exclusive* breastfeeding in Norway declines rapidly from 3 months onward (Figure 2.1). The mean volume of breastmilk given to the child is surprisingly similar in exclusively and partially breastfed infants up till 5 month of age (see Tables 2.2 and 2.3). Thus, the child may receive almost the same exposure to substances per kg body weight from the mother whether being exclusively or partly breastfed, at least up to a certain point in time.

This chapter attempts to tie all previous chapters and sections together into a whole and address benefits from breastmilk and risks from contaminants in breastmilk in a systematic way, taking factors relevant for Norwegian conditions into consideration. As there are many threads to be woven together, the following illustration might serve as a reading map:

The chapter starts with a go-through of guidelines for conducting benefit and risk assessments (section 10.1) and performing a grading (section 10.2). Then follows the actual grading, first of the documentation on positive health effects described in chapter 6, than a grading of the

documentation on negative health effects described in chapter 7. However, the grading alone is not sufficient for a benefit and risk assessment. Additional aspects also have to be taken into consideration (section 10.3). Additional considerations include e.g. exposure levels in Norway and infant formula as an alternative to breastmilk. Finally, all these aspects will be merged into a benefit and risk assessment (section 10.4) which leads up to the conclusion (chapter 11).

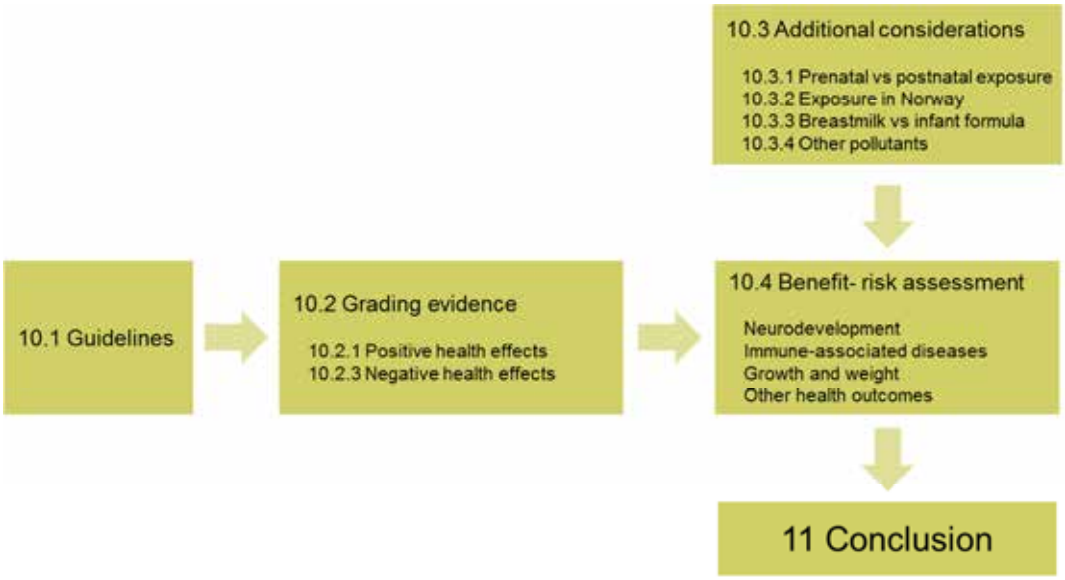

Figure 10.1: A reading guide to chapter 10 and 11.

10.1 A health-based risk-benefit assessment – guidelines and grading

10.1.1 Benefit and risk assessment guidelines

In 2010, the EFSA Scientific Committee developed "Guidance on human health risk-benefit assessment of foods" (EFSA, 2010a). They recommended a stepwise approach for the risk-benefit assessment, i.e.

> i) an initial assessment, addressing the question whether the health risks clearly outweigh the health benefits or vice versa,
>
> ii) a refined assessment, aiming at providing semi-quantitative or quantitative estimates of risks and benefits at relevant exposure by using common metrics, and
>
> iii) a comparison of risks and benefits using a composite metric such as disability-adjusted life years (DALYs) or quality-adjusted life years (QALYs) to express the outcome of the risk-benefit assessment as a single net health impact value.

Furthermore, the EFSA guidance document stated the following:"The outcome of each step of the assessment should also include a narrative of the strengths and weaknesses of the evidence base and its associated uncertainties. The overall magnitude of uncertainty associated with a risk-benefit assessment may often be large. This should not be regarded as implying a failure of the assessment; on the contrary, it provides essential information for decision-making and helps in identification of data needs."

This report focuses on step i) and ii). The common metrics will be the health outcomes. Employing step iii) has been beyond the capacity of the working group. To our knowledge no-one has worked with DALYs or QALYs and breastmilk in a risk-benefit perspective in healthy, born at term infants.

10.1.2 Grading the evidence – guidelines

There are several methods in use for grading evidence in systematic literature reviews. The different methods became apparent in the reviews used as a basis for our benefit assessment.

When grading the results of the risk assessment part of this work, we will use the grading system developed by the 2007 World Cancer Research Fund (WCRF) report (World Cancer Research Fund, 2007) and adopted by the Nordic Nutrition Recommendation project group (NNR) as a starting point. This grading system is especially developed for a situation where observational studies form the basis, as in this assessment. Evidence is classified as convincing (grade 1), probable (grade 2), limited-suggestive (grade 3) and limited – no conclusion (grade 4) depending on the number and quality of supporting, non-supporting and contradicting studies.

As described in chapter 7, the majority of the papers reviewed showed no statistically significant association between contaminant exposure from breastmilk and the health outcome in focus. Thus, if many studies show no association, a discussion arises, whether lack of association is equivalent to convincing evidence of no effect. We have tried to keep in mind that "Absence of evidence is not evidence of absence". As the method for systematic literature reviews in NNR is inspired by the approach used in the extensive work performed in the WCRF report from 2007 it is relevant to see how WCRF handles the issue.

The WCRF introduces the expression "substantial effect on risk unlikely" when there is evidence to support a judgement that a particular exposure is unlikely to have a substantial causal relation to the outcome. The evidence should be robust enough to be unlikely to be modified in the foreseeable future as new evidence accumulates.

Box 2. Criteria for assigning grade of evidence (modified from WCRF) for the three category quality grading system.

This box lists the criteria modified from the WCRF cancer report that have been connected to the three category quality grading system developed by the AHQR. The grades shown here are 'convincing', 'probable', 'limited — suggestive', 'limited — no conclusion'.

Convincing (High)

These criteria are for evidence strong enough to support a judgement that there is a convincing causal relationship or absence of relationship. A convincing relationship, or absence of relationship, should be robust enough to be highly unlikely to be modified in the foreseeable future as new evidence accumulates. All of the following criteria are generally required:

- Evidence from more than one study type (RCT, prospective cohort or nested case-control studies). For some outcomes (e.g. some riskfactors) evidence from several RCT may be sufficient.
- Evidence from at least two independent cohort studies (cf above).
- No substantial unexplained heterogeneity within or between study types or in different populations in relation to the presence or absence of an association or the direction of effect.
- Several good quality studies (quality grading category A) with consistent findings to exclude with confidence the possibility that the observed association, or absence of association, results from random or systematic error, including confounding, measurement error, and selection bias.
- Presence of a biological gradient ('dose response') in the association. Such a gradient need not be linear or even in the same direction across the different levels of exposure, so long as this can be explained plausibly.
- Strong and plausible experimental evidence, either from human studies or relevant animal models, that typical exposures in humans can lead to relevant outcomes.

Probable (Moderate)

These criteria are for evidence strong enough to support a judgement of a probable causal relationship. All the following criteria are generally required:

- Evidence from at least two independent cohort studies, or at least five case-control studies. For some outcomes (e.g. some riskfactors) evidence from a few RCT may be sufficient
- No substantial unexplained heterogeneity between or within study types in the presence or absence of an association, or the direction of effect.
- Several good quality studies (quality grading category A and B) with consistent findings to exclude with confidence the possibility that the observed association, or absence of association. results from random or systematic error, including confounding, measurement error, and selection bias.
- Evidence for biological plausibility, in case of an observed association.

Limited — suggestive (Low)

These criteria are for evidence that is too limited to permit a probable or convincing causal, or absence of causal, relationship, but where there is evidence suggestive of a direction of effect. The evidence may have methodological flaws, or be limited in quantity, but shows a generally consistent direction of effect. All the following criteria are generally required:

- Evidence from at least two independent cohort studies or at least five case-control studies.
- The direction of effect is generally consistent though some unexplained heterogeneity may be present.
- Several studies of at least moderate quality (quality grading category B).
- Evidence for biological plausibility

Limited — no conclusion (Insufficient)

Evidence is so limited that no firm conclusion can be made. A body of evidence for a particular exposure might be graded 'limited — no conclusion' for a number of reasons. The evidence might be limited by the amount of evidence in terms of the number of studies available, by inconsistency of direction of effect, by poor quality of studies (for example, lack of adjustment for known confounders), or by any combination of these factors. Most of the studies are in the quality grading category C or there are 2 or more high (A) or moderate (B) quality studies with contradicting or null results.

Box 3: "Substantial effect of risk unlikely" as defined by WCRF.

- Evidence from more than one study type
- There is evidence from at least two independent prospective cohort studies
- Summary estimate of effect close to 1.0 for comparison of high versus low exposure categories
- No substantial unexplained heterogenicity within or between study types or in different populations
- Good quality studies to exclude, with confidence, the possibility that the absence of an observed association results from random or systematic error, including inadequate power, imprecision or error in exposure measurement, inadequate range of exposure, confounding, and selection bias
- Absence of a demonstrable biological gradient ("dose-response")
- Absence of strong and plausible experimental evidence, either from human studies or animal models, that typical human exposures lead to relevant cancer outcomes

This report will discuss grading that falls in the "substantial effect on risk unlikely"-category defined as above if relevant.

10.2 Grading of evidence

The grading of evidence on the positve and negative side will have one important difference: when grading the positive health effects of breastmilk, it is the documentation for the *breastmilk as such* which is evaluated, while the grading on the negative side is an evaluation of the documentation for negative health effects, or *hazard*, of *substances* in breastmilk.

10.2.1 Grading of evidence – postive health effects

As described in chapter 6, positive health effects associated with consumption of breastmilk in this report is based on conclusions from systematic reviews and meta-analyses published within the last 10 years. When grading the evidence on the benefit side, we will thus have to perform the "excercise" of grading the sum of others work, some of whom have not graded their results and some using grading, but various grading systems. An element of "best judgement" is thus unavoidable from VKM's side, as there to our knowledge are no international guidelines for grading on the basis of reviews and meta-analyses.

While the reports by Ip *et al*. (2007), WHO (2007) and SACN (2011) have no grading of evidence within their reports, grading was used in both the van Rossum *et al*. (2005) and Hörnell *et al*. (2013). The recent WHO-review (2013) uses statistical models and odds ratios to come to conclusions.

Table 6.1 summarises most of the reported positive health effects of breastfeeding from the six systematic reviews and meta-analyses. The health outcomes are multiple and represent various disease categories, such as infectious diseases, immune response-associated diseases, metabolic syndrome (e.g. overweight/obesity and diabetes), cognitive development, malignant disease and sudden infant death syndrome.

In the following grading of evidence most attention will be given to those health outcomes which also have been studied from a risk point of view, i.e. neurodevelopment, immune response-associated diseases and growth and overweight/obesity. A short paragraph on biological plausibility will be given if the grade of evidence is convincing or probable or otherwise relevant.

10.2.1.1 Neurodevelopment

Four out of the six systematic reviews and meta-analyses on positive health effects of breastfeeding described in chapter 6 studied outcomes related to cognitive development (van Rossum, 2005; WHO, 2007; Hornell, 2013; WHO, 2013). All four reviews found a positive impact of breastfeeding on child neurodevelopment, van Rossum *et al*. concluding with there being probable evidence of improved intellectual and motor development. However, socio-economic factors, parental intelligence and HOME score are vital confounding factors. Taking these factors into account, Hörnell *et al*. concluded that there is a probable evidence that breastfeeding is beneficial for IQ and developmental scores of children, i.e. increasing scores with increasing duration (Hornell et al., 2013).

WHO (2013) found an increase in test scores both in the pooled analysis of all studies, as well as in the high-quality studies included. They concluded that there is a strong evidence of causal effect of breastfeeding on increased IQ, although the magnitude of this effect seems to be modest (WHO, 2013).

In the PROBIT Study from Belarus, at 6.5 years of age, the intervention group had higher means on all of the Wechsler Abbreviated Scales of Intelligence measures (section 6.1.2). Teachers' academic ratings were significantly higher in the intervention group for both reading and writing. When pooling their data and treating them as an observational study, there was still a higher IQ in the children breastfed for 6 vs 3 months, but the difference did not reach statistical significance.

The biological plausibility for an association between breastmilk and neurodevelopment is connected to specific breastmilk constituents which may offer developmental advantages. They include long-chain polyunsaturated fatty acids (LCPUFA) and particularly DHA (see section 3.4). Isaacs *et al*. reported that breastfeeding was positively related to brain volume and – among male subjects – increased amounts of white matter was also related to breastfeeding (cited in WHO, 2013), thus suggesting that breastmilk promotes structural changes in the brain. In a recent publication from Canada, exclusive breastfeeding was associated with the brain's cortical thickness – in addition to replicating the association between breastfeeding and general intelligence (Kafouri et al., 2013).

As described in section 3.4.4, breastmilk influences the gut microbiota differently from infant formula, and this may also be relevant for neurodevelopment.

However, the suggested biological mechanisms must be considered as hypotheses; none of the suggestions are so far supported by unequivocal evidence.

VKM concludes that the evidence is *convincing* for a positive effect of breastmilk on neurodevelopment. The optimal length of exclusive and partial breastfeeding associated with the positive effect remains to be settled.

10.2.1.2 Immune response-associated diseases

Infections

A reduced risk of lower respiratory tract infections, especially the severe ones, as well as of gastroenteritis and otitis media in breastfed versus non-breastfed children is well documented (Agostoni et al., 2009; Ip et al., 2009; van Rossum et al., 2005). The reduced risk is demonstrated both when comparing exclusive with partial breastfeeding or formula feeding and when comparing different lengths of exclusive or partial breastfeeding with formula feeding.

In the recent systematic literature review by Hörnell *et al*. (2013) it is concluded that the evidence is convincing that breastfeeding protects infants in industrialised countries against overall infections, acute otitis media and gastrointestinal and respiratory tract infections. The magnitude of the effect varies depending on the specific outcome and the exclusiveness of breastfeeding (Hornell et al., 2013).

The biological plausibility for the protective effect of breastmilk on infectious diseases is strong (see section 3.4).

All the reviews agree that the protection from infections lasts as long as the child is breastfed. The large Generation R study found that exclusive breastfeeding for 4 months and partial breastfeeding thereafter reduced the risk significantly up to 1 year of age (Duijts et al., 2010). It is unclear whether the effects last beyond the partly breastfeeding period.

VKM concludes that the evidence is *convincing* for a protective effect of breastmilk on infections, at least as long as the child is exclusively or partly breastfed.

Asthma and wheezing, allergies and atopic dermatitis

The studies on immune-assosiated diseases show heterogeneous results. Both for asthma, allergies and atopc dermatitis (see section 6.3), the review by van Rossum concluded that there is probable evidence, while Hörnell *et al*. concluded that there is limited evidence for a protective effect and no conclusion can be given.

In the PROBIT Study a reduced prevalence of atopic eczema was found at 1 year when treating the data as an RCT study. No differences in allergy incidence were observed between the intervention and control groups when the children were 6.5 years old irrespective of how they handled the data statistically (section 6.1.2). The results may indicate that the protective effect is strongest in early life. It must be kept in mind, though, that *all* children in the PROBIT study were breastfed initially.

The immunoregulatory properties of breastmilk form a strong biological plausibility for a positive impact of breastmilk on immune response-associated diseases.

As described in detail in section 3.4, numerous components of breastmilk not only possess innate and adaptive defence properties, but may also aid the nursed infant's immune development, including generation of immunological tolerance with appropriately controlled inflammatory reactions.

VKM is aware of the challenges connected to diagnosis of immune response-associated diseases and that this may vary between studies. VKM is also aware that the heterogenicity of results may be related to the differences in breastfeeding length reported between studies. An explanation might be that there is an unknown, but optimal length of breastfeeding in relation to immune response-associated diseases, after which the impact of breastfeeding fades.

VKM concludes that the evidence is *limited, and no conclusion* can be given for a protective effect of breastmilk on asthma, wheezing, allergies and atopic dermatitis.

Type 1 diabetes

As pointed out in chapter 6, available evidence suggests that breastfeeding may reduce the risk of type 1 diabetes mellitus. Ip *et al*. concluded that the risk of developing type 1 diabetes is significantly reduced in breastfed infants, van Rossum *et al*. (2005) indicated "possible

evidence" for a protective effect from breastfeeding, while Hörnell *et al*. (2013) indicated "probable evidence".

Type 1 diabetes is characterised by a selective loss of insulin-producing B cells in the pancreatic islets in genetically susceptible individuals. Evidence suggests that B cell autoimmunity may be induced early in life. The biological plausibility for an association between breastfeeding and type 1 diabetes may be due to breastmilk *per se* or to the associated delayed introduction of gluten and/or cow's milk protein (see section 6.2.3).

Furthermore, the positive effect of breastmilk has been suggested to be based on the fact that breastmilk strengthens the child's immune system. Several anti-microbial substances in human milk may protect the child from for instance enterovirus infections, and as a consequence from B cell autoimmunity, which progressively can lead to the development of type 1 diabetes. Another possible protective mechanism is the more rapid decrease in gut permeability that breastfed children exhibit compared to children who are formula fed during the first months of life. Conversely, the early introduction to cow's milk proteins may increase intestinal permeability, cause inflammation of the intestinal mucosae, and deregulate the immune response to cow's milk protein (Patelarou et al., 2012).

VKM concludes that the evidence is *probable* for a protective effect of breastmilk on risk of type 1 diabetes. The optimal length of exclusive and partial breastfeeding associated with the reduced risk remains to be settled.

Crohn's disease and ulcerative colitis

Crohn's disease is included in the reviews by van Rossum *et al*. (2005) and Hörnell *et al*. (2013). The first concluded that there is "possible evidence" for a protective effect of breastmilk while the latter concluded that the evidence is "probable". As to ulcerative colitis, van Rossum *et al*. concluded that the evidence is insufficient and Hörnell *et al*. concluded that the evidence for a protective effect from breastfeeding is "probable".

The suggested biological plausibility for an association between breastmilk and inflammatory bowel disease may be attributed to the immunomodulatory properties of human milk. Also, breastmilk oligosaccharides supporting the growth of beneficial intestinal microorganisms might be of importance (Klement et al., 2004).

VKM concludes that the evidence is *limited suggestive* for a protective effect of breastmilk on risk of Crohn's disease and ulcerative colitis.

Coeliac disease

Coeliac disease was reviewed by Hörnell *et al*. (2013) and the conclusion was that there was "probable evidence" for breastfeeding as a protective factor for coeliac disease, or at least for delaying its clinical presentation. SACN COT (2011) stated that the evidence currently available is not strong enough to make specific recommendations about the appropriate timing of introduction of gluten.

The biological plausibility for an association between coeliac disease and breastmilk may be linked to the presence of immunomodulating factors in breastmilk, influencing tolerance to gluten antigen.

VKM concludes that the evidence is *limited suggestive* for a protective effect of breastmilk on risk of coeliac disease.

10.2.1.3 Growth, overweight and obesity

All the systematic reviews and meta-analyses on positive health effects of breastmilk, described in chapter 6, have studied overweight and obesity, and they all conclude that breastmilk reduces the risk of overweight and obesity in childhood. In the systematic literature review by Hörnell *et al*. (not including the 2013 PROBIT Study on overweight (Martin et al., 2013)) it is concluded that growth in infancy (length and height) varied only a little between those exclusively breastfed for 4 months or 6 months. Nonetheless, it is concluded that there is probable evidence that exclusive breastfeeding for more than 4 months is associated with slower weight gain during later infancy compared with those exclusively breastfed for less than 4 months. The evidence is convincing that longer duration of exclusive or any breastfeeding is associated with a protective effect against overweight and obesity in childhood and adolescence. With regard to the association with overweight/obesity in adulthood, due to a scarcity of studies, it is judged that the evidence is limited suggestive for a protective effect of breastfeeding (Hornell et al., 2013).

The latest WHO review stated that a significant reduction in body weight in children was found both in the pooled analyses of all studies (24% reduction) as well as in the high quality studies (12% reduction). The protective effect was especially notable in childhood and through the teens till the age of 19. The report concluded that breastfeeding may provide some protection against overweight and obesity, but residual confounding cannot be ruled out (WHO, 2013).

The PROBIT Study found no difference in risk of overweight and obesity between the different breastfeeding groups at the ages of 6.5 and 11 years, irrespective of how they organised their data (i.e. using the RCT design or observational design, see 6.1.2) and irrespective of length of exclusive breastfeeding. However, again it must be kept in mind that *all* PROBIT children were breastfed initially.

As to biological plausibility, several mechanisms have been proposed for a protective effect of breastfeeding against overweight and obesity, although they must be considered to be hypotheses as none of the suggestions are so far supported by unequivocal evidence. Increased protein intake in early infancy has been suggested to be an important risk factor for later overweight and obesity – and knowing that infant formula in general has higher protein content than breastmilk (Nommsen-Rivers and Dewey, 2009) – this could contribute to some of the differences found in growth between breastfed and formula fed children. A high-protein diet stimulates insulin-like growth factor 1 (IGF-1) and insulin release in infancy, factors involved in growth the first years of life (Rzehak et al., 2013; Socha et al., 2011). Another path to explain the differences in risk between formula fed and breastfed infants goes by the gut microbiota. Type of feeding (breastmilk or infant formula) plays important roles in determining the microbiota composition, see section 3.4.4 and Morelli *et al*., 2008 (Morelli, 2008).

Yet another hypothesis to explain differences in risk of overweight between breastfed and formula fed children goes by the infant's ability to self-regulate intake: there is evidence that infants who are bottle-fed in early infancy, irrespective of the content being formula or expressed breastmilk, are more likely to empty the bottle. This tendency to finish a portion seems to continue into later childhood (Li et al., 2008; Li et al., 2010; Li et al., 2012). This implies a higher caloric intake and may explain the increased risk.

VKM concludes that the evidence is *convincing* for a protective effect of breastfeeding on risk of overweight and obesity in childhood and adolescence. The optimal length of

exclusive and partial breastfeeding associated with the reduction in risk remains to be settled.

10.2.1.4 Type 2 diabetes

As to type 2 diabetes, Ip *et al*. (2007) and SACN (2011) indicated a protective effect from breastmilk, while Hörnell *et al*. (2013) concluded that there is a "probable" reduced risk. WHO (2013) concluded that further studies are needed. Few studies have investigated breastmilk in relationship to risk of type 2 diabetes in adults.

The biological plausibility for an association between breastmilk and type 2 diabetes may be the presence of long-chain polyunsaturated fatty acids (LCPUFAs) in breastmilk. Breastmilk increases the amount of these fatty acids in skeletal muscle membrane, which is inversely related to fasting blood glucose. Differences in insulin secretion represent another possible mechanism; the concentration of insulin in formula fed infants is higher than in breastfed, and may lead to failure of the B cells, and subsequently type 2 diabetes. Also, since obesity is an important determinant of type 2 diabetes, a protective effect of breastfeeding on obesity may represent another explanation (WHO, 2013).

VKM concludes that the evidence is *probable* for a protective effect of breastmilk on risk of type 2 diabetes. The optimal length of exclusive and partial breastfeeding associated with the reduction in risk remains to be settled.

10.2.1.5 Childhood cancer

As described in section 6.2.5, some studies suggest a reduced incidence of some cancers among breastfed individuals. Ip *et al*. (2007) and SACN (2011) indicated that there is a reduced risk, while van Rossum *et al*. (2005) and Hörnell *et al*. (2013) concluded that the evidence is limited suggestive.

One possible biological plausibility for an association between breastmilk and malignancies may be the presence of substances with antitumor activity, like HAMLET formations (described in section 3.4.1).

VKM concludes that the evidence is *limited suggestive* for a protective effect of breastmilk on risk of childhood cancer.

10.2.1.6 Sudden infant death syndrome (SIDS)

Breastfeeding appears to be associated with a reduced risk for SIDS. Ip *et al*. (2007) indicated that there is a reduced risk, while van Rossum *et al*. (2005) concluded that the evidence is "insufficient". A later meta-analysis published in 2011 (Hauck *et al*.), concluded that breastfeeding is protective and the effect is stronger when breastfeeding is exclusive.

SIDS may have several causes, airway infection being one predisposing factor. Breastfed children seem to have a different sleeping pattern with lower arousal thresholds than formula fed infants, which may provide one mechanism for protection against SIDS.

VKM concludes that the evidence is *limited suggestive* for a protective effect of breastmilk on risk of sudden infant death syndrome.

10.2.1.7 Cardiovascular diseases

All reviews included in the present assessment agreed that there is no convincing evidence that breastfeeding influences cardiovascular morbidity and mortality.

VKM concludes that the evidence is *limited and no conclusion* can be given for a protective effect of breastmilk on risk of cardiovascular diseases.

10.2.1.8 High blood pressure

WHO (2007) and SACN (2011) concluded that blood pressure later in life is reduced (systolic and diastolic) in breastfed children. Van Rossum *et al*. (2005) indicated "convincing evidence" while Hörnell *et al*. (2013) indicated "probable evidence". The WHO (2013) systematic review found a small protective effect of breastfeeding against increased systolic, but not diastolic blood pressure. The effect on systolic blood pressure was especially pronounced through childhood until the age of 19.

The suggested biological plausibility for an association between breastmilk and blood pressure is related to differences in sodium content between formula and breastmilk. This might be a mechanism for programming of later blood pressure, but evidence for such a mechanism is scarce. Different long-chain polyunsaturated fatty acids are present in breastmilk which until recently were not present in formula. As these are important structural components of the vascular endothelium, they may partly explain an eventual favourable effect of human milk on later blood pressure (WHO, 2013).

VKM concludes that the evidence is *probable* for a protective effect of breastmilk on later risk of high blood pressure. The optimal length of exclusive and partial breastfeeding associated with the reduced risk remains to be settled.

10.2.1.9 Serum cholesterol

Long-term reduction in serum cholesterol is included in the review from WHO (2007) which reported a reduced mean difference in serum cholesterol between breastfed and non-breastfed which lasts into adulthood. Also SACN (2011) indicated reduced serum cholesterol from breastmilk and Hörnell *et al*. (2013) concluded that there is a "probable" reduction. A significant reduction in serum cholesterol among adults was not found in the updated systematic review from WHO (2013).

VKM concludes that the evidence is *limited and no conclusion* can be given for an association between breastmilk and reduced serum cholesterol.

10.2.2 Summary grading of evidence, positive health effects

Table 10.1 summarises the results from the systematic reviews and meta-analyses of the positive health effects associated with consumption of breastmilk, and includes an evaluation of the strength of evidence from VKM.

Table 10.1: Summary of the strength of evidence in children for positive health effects associated with consumption of breastmilk. The Table is identical to Table 6.1 apart from the addition of the column with conclusions from VKM to the very right.

Main results in infants	WHO, 2007	Ip, 2007	van Rossum, 2005	SACN, 2011	Hörnell,2013 (NNR)	WHO, 2013	VKM
Intelligence test score	↑ MD 4.9 (2.97; 6.92)[a]	-	-	-	Probable ↑	↑ MD 3.45 (1.92; 4.98)[a] ↑ MD 2.19 (0.89; 3.50)[b]	Convincing ↑
Intellectual and motor development	-	-	Probable evidence ↑	-	-	-	No conclusion
Otitis media	-	↓	Convincing evidence ↓	-	Convincing ↓	-	Convincing ↓
Gastrointestinal infections	-	↓	Convincing evidence ↓	-	Convincing ↓	-	Convincing ↓ *
Respiratory infections	-	-	Possible evidence ↓	-	Convincing ↓	-	Convincing ↓
Severe lower respiratory tract infections	-	↓	-	-		-	Convincing ↓*
Atopic disease	-	-	Probable evidence ↓	-	Limited, no conclusion	-	Limited, no conclusion
Atopic dermatitis	-	↓	Eczema, Probable evidence ↓	-	Limited, no conclusion	-	Limited no conclusion
Asthma (young children)	-	↓	Probable evidence ↓	-	Limited, no conclusion	-	Limited, no conclusion
Wheezing	-	-	Probable evidence ↓	-	Limited, no conclusion	-	Limited, no conclusion
Obesity	↓ OR 0.78 (0.72; 0.84)[c]	↓	Convincing evidence ↓	↓	Convincing ↓	↓ OR 0.76 (0.71; 0.81)[c] ↓ OR 0.88 (0.83; 0.93)[d]	Convincing
Coeliac disease	-	-	-	-	Probable ↓	-	Limited, suggestive
Type 1 diabetes	-	↓	Possible evidence ↓	No evidence	Probable ↓	-	Probable ↓
Type 2 diabetes	↓ OR 0.63 (0.45; 0.89)[c]	↓	-	↓	Probable ↓	↓ OR 0.66 (0.49; 0.89)[c]	Probable ↓
Childhood cancer	-	↓	Possible evidence ↓	↓[1]	Limited, suggestive ↓	-	Limited, suggestive ↓
SIDS	-	↓	Insufficient evidence	-	-	-	Limited, suggestive**
Cardiovascular diseases	-	Not clear	No evidence	No evidence[2]	-	-	Limited, no conclusion
Crohn's disease	-	-	Possible evidence ↓	-	Probable ↓	-	Limited, suggestive
Ulcerative	-	-	Insufficient	-	Probable ↓	-	Limited,

Main results in infants	WHO, 2007	Ip, 2007	van Rossum, 2005	SACN, 2011	Hörnell,2013 (NNR)	WHO, 2013	VKM
colitis			evidence				suggestive
High blood pressure	↓ Systolic MD-1.2mmHg (-1.72; -0.70)[a] ↓ Diastolic MD-0.49mmHg (-0.87, -0.11)[a]	-	Convincing evidence ↓	↓	Probable ↓	↓ Systolic MD-1.02mmHg (-1.45; -0.59)[a] ↓ Systolic MD-0.71mmHg (-1.24; -0.19)[b] ↓ Diastolic MD-0.37mmHg (-0.71; -0.04)[a] ↓ Diastolic MD-0.27mmHg (-0.64; 0.09)[b]	Probable ↓
Serum cholesterol	Adulthood ↓ MD-0.18mmol/L (-0.30; -0.06)[a] Children and adolescents NS	-	-	↓	Probable ↓	No evidence	Limited, no conclusion

[a]Mean difference (MD) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies.
[b]Mean difference (MD) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies, restricted to studies considered high quality
[c]Odds ratio (OR) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies.
[d]Odds ratio (OR) and 95% confidence intervals by meta-analysis comparing breastfed vs. not-breastfed subjects in different studies, restricted to studies considered high quality.
[1]Reduced risk for acute lymphoblastic leukemia, Hodgkin's disease and neuroblastoma.
[2]SACN 2011 has looked into cardiovascular mortality whereas the others have reported morbidity.
- (=Not investigated).
MD: Mean difference.
*Including results from Duijts *et al*., 2010.
**Including results from Hauck *et al*., 2011.
↓Reduced risk.
↑Increased score.

10.2.3 Grading of evidence – negative health effects

To comply with the terms of reference, the assessment of risks is based on findings from a full-scale systematic literature search, see section 7.1. To assess the relevance and quality of included studies, a rating system based on the NNR5 AMSTAR was applied, modified for our purposes, see Appendix 2. The quality assessment resulted in no studies in category A, while 24 studies qualified for category B. The category C-studies were not considered further.

A short paragraph on biological plausibility will be given if the grade of evidence is convincing or probable or otherwise relevant. As stated in the introduction to section 10.2, the papers which follow have investigated possible negative health effects of breastmilk with focus on *substances* in breastmilk, and not breastmilk as such. Our grading of the evidence

thus has similarities to a hazard characterisation, i.e. how well it is documented that environmental contaminants in breastmilk are a cause of concern.

10.2.3.1 Neurodevelopment

PCBs are the most extensively investigated POPs with regard to possible negative effects on neurodevelopment. Also dioxins, DDE and HCB have been investigated. Since these substances generally show high correlation in breastmilk, are all fat-soluble, and since no causative associations can be deduced from the cohort studies, VKM has pooled the studies on PCBs, dioxins, DDT/DDE and HCB when grading the evidence, whereas mercury is addressed separately.

The biological rationale for a detrimental effect of POPs on neurodevelopment is described in chapter 5.

Eight of the cohorts included in the present benefit and risk assessment (published in 16 papers, 14 including POPs and two including mercury) have looked particularly on breastmilk exposure to contaminants and risk of impaired neurodevelopment. The most consistently used tests are the Bayley Scales of Infant Development up till 2 years of age, and the McCarthy Scales of Children's Abilities in the 4 to 7-year olds. The Kaufman Assessment Battery for Children was used on 4-year olds in two cohorts and a follow-up at 6 years in one cohort. The same tests have been administered at similar ages in several of the studies. No association between neurodevelopment (cognitive or motor abilities) and postnatal exposure to POPs was found in three cohorts (eleven out of 15 papers, see Table 7.14).

A positive effect of breastmilk was however noted in several of the studies (see section 7.2.4).

In three papers from three cohorts, various negative associations with PCB exposure were reported. In the Dutch cohort (summarised in section 7.3), indications of a negative impact of contaminants on neurodevelopment were transient (observed at 7 months but not at 18 months, Bayley Scales of Infant Development) and not found at later follow-up when assessing developmental milestones and intelligence (Kaufman Assessment Battery, McCarthy Scales). In the German cohort, no associations between postnatal PCB exposure and development at 7, 18 and 30 months (Bayley Scales of Infant Development) were seen, but at 42 months, there was association with decreased intelligence scores assessed by the Kaufman Assessment Battery. In a later follow-up at 6 years of age, the negative effect of postnatal PCB exposure was no longer significant.

The last study (the Canadian Inuit cohort), using a PBPK model to estimate exposure, assessed development (Bayley Scales of Infant Development) at 11 months of age and found that activity level, measured by non-elicited activity, was best predicted by postnatal exposure, with the strongest association obtained for simulated PCB levels during the fourth month of life, indicating this as a sensitive period in neurodevelopment. No follow-up has been published, and only exclusive breastfeeding was used in the exposure model.

Investigations of Hg in breastmilk and neurodevelopment is limited to two papers from one cohort from the Faroe Islands and showed a positive association between exposure via breastmilk and milestone development, in spite of substantially higher contaminant concentrations (PCBs, DDE and Hg) in the breastmilk. Studies from this cohort have however clearly indicated that children are more susceptible to prenatal methyl-Hg exposure than to postnatal exposure via breastmilk.

The biological plausibility for the neurotoxicity of POPs such as dioxins and dl-PCBs, non-dl-PCBs and DDT is well documented, but is less clear for HCB (section 5.2).

The transient negative associations in the Dutch cohort did not show a dose-response relationship. In the German cohort, the effect was shown by linear regression analyses, implying an underlying dose-response relationship. In the Canadian Inuit cohort there appeared to be a dose-response between the simulated exposure (unadjusted for confounders) at 4 months and the outcome.

Mean serum PCB-levels were approximately 1.4-fold higher in the German cohort than in the Dutch and Canadian cohorts. The cohorts reporting no effects had levels in the same range as the Dutch, with the exception of the INMA mainland cohorts, which had substantially lower exposure, and in the same range as in Norway.

Two studies considered the possibility of postnatal critical windows and estimated serum concentrations in infants. However, only one of the studies (the Canadian Inuit cohort) had findings indicating an effect of POPs in breastmilk – the other study (the INMA mainland cohort) had no findings. As explained above, the contaminant exposure level was much lower in the INMA mainland cohort than in the Canadian cohort, possibly explaining why no effect was observed.

The above summary of findings shows that in four out of seven cohorts, no association between POPs in breastmilk and neurodevelopment was found. In two cohorts, the association was transient, as an association was found at low age, but was no longer significant at later follow-up. The last study used PBPK modelling and found an association in 11-month old infants, but follow-up data have not been published. These overall results led the VKM committee into discussions of how to grade the evidence in a hazard characterisation context. It was discussed if the results qualified for a conclusion of "substantial effect on risk unlikely". However, there is a biological rationale for POPs having harmful effects on neurodevelopment (described in section 5.2), this effect has been confirmed in animal studies, and there was a transient dose-response relationship of effect up to a certain point in time in two of the studies. Thus the evidence does not qualify for this conclusion.

VKM concludes that the evidence is *limited suggestive* for a negative effect from POPs in breastmilk on neurodevelopment. The concentration of POPs in breastmilk, combined with the length of exclusive and partial breastfeeding associated with the transient negative effects, remains to be settled. As to mercury, this compound was only investigated in one cohort and VKM concludes that the evidence for an effect of exposure from breastmilk is limited and no conclusion can be given.

10.2.3.2 Immune response-associated diseases (thymus weight, vaccine antibody titer, middle ear infections, asthma and wheezing)

Associations between contaminants in breastmilk and later immune response-associated parameters have been studied in four cohorts published in five papers (Table 7.15). Widely different outcome measures associated with function of the immune system were investigated in the five included studies (thymus weight, vaccine antibody titer, prevalence of middle ear infections, asthma and wheezing), and they are not directly comparable.

In the Faroe Islands cohort 3, Grandjean *et al.* (2010) found that an apparent association between breastfeeding duration and increased serum IgE in children was weakened and no longer significant after adjustment for the concomitant PCB exposure. A history of asthma or atopic dermatitis was not associated with the duration of breastfeeding. The results indicate that PCBs in breastmilk may cause an IgE increase in children and thus interact with beneficial effects from breastfeeding (Grandjean et al., 2010).

Also, in the Faroe 3 cohort there was an inverse association between PCB exposure and vaccine antibody concentrations at 7 years, indicating that the contaminants reduce the efficacy of vaccines (Heilmann et al., 2010). In the Dutch cohort, there was no association between postnatal PCB and dioxin exposure and vaccine antibody titer to mumps, measles and rubella. The Dutch study found an increase in middle ear infection, but in the same children reduced prevalence of allergic disease with increasing PCB and dioxin exposure in the children at age 4. The INMA (Menorca) cohort found no association with asthma and wheezing and postnatal exposure to DDE/DDT. The Slovakian cohort had thymus size as endpoint and found a small but significant decrease in thymus weight between highly and moderately PCB exposed when the child was 6 months but not at 16 months.

The mean serum PCB-153 level was more than twice as high in the Faroe 3 cohort as in the Dutch, Slovakian and the INMA (Menorca) cohorts. The Menorca cohort addressed DDE/DDT (not PCBs), and the mean level of cord serum DDE was twice as high as those of the Faroe 3 cohort.

Biological plausibility for immunological effects of PCB and PCDD/PCDF include morphological changes in organs related to the immune system, as well as functional impairment of humoral- and cell-mediated immune responses. Reduced lipopolysaccharide-induced proliferative response in splenocytes, reduced antibody secretion and impaired neutrophil function are associated with the ndl-PCBs as these effects seem to be independent of the Ah receptor. Examples of Ah receptor-mediated adverse effects of dioxins and dl-PCBs seem to be disruption of the endothelial barrier function, activation of oxidative stress-sensitive signalling pathways, and induction of proinflammatory events (EFSA, 2005a). Immune response-associated disorders are probably most likely to occur in a sensitive perinatal or postnatal window of development (section 10.3.1).

VKM concludes that the evidence is *limited and no conclusion* can be given for a negative effect from POPs in breastmilk on thymus weight, vaccine antibody titer, middle ear infections, asthma and wheezing in children.

10.2.3.3 Growth and weight

Associations between POPs in breastmilk and later growth and development have been studied in three cohorts published in four papers, see Table 7.16. Two of the cohorts (the North Carolina and Michigan cohorts) found no associations between PCB exposure from breastmilk and child height or weight development, while the Faroe 2 cohort study noted a somewhat *lower* weight and height in 18-month old children with increasing PCB exposure. However, as pointed out earlier, a slightly reduced growth in breastfed versus formula fed children is often observed, and may not solely be ascribed to contaminant exposure even though PCB exposure is higher in breastfed than formula fed children (Nommsen-Rivers and Dewey, 2009). The North Carolina cohort also investigated pesticide exposure from breastmilk and growth and found no associations. In addition, one study in the Canadian cohort investigated associations between PCBs and HCB exposure from breastmilk and thyroid-related parameters (which again can affect growth), and found no associations.

The time point for assessing effect on growth and sexual maturation varied between the studies as illustrated in Table 7.16, but the outcome measurement, height and weight, were the same in three of the four studies, and thus there is comparability of the outcome between the studies.

The Michigan cohort had mean PCB-153 level 1.5-fold that of the North Carolina cohort, and the mean PCB-153 level was in the same range in the Canadian cohort. The Faroe 2 cohort had higher PCB-exposure, and the mean PCB-153 was more than 5-fold that of the North Carolina cohort.

In animal experiments, the biological plausibility for an association between a number of environmental contaminants and increased risk of overweight and obesity have been suggested, especially within the group of chemicals with endocrine activity. Such compounds (e.g. PCBs, DDT, phthalates, BPA etc.), are environmental contaminants that can mimic or block hormonal actions by activation or repression of e.g. estrogenic, androgenic and thyroid hormone receptors or changing homeostasis by altering metabolism of hormones. Several of these compounds may modulate lipid metabolism and adipogenesis, contributing to obesity initiation and/or exacerbation. It has been hypothesized that obesogenic chemicals provided by breastmilk may contribute to increased risk of overweight and obesity later in life (Janesick and Blumberg, 2011).

VKM concludes that the evidence is *limited and no conclusion* can be given for an association between POPs in breastmilk and weight or height in children.

10.2.3.4 Thyroid parameters

Only one of the included studies investigated thyroid parameters as outcome, and no association was found between PCBs or HCB in breastmilk and thyroid hormones.

VKM concludes that the evidence is *limited and no conclusion* can be given for an association between POPs in breastmilk and parameters related to thyroid hormones.

10.2.4 Summary grading of evidence, negative health effects

Table 10.2 summarises the results from the literature review of the contaminants in breastmilk, and includes an evaluation of the strength of evidence from VKM. Findings of a positive association between breastmilk and health outcome in the cohorts where negative health effets were investigated are not included in Table 10.2, but are described in section 7.2.4.

Table 10.2: Summary of the strength of evidence for an association between children's health outcomes and suggestive contaminant exposure through breastmilk.

Outcome	Exposure	Number of participants* (cohorts)	Association/effect	Number of papers rated	VKM Strength of evidence
Neurodevelopment	PCBs, dioxins, DDE, HCB	4725 (6, Michigan, North Carolina, Canada, Germany, The Netherlands, Spain (mainland and Menorca))	Adverse effect **(3, thereof 2 with transient effects)** NS **(11)**	6 rated B+[1] 8 rated B[2]	Limited, suggestive
Neurodevelopment	Hg	1022 (1, Faroe 1)	Adverse effect (0) NS **(2)**	2 rated B[3]	Limited, no conclusion

Outcome	Exposure	Number of participants* (cohorts)	Association/effect	Number of papers rated	VKM Strength of evidence
Immunological parametres	PCBs, dioxins, DDE, DDT	2690 (4, Faroe 3, Slovakia, The Netherlands, Spain, Menorca)	Adverse effect **(4)** NS **(1)**	1 rated B+[4] 4 rated B[5]	Limited, no conclusion
Growth and puberty signs	PCBs, DDE	1353 (3, Faroe 2, Michigan, North Carolina)	Adverse effect **(1)** NS **(2)**	3 rated B[6]	Limited, no conclusion
Thyroid parametres	PCBs, HCB	204 (1, Canada)	Adverse effect (0) NS **(1)**	1 rated B[7]	Limited, no conclusion

*Participants at inclusion. For participants at follow-up, see Summary Tables.
[1]Gascon et al., 2013; Vreugdenhil et al., 2002 (1); Vreugdenhil et al., 2002 (2); Patandin et al., 1999; Lanting et al., 1998; Koopman-Essebom et al., 1996.
[2]Forns et al., 2012; Verner et al., 2010; Winneke, *et al*., 2005; Walkowiak et al., 2001; Huisman et al., 1995; Gladen&Rogan, 1991; Rogan&Gladen, 1991; Jacobsen et al., 1990.
[3]Jensen et al., 2005; Grandjean et al., 1995.
[4]Weisglas-Kuperus et al., 2000.
[5]Jusko et al., 2012, Grandjean et al., 2010, Heilmann et al., 2010, Sunyer et al., 2006.
[6]Grandjean et al., 2003; Gladen et al., 2000; [11]Jacobsen et al., 1990.
[7]Dallaire et al., 2009.
NS=no significant association.

10.3 Additional considerations

The literature grading (sections in 10.2) will form the basis of the benefit and risk assessment (in section 10.4). Several additional considerations have to be brought into the discussion to comply with the mandate, i.e. "…to evaluate the benefits and potential risks related to breastmilk *based on Norwegian data* on prevalence of breastfeeding and concentrations of environmental contaminants in breastmilk". These additional considerations will be presented here.

10.3.1 Timing of exposure – critical windows of susceptibility and prenatal versus postnatal exposure

A number of the papers evaluated for risk of negative health effects found assiciations with prenatal exposure to contaminants which were not observed postnatally (Tables 7.5-7.13). It is now widely accepted that the timing of chemical exposures to the developing organism may be of critical importance for the health outcome (Brown et al., 2008; Selevan et al., 2000). An impaired outcome may become manifest during the particular life stage of exposure or at later life stages. The most critical period of development is recognised to be during organogenesis and organ development. The rapid growth of the foetus implicates high activity in cellular processes which needs to be tightly regulated. At this stage there is also immature capacity to handle and detoxify xenobiotics and limited capacity of homeostatic regulation. Hence, the unborn foetus is generally recognised as the most vulnerable being in the population regarding adverse effects from exposure to environmental contaminants or other xenobiotics such as drugs.

The development of organs and physiological functions, including biotransformation and elimination of xenobitics, in many cases extends into the neonatal and postnatal period and early childhood. Consequently, these periods are also regarded as life stages where susceptibility to chemicals may be increased. Especially the nervous- and immune system

undergo substantial development after birth, and have been assumed to be sensitive to chemical exposures also in the postnatal period. It is currently not known whether the nervous system is as sensitive to chemicals postnatally as it is prenatally, or whether there are particular sensitive time periods in infancy. In the absence of such knowledge, a conservative approach would be to assume that the infant is as sensitive as the foetus. It would also be a conservative approach to assume that children, as long as they are growing and developing, are more sensitive than adults. In toxicological risk assessments the higher sensitivity at certain life stages, such as the fetal period, infancy and childhood, is taken into consideration by application of uncertainty factors[44] to account for differences in toxicokinetics and toxicodynamics between population groups (Brown et al., 2008).

It has been questioned whether the substantial exposure to some lipid-soluble contaminants from breastmilk, which may result in a temporary several fold increase in body burden of the contaminant in infants compared with that at birth (see section 5.2.5), has been adequately taken into consideration in risk assessments of such substances. The included studies in this benefit and risk assessment have separate measures of pre- and postnatal exposure and associations with the health outcomes. Effects from prenatal exposure can be assessed by e.g. investigating association between infant and/or maternal exposure levels at birth and health outcomes later in life. However, if postnatal exposure is also of importance, it may influence the associations with prenatal exposure. The postnatal exposure via breastmilk is determined by the maternal level/breastmilk concentration and duration and degree of breastfeeding, but associations between postnatal exposure and health outcome can be influenced by prenatal exposure. Although such influence can be adjusted for in statistical analyses, residual influence cannot be ruled out. However, focusing on studies separating pre-and postnatal exposure measures was in view of VKM the best approach available for this benefit and risk assessment.

10.3.2 Exposure in Norway

Breastmilk consumption in Norwegian infants – comparison with exposure levels in papers investigating positive health effects from breastmilk

Chapter 2 describes in detail the data available on breastfeeding prevalence, degree and duration in Norway. The Figures 2.3-2.5,, which compare rates of breastfeeding between European countries, illustrate what has been stated many times in this report: that breastfeeding duration in Norway is exceptially long. The breastfeeding prevalence is confirmed by nationally representative surveys showing that 80% of mothers breastfeed at 6 months and 46% still breastfeed at when the child is 12 months old.

The two most recent systematic reviews for positive health effects of breastmilk include studies with different durations and exclusivity of breastfeeding. The length of breastfeeding in the studies included in these reviews varying from zero months to 1 year. Few studies treated breastfeeding duration as a continuous or ordinal variable with several categories, thus allowing dose-response analyses. Breastfeeding degree (exclusive, predominant or partial) has rarely been assessed. Thus a direct comparison of breastmilk consumption in Norway with the degree and duration of breastfeeding in the systematic reviews is not possible.

[44]Uncertainty factors (also called assessment factors) have been used to account for uncertainty in the extrapolation of the data derived from animals to humans, and to account for variations among humans. Usually, a default factor of 10 is used for extrapolation from animals to humans and a factor of 10 is used for variation among humans (100 altogether).

Exposure of contaminants from breastmilk in Norwegian infants – comparison with exposure levels in papers investigating negative health effects from contaminants in breastmilk

Possible negative effects from contaminants in breastmilk observed in the included cohorts are assumed to be related to exposure levels, which are determined by contaminant concentrations in the breastmilk and breastfeeding duration. In this chapter exposure levels in the included cohort are therefore compared with current exposure levels in Norway.

The present-day exposures to PCB-153, dioxins and dl-PCBs, DDE and HCB in breastfed infants in Norway are described in chapter 8.There are two main differences between the included cohorts and present-day Norwegian conditions:

a) Present-day mean concentrations of contaminants in Norway (breastmilk PCB-153 39 ng/L and DDE 50 ng/L) are substantially lower than in all of the included studies except one (The INMA mainland cohort)
b) Norwegian women continue to partial breastfeeding longer (mean duration 10.3 months) than women in the included cohorts, except in the Faroe Island and possibly in the Canadian cohorts

Contaminants concentration in breastmilk: All but one of the included cohorts had mothers (and consequently breastmilk) with substantially higher mean contaminant concentrations than the present-day levels in Norway. Based on the comparison of the maternal PCB-153 levels (see Table 7.3), the maternal body concentration of PCBs in the Faroe Islands cohorts was 9 to 12-fold higher than the present-day Norwegian. The Slovakian, German, Dutch, Michigan, Canadian (Inuit) and the INMA (Menorca) cohorts had about 3 to 4 times higher mean maternal levels than the present levels in Norway, whereas the mean contaminant levels in the North Carolina cohort were more than twice the levels of today's exposure in Norway. The INMA mainland sub-cohorts had, however, maternal PCB levels comparable to the present-day Norwegian levels.

The DDE levels were more than 10 times higher in the Faroese cohorts and in the INMA sub-cohort from Menorca than the present mean level in Norway (Table 7.3). The INMA mainland sub-cohorts had maternal DDE level more than 2.5-fold the Norwegian level.

The maternal mercury level in the Faroe Island cohorts was substantially higher than in Norway (Table 7.3).

Duration of breastfeeding: In the Faroe Islands cohorts the rate of breastfeeding was high and duration was longer than in most of the other cohorts. As an example, in the Faroe 2 cohort, the mean exclusive breastfeeding duration was 3.5 months and the total mean duration of partial breastfeeding was 7.8 months. As many as 18.2% were exclusively breastfed for 6 months. In Norway, 9% are exclusively breastfed for 6 months, mean duration of any breastfeeding is 10.3 months[45], and 46% are still breastfed at 12 months. Also in the Canadian cohort the mean length of exclusive breastfeeding was long (156 days, corresponding to 5.1 months). However, no information on duration of partial breastfeeding was included in this cohort, and since only exclusive breastfeeding was fed into the pharmacokinetic model used in the study, this may represent an underestimation of the real exposure.

In the Michigan cohort mean duration of any breastfeeding was 30 weeks, and in the INMA cohorts the mean duration of exclusive breastfeeding was 3.8 months and partly breastfeeding 6.1 months.

[45] Based on calculations by VKM on data from Småbarnskost 2007.

The duration of breastfeeding in the North Carolina cohort was reported in four intervals: short (0-9 weeks), medium (10-19 weeks), long (20 weeks) and very long (weaning after 49 weeks) in the Dutch cohort in three intervals zero, short (6-16 weeks) or long (>16 weeks), and also in the German cohort in three intervals, short (≤2 weeks), medium (>2 weeks to 4 months) and long (>4 months). Based on the reported intervals it appears that mean or median breastfeeding duration in these cohorts was shorter than in Norway. The Slovakian cohort did not report (although they recorded), length of breastfeeding duration, and could thus not be compared with the breastfeeding duration in Norway.

Body burden: The amount of POPs in the body is related to possible negative effects of these compounds. In a breastfed infant this amount (body burden or body concentration) increases throughout the nursing period, however, it increases more during the first 4 to 6 months of breastfeeding than in the period 6 to 12 months (section 5.2.5). Since breastfeeding duration in the Faroe Islands cohorts was in the same range as the current Norwegian practice, while the breastmilk PCB levels were higher, the mean PCB concentration in the infant body was probably substantially higher in the infants in all age groups in the Faroese cohorts than in Norway at present. A similar situation was probably the case in the Canadian cohort since they had a long duration of exclusive breastfeeding, and assuming that partial breastfeeding continued equally long in the Canadian cohort as in the Faroese cohorts and in Norway.

The generally longer duration of breastfeeding in Norway compared with the other included cohorts from Michigan, Germany, Spain, the Netherlands and North Carolina, combined with the higher PCB levels in the mothers in these cohorts (giving higher levels in infants at birth and higher levels in breastmilk), imply that the body concentrations were higher in infants in these cohorts than in present day Norwegian children the first few months after birth. The mean body concentration in Norwegian infants will generally not reach similar peak body concentrations as infants in the included cohorts, even with a substantially longer breastfeeding period. Provided that the body concentration is approximately doubled by 4 months of breastfeeding, and the mean POP levels in the cohorts are more than 2-fold the Norwegian (generally 3 to 4-fold), it takes approximately 5 months of breastfeeding before Norwegian infants reach similar accumulated body concentration as the infants in the included cohorts had at birth. Even after 2 years of breastfeeding, the mean accumulated body concentration in Norwegian infants will not reach a concentration similar to the peak concentration in the included cohorts. This is illustrated in Figure 10.2 The cumulative amount per kg body weight on top of the body concentration at birth, which is displayed as a function of breastfeeding duration, is an approximation of the body concentration during the breastfeeding period. Infants in the INMA mainland sub-cohorts, might on the other hand experience lower PCB body concentrations than Norwegian infants at the end of the nursing period, because PCB levels in the INMA-mainland cohort mothers were similar to the Norwegian, but the breastfeeding duration was shorter.

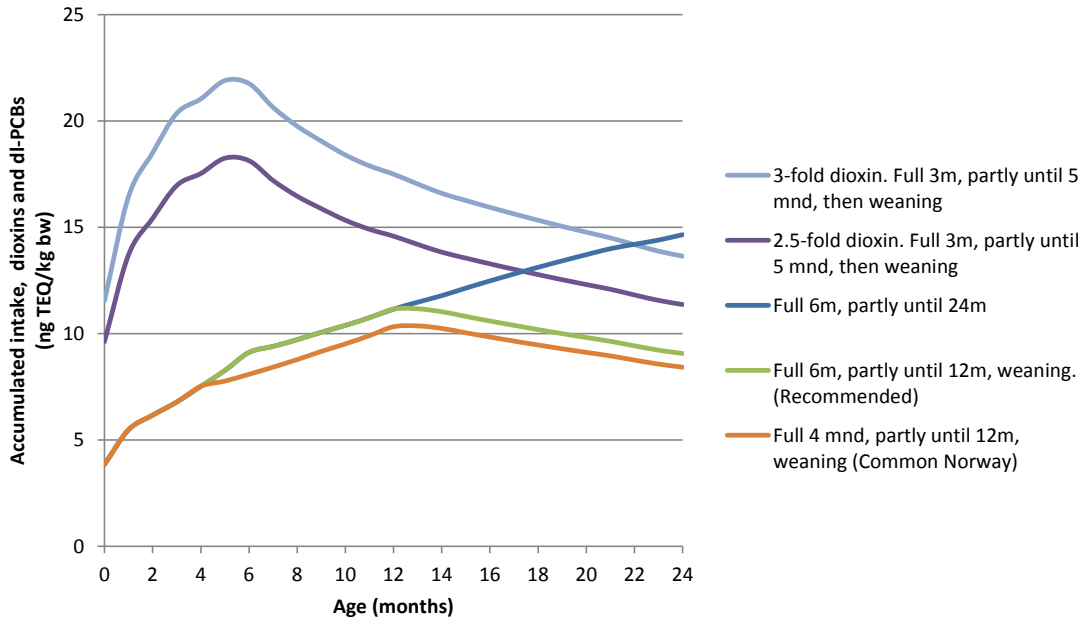

Figure 10.2: Scenarios of estimated body concentration (cumulative amount per kg body weigth received by the infant on top of the body concentration at birth) of dioxins and dl-PCBs in breastfed infants with varying maternal level and with varying duration of breastfeeding. For calculations the mean concentration (11 pg TEQ/g fat) of dioxins and dl-PCBs in breastmilk (3.5% fat) in Norway and 2.5 and 3-fold this level was used. Concentration in the infant's body at birth was considered similar as in the mother, which was calculated from the concentration in breastmilk, 35% fat in the body and bw 70 kg. This can be considered as an overestimate in the infant, because the newborn infant has substantially lower fat percentage. Infant bw was according to WHO (Table 8.1 in this document). Breastmilk consumption was according to Table 2.2 in this document for exclusive breastfeeding and 500 ml/day for partially breastfeeding. This volume for partially breastfeeding is considered an overestimate (see section 8.1). Weaning was estimated to occur over two months, 250 ml/day weaning month one and 125 ml/day weaning month two.

Based on the discussion above, it can be concluded that the total PCB exposure in the included cohorts was generally substantially higher than in Norway, even with a longer breastfeeding duration in Norway. In this rough estimate of body concentration (cumulative amount per kg body weight received by the infant on top of body concentration at birth) in order to compare exposure in infants in Norway with infants in the included cohorts, it has not been taken into account that the concentration of contaminants in breastmilk decreases during the nursing period (section 5.2.5) and thus represents an overestimation of the situation among breastfed infants. Another factor that has not been taken into consideration which affects the serum concentration of contaminants in infants is the change in fat percentage in infants from birth to childhood.

The highest body concentration in Norwegian infants is reached later in infancy than in the cohorts from Michigan, Germany, Spain, the Netherlands and North Carolina because the breastfeeding period is longer in Norway. As mentioned in section 10.3.1, it is not known if infants are equally sensitive to contaminant exposure in the period they reach highest body concentration (around weaning) as in the prenatal or early postnatal period. Assuming equal sensitivity is a conservative approach, meaning that it overestimates rather than underestimates the risk.

Norwegian contaminant exposure estimates and tolerable intake levels

The estimated contaminant exposures from breastmilk in Norwegian infants (chapter 8) indicate that in all infant age groups the exposure to dioxins and dl-PCBs is substantially higher (10 to 30-fold at mean exposure) than the tolerable intake at 14 pg/TEQ/kg bw/week (sections 5.2.1 and 8.5).

Exceeding the tolerable intake in infants does not necessarily imply that the concentration in the infant body reaches a level of concern, and therefore the tolerable intakes of dioxins and dl-PCBs are not directly applicable for infants (explained in section 5.2.5). The tolerable intake of dioxins and dl-PCBs has been set to ensure that the contaminant concentration in the mother (the maternal whole body concentration, expressed per kg body weight) is below the highest concentration that is considered safe to the foetus. This is the body concentration associated with intake similar to the tolerable intake. Due to the rapid growth of the the infant a dilution of contaminants takes place with a slower increase in body concentration.

The dietary intake of dioxins and dl-PCBs, as recently estimated in the MoBa cohort is below the TWI[46] for more than 97% of the participating Norwegian pregnant women (Caspersen et al., 2013). The median, 95-percentile, 97-percentile and 99-percentile weekly exposure to dioxins and dl-PCBs in MoBa was 3.9, 9.9 and 18.5 pg TEQ/kg body weight, respectively (Caspersen et al., 2013). This indicates a low risk associated with prenatal exposure among infants in Norway.

There is no established tolerable intake level of PCB-153 or sum6 PCBs. A PTDI at 20 ng/kg bw/day for all 209 PCB congeners was proposed at the "2nd PCB workshop" in Brno (Czech Republic, May 2002) and has been used in France, the Netherlands, and Norway as a guideline value (AFSSA, 2007; Baars et al., 2001; VKM, 2008). This value was based on maternal body burden and postnatal neurodevelopmental and immune effects in children, and corresponds to 10 ng PCB-6/kg body weight per day. The BMDL-calculations are based on the Michigan cohort (Jacobson and Jacobson, 2002) and the Faroese cohort 1 (Grandjean et al., 2003), and thus exposure from breastmilk is to some extent already taken into account in the PTDI for total PCBs. Based on dietary intake from the Norwegian MoBa cohort (Caspersen et al., 2013), the median and 95-percentile dietary intake of PCB6 was 2.58 and 8.31 ng/kg bw/day, respectively, and thus below 10 ng PCB-6/kg body weight per day. However, the 97.5-percentile intake was 15.48 and the 99-percentile intake was 26.22 ng/kg bw/day, indicating that less than 5% of the Norwegian infants may have PCB exposure higher than the PCB-6 intake corresponding to the PTDI suggested for total PCB.

For sum DDT, the provisional tolerable daily intake (PTDI) is 0.01 mg/kg bw (10000 ng/kg bw/day). Depending on infant age, there is a margin of 33 and 130 between mean daily intake of DDE in breastfed infants and the PTDI. The PTDI is 15-fold higher than the 95-percentile exposure for 1-month old infants. Since *p.p'*-DDE is the major constituent of sum DDT, calculating exposure to sum DDT would not have affected this margin substantially.

The estimated HCB exposure from breastmilk was in all infant age groups lower than the health based guidance value of 170 ng/kg bw/day suggested by WHO.

10.3.3 Breastmilk versus infant formula

Infant formula contains all the nutrients a child needs to grow and develop (described in chapter 4). However, infant formula contains none of the enzymes (e.g. lipase, lysozyme) that

[46]The TWI for dioxins and dl-PCBs is 14 pg TEQ/kg body weight/week.

makes breastmilk more digestible, no immune-boosting substances like immunoglobulins, hormones and growth factors, formerly no DHA (this has been added recently) or cholesterol important for brain development. Furthermore, infant formula has lower bioavailability of minerals, to mention some of the main differences. With all the recent knowledge accumulating as to the impact of the gut microbiome for health, it has become apparent that the carbohydrate profile of breastmilk has advantages over infant formula also in this respect (Sela and Mills, 2010).

Infant formula also contains contaminants, although with a somewhat different profile and with lower levels of the POPs than in breastmilk. As indicated in sections 5.3 and 5.4, infant formula may contain both process-generated substances like acrylamide, PAHs, 3-MCPD and furans, and substances migrating from food contact materials like BPA and phthalates. The latter substances are also present in breastmilk, but possibly at lower concentrations. Infant formula has been found to have somewhat higher concentration of lead than breastmilk. There are indications that the daily intakes of manganese and molybdenum are much higher from infant formulas than from breastmilk, but eventual implications are unknown. Thus we provide the infant with unwanted substances irrespective of feeding path.

Possible outbreaks from microbiological hazards in the infant formula itself or due to contaminated water are an issue in developing countries, but no such outbreaks have been registered in Norway.

The main difference between the unwanted substances provided by breastmilk and those in infant formula is that the contaminants with higher levels in breastmilk are more persistent, meaning they have a longer half-life and accumulate in the fat compartments of the body, while most of the unwanted substances dominating in infant formula have a shorter half-life and do not accumulate to the same degree. The net impact on child health of the unwanted substances in infant formula is not known.

10.3.4 Other pollutants not found in the literature search

Although most studies have focused on one pollutant (or pollutant group) and one outcome at a time, in reality, the contaminant profile of breastmilk mirrors the concentration in the mother's body. VKM takes the standpoint that one measured contaminant in breastmilk may be used as a marker of most of those not measured, since they are characterised by being mobilised through the physiological production of breastmilk. Thus the combined effect of multiple contaminants in breastmilk is in reality being studied, although the number of analysed components may be limited.

This argumentation also applies to the many studies on positive health effects of breastmilk included in chapter 6. Hidden behind all the positive findings there also lies a mixture of contaminants in unknown doses.

Some recent studies not addressing breastmilk directly, point to fluorinated compounds in particular, as contaminants where postnatal exposure from breastmilk may be of significance (see section 7.2.5).

10.4 Benefit and risk assessment of breastmilk, discussion by health outcome

The aim of the following sections is to bring all information presented in previous chapters and sections into a balanced discussion of benefits and risks associated with breastfeeding and

health outcomes, with special emphasis on the impact of duration of breastfeeding. It is important to bring the methodological challenges into the benefit and risk assessment (see chapter 9), because even well-designed studies are subject to methodological limitations that are unavoidable in observational research.

The sections are organised according to health outcomes, starting with those outcomes that have been studied both from a benefit and risk point of view; i.e. neurodevelopment, immune response-associated diseases and growth/overweight/obesity.

VKM is aware of the many methodological challenges connected to interpretation of results from epidemiological observational studies (chapter 9). All studies included in the present assessment were graded B, implying that they had some limitations. However, if outcome measurements are comparable and all point in the same direction, we comply with the established guidelines that a conclusion can be drawn.

10.4.1 Neurodevelopment – benefit and risk assessment

A child's cognitive development is considered a sensitive endpoint for development of the nervous system. Studies of cognitive development in children focus on information processing, conceptual resources, perceptual skill, language learning and other aspects of brain development. Standard IQ tests or school grades are two of many ways to score development (types of tests used are described in Appendix 7). Early life influence on the nervous system can affect cognitive and motoric skills later in life.

Motor development and cognitive development may be fundamentally interrelated and display equally protracted developmental timetables. When cognitive development is perturbed, as in a neurodevelopmental disorder, motor development is often adversely affected as well (Diamond, 2000).

Neurodevelopmental deviations like reduced IQ, distractibility, anti-social behaviour and inattention have been subject to a number of the included studies both on the benefit and risk side. The neurodevelopmental outcomes related to breastmilk consumption in these studies are probably within the normal variation of what is expected for cognitive development. Instead of prevalence data on disorders connected to severe aberrant cognitive development, such as ADHD, autism spectre disorders, mental retardation, etc., the impact of altered IQ distribution is presented below, since it is one of the neurodevelopmental markers most often measured.

IQ distribution as a marker of cognitive development

Figure 10.3 illustrates that a five point gain or loss in IQ might not affect an individual's life-quality, but may be of importance at the population level, by increasing the proportion of people having mild disabilities, and decreasing the proportion of specially gifted people, or vice versa.

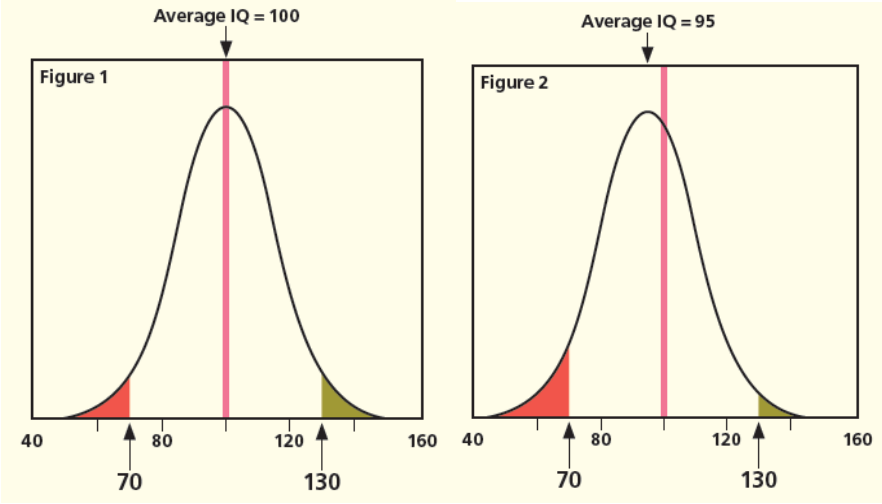

Figure 10.3: Impact of a five point change in IQ in a population. Source: Schmidt, 2013.

Weighing the evidence, benefit and risk assessment of breastmilk versus risk of impaired cognitive development

Grading the scientific evidence on the benefit side (section 10.2.1 and Table 10.2.1), VKM concluded that the evidence is convincing for a positive effect of breastmilk on neurodevelopment. The optimal length of exclusive and partial breastfeeding associated with the positive effect remains to be settled.

Grading of the scientific evidence on the risk side (section 10.2.3), VKM concluded that the evidence is limited suggestive for a negative effect from POPs in breastmilk on neurodevelopment. The length of exclusive and partial breastfeeding associated with the transient negative effect remains to be settled.

For mercury, the evidence is limited and no conclusion can be given.

The grading of the scientific evidence for negative health effects from POPs in breastmilk on neurodevelopment was based on seven cohorts, with findings in three cohorts (Dutch, German and Canadian cohorts). The findings were transient in the Dutch and German cohorts.

The rough estimates of body concentration of specific POPs in infants in Norway and in infants from cohorts with higher mean POP levels indicate that Norwegian children generally will not reach similar body concentrations as infants in the Canadian, German and Dutch cohorts (section 10.3.2). Furthermore, the infants in the Dutch and German cohorts had peak body concentrations earlier in life than Norwegian infants. Whereas it is known that the unborn child is especially sensitive to substances that can affect neurodevelopment, it is not known if there are equally sensitive postnatal periods. Assuming equal sensitivity is a conservative approach which most likely overestimates the risk when the highest body concentration (i.e. concentration in blood) is reached as late in infancy as 12 months.

A specific confounder challenge for interpretation of studies on neurodevelopment is the lack of HOME score measurements. Where it had been included, several of the papers came to the conclusion that a stimulating home environment, which was also associated with higher rate of breastfeeding, outbalanced an eventual negative effect of contaminants in breastmilk. HOME score measurements were used in 7 of the 14 papers investigating associations between POP's in breastmilk and neurodevelopment, including the two papers which reported transient findings (the Dutch and German cohorts).

The outcomes investigated within the category negative health effects from POPs in breastmilk and neurodevelopment used a number of different tests (Appendix 7). Furthermore, the timepoint of investigation varied from some weeks to teenage years. The same tests have, however, been administered at similar ages in several of the studies.

Several of the studies investigating associations between contaminants in breastmilk and neurodevelopment (in particular in the Dutch and Faroese cohorts) found an overall beneficial effect of breastmilk *per se*, implying that the beneficial effect was negatively confounded by dioxins and dl-PCBs in breastmilk. This indicates that possible negative effects from the contaminants may be camuflaged by beneficial effects of breastmilk itself.

Taking the present-day levels of contaminants in breastmilk and the long duration of breastfeeding (12 months) in Norway into account, the following conclusion is drawn:

VKM concludes that the benefits of breastmilk clearly outweigh the possible risk of impaired neurodevelopment from contaminants in breastmilk. It is still not fully known whether contaminants in breastmilk may confound the positive effect of breastmilk *per se*, i.e. whether breastmilk would be even more beneficial for neurodevelopment if the levels of contaminants were lower.

10.4.2 Immune response-associated diseases – benefit and risk assessment

Immune response-associated diseases include among others infections, food allergy, atopic dermatitis/eczema, allergic rhinitis and allergic asthma. Infancy is a time of increased disease susceptibility and severity, and ear infections, gastroenteritis and respiratory tract infections are quite common.

The typical progression of allergic diseases is often referred to as the allergic or atopic march, and is initiated by food allergy, with or without associated atopic dermatitis/eczema in infancy or early childhood, followed by allergic asthma and/or allergic rhinitis later in childhood as sensitization with IgE antibodies develops. Reliable and comparable prevalence data on infectious diseases in infancy and other immune response-associated diseases are scarce, so the data below should be considered as suggestive.

In the work with this benefit and risk assessment it has been challenging to find comparable endpoints within the category of "immune response-associated diseases". VKM is aware that allocating studies into such a broad category has limitations when benefits and risks are to be compared.

As there were other health outcomes categorized under the "immune response-associated disease umbrella" in this report, such as type 1 diabetes, coeliac disease and ulcerative colitis and Crohn's disease, with total lack of comparability with outcomes investigated for in the contaminant papers, these will be assessed in section 10.4.4.

Prevalence of infections, food allergy, atopic dermatitis/eczema, allergic asthma and allergic rhinitis in infancy and childhood

A high proportion of infants will experience gastrointestinal or lower respiratory tract infections within their first year of life. Prevalence is difficult to estimate as only a proportion of the patients will visit a doctor or other health personnel. There are also wide variations in prevalence rates for acute otitis media from different studies because of varying diagnostic criteria and selection of patients. In a study from 2010, episodes of acute otitis media confirmed by a doctor are reported to be 28.6% at 1 year of age.

The prevalences of food allergy, atopic dermatitis/eczema, allergic asthma and allergic rhinitis in infancy and childhood are challenging to determine – reported rates vary from country to country and study to study. As with infections, prevalence is difficult to estimate as only a proportion of the patients will visit a doctor or other health personnel.

Weighing the evidence, benefit and risk assessment of breastmilk versus risk of infections, food allergy, atopic dermatitis/eczema, allergic asthma and allergic rhinitis in infancy

Grading the evidence on the benefit side, VKM concluded that the evidence is convincing for a protective effect of breastmilk on infections, at least as long as the child is breastfed (section 10.2.1 and Table 10.1).

VKM concluded that the evidence is limited and inconclusive for a protective effect of breastmilk on asthma and wheezing, allergies and atopic dermatitis.

Grading the evidence on the risk side, VKM concluded that the evidence is limited and inconclusive for a negative effect from POPs in breastmilk on thymus weight, vaccine antibody titer, middle ear infections, asthma and wheezing.

The grading of the scientific evidence for negative health effects from POPs in breastmilk on immune response-associated diseases was based on four cohorts, with findings of increased risk in three cohorts (Faroe Island 3, Dutch and Slovakian cohorts).

The Faroe 3 cohort had approximately 9-fold higher concentrations of PCBs than the present-day Norwegian levels (Table 7.3). The Slovakian and Dutch cohorts had about 3 to 4 times higher mean maternal levels than the present levels in Norway. In the INMA (Menorca) cohort DDE was addressed, and the mean levels were 100-fold higher than in Norway.

The rough estimates of body concentration of specific POPs in infants in Norway and in infants from cohorts with higher mean POP levels indicate that Norwegian children generally will not reach similar body concentrations as infants in the Faroese, Slovakian[47] and Dutch cohorts (section 10.3.2). Furthermore, the infants in the Dutch cohort probably had peak body concentration earlier in life than Norwegian infants. No comparison with the Slovakian cohort could be performed, since breastfeeding duration was not given.

In the risk assessment of other immune response-associated diseases than infections, a major challenge was the heterogeneity within each main group of outcomes, e.g. immunologically or allergy related diseases, where all five papers included in reality measured widely different outcomes, and different parameters of similar outcomes.

Four of the five studies investigating association between POPs in breastmilk and immunological parameters also found an association between *prenatal* contaminant exposure and immune response-associated diseases. The nervous- and immune systems undergo substantial development after birth, and have been assumed to be sensitive to chemical exposures also in the postnatal period. In the absence of knowledge, a conservative approach would be to assume that the infant is as sensitive as the foetus.

In two of the four studies reporting negative associations between POPs in breastmilk and immune response-associated diseases, the negative associations were counteracted by breastfeeding. In one paper from the Faroe Island 3 cohort it was shown that an apparent

[47]No direct comparison with the Slovakian cohort could be performed, since breastfeeding duration was not given. However, since the mean level was 3 to 4-fold the Norwegian, it can be assumed that with a common breastfeeding duration as in e.g. Germany, the body concentration in infants was substantially higher than in Norway.

association between breastfeeding duration and increased serum IgE in children was weakened and no longer significant after adjustment for the concomitant PCB exposure. In a paper from the Dutch cohort, breastfeeding duration counteracted the increased odds ratio for middle ear infection. It is not known whether POPs in breastmilk may confound the positive effect of breastmilk *per se*, i.e. whether breastmilk would be even more beneficial for the immunodevelopment if the levels of contaminants were lower.

Taking the present-day levels of contaminants in breastmilk and the long duration of breastfeeding (12 months) in Norway into account, the following conclusion is drawn **:**

VKM concludes that the benefits of breastmilk in terms of defence against infections clearly outweigh the possible risk of reduced resistance to infections from contaminants in breastmilk, at least as long as the child is breastfed.

No conclusion can be drawn on other immune response-associated diseases due to inconclusive results on the benefit side and few and disperse studies on the risk side.

10.4.3 Growth, overweight and obesity – benefit and risk assessment

In adults, raised body mass index (BMI) is a major risk factor for cardiovascular diseases, diabetes, musculoskeletal disorders and some cancers (endometrial, breast, and colon). Childhood obesity is associated with a higher risk of adult obesity, disability in adulthood and shortened life span (WHO fact sheet accessed July 2013).

The influence of early life factors on later body weight and metabolic diseases has generated increasing interest in recent years (Shamir et al., 2013). There is evidence that obesity risk may begin early in life, during pregnancy or in early childhood and that rapid weight gain, especially in the first few months of life, is associated with obesity later in life (Ong et al., 2000).

Prevalence

In Norway, approximately 20% of adult men and 17% of adult women were considered to be obese in 2005.

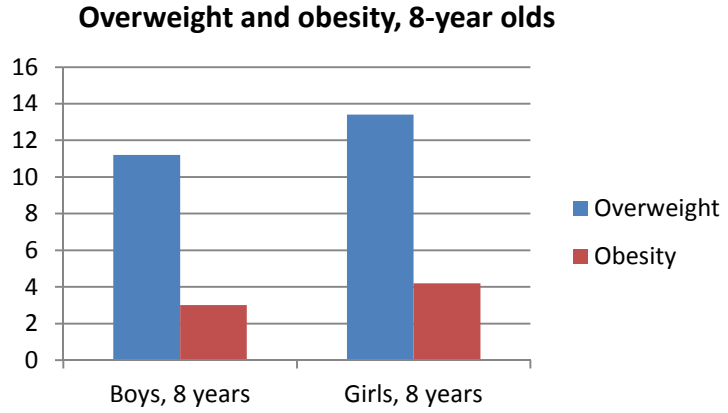

Figure 10.4: Overweight and obesity in Norwegian 8-year olds by gender.

Data from 2012 show that approximately 16% of Norwegian 8-year olds are overweight or obese. Figure 10.4 shows that 3.5% of these were obese and 12.3% were overweight (Source: Fact sheet from the Norwegian Institute of Public Health[48]). Overweight, but not obesity, was more prevalent among girls than boys in this age group.

Weighing the evidence, benefit and risk assessment of breastmilk versus risk of impaired growth, overweight and obesity

Grading the evidence on the benefit side, VKM concludes that the evidence is convincing for a protective effect of breastmilk on risk of overweight and obesity in childhood. The optimal length of exclusive and partial breastfeeding associated with the reduction in risk remains to be settled, although there are indications that exclusive breastfeeding for more than 4 months is associated with slower weight gain during later infancy compared with those exclusively breastfed for less than 4 months.

Grading the evidence on the risk side, VKM concludes that the evidence is limited and inconclusive for an association between POPs in breastmilk and altered weight or height in children.

The grading of the scientific evidence for negative health effects from POPs in breastmilk on growth and weight was based on three cohorts, with negative findings in one, pointing towards reduced (and not increased) weight with increasing PCB exposure (Faroe Island 2). Infants in the Faroese cohorts are expected to have a substantially higher level of contaminants in blood both at birth as well as early and late in the nursing period than Norwegian infants.

Taking the present-day levels of contaminants in breastmilk and the long duration of breastfeeding (12 months) in Norway into account, the following conclusion is drawn**:**

VKM concludes that the reduced risk of overweight and obesity associated with breastfeeding clearly outweighs the possible risk presented by contaminants in breastmilk.

10.4.4 Other health outcomes – benefit and risk assessment

Table 6.1 summarises most of the reported beneficial health effects of breastfeeding from six systematic reviews and meta-analyses. The following health outcomes have only been studied from a benefit point of view: Coeliac disease, type 1 and 2 diabetes, childhood cancer, Sudden Infant Death Syndrome, cardiovascular diseases and its risk markers (high blood pressure and serum cholesterol), Crohn's disease and ulcerative colitis.

As these outcomes only have been studied from a benefit side they do not fulfill the criteria for being included in the benefit risk assessment; common health outcomes was to be the comparison metrics (see section 10.1). However, when approaching the issue of benefits and risks of breastmilk in a broader perspective, looking beyond the strict criteria set up by international bodies like EU, it is important to have all these other health outcomes in mind.

Adding to this comes all the aspects of breastfeeding omitted in this report and stated in section 1.1, e.g. the many positive psychological aspects of breastfeeding both for mother and child, or the positive impact of breastfeeding on maternal physical health.

[48] http://www.fhi.no/eway/default.aspx?pid=239&trg=List_6212&Main_6157=6263:0:25,6306&MainContent_6263=6464:0:25,6308&List_6212=6218:0:25,6320:1:0:0:::0:0.

Type 1 diabetes, type 2 diabetes and high blood pressure

VKM concluded that the evidence is probable for a protective effect of breastmilk on risk of type 1 diabetes, type 2 diabetes and high blood pressure. The optimal length of exclusive and partial breastfeeding associated with the protective effect on reduced risk remains to be settled.

Crohn's disease, ulcerative colitis, coeliac disease, childhood cancer and sudden infant death syndrom

VKM concluded that the evidence is limited suggestive for a protective effect of breastmilk on risk of Crohn's disease, ulcerative colitis, coeliac disease, childhood cancer and sudden infant death syndrom.

11 Conclusions: benefit and risk assessment of breastmilk and infant health in Norway

In the wake of intermittent scientific and public concern about contaminants in breastmilk, a debate has arisen among experts. Although the experts agree that breastfeeding is beneficial, there are discussions about the advisable length of breastfeeding. As a basis for future advice on these issues the Norwegian Scientific Committee set out to perform a benefit and risk assessment of the health impact of breastmilk on infant health in Norway. The Committee concludes as follows.

Breastfeeding in Norway

- Norwegian women characteristically have a long duration of partial breastfeeding: 80% breastfeed till the child is 6 months of age and 46% still breastfeed when the child is 12 months old.

Nutrients and immunological components in breastmilk and infant formula

- *Breastmilk* is physiologically tailored to meet the needs of a full-term newborn. Provided that the nutritional needs of the mother are met during pregnancy and breastfeeding, breastmilk meets all the nutritional requirements of the infant in the first months of life, with the exception of vitamin D. Breastmilk also contains a number of specialised components, including factors with anti-microbial and anti-inflammatory properties as well as constituents stimulating the maturation of the infant's immune system.
- *Infant formula* covers all the established nutritional needs necessary for normal infant growth and development, but lacks the many additional protective and immune-maturing substances present in breastmilk.

Positive health effects of breastmilk

- Both exclusive and partial breastfeeding have health benefits for the infant that extend into childhood and, possibly, adolescence and adulthood.
- The evidence is *convincing* that breastfeeding protects against infections.
- The evidence is *convincing* that breastfeeding has a protective effect against later risk of overweight and obesity.
- The evidence is *convincing* that breastmilk enhances neurodevelopment.
- The evidence is *probable* that breastfeeding has a risk-reducing effect on type 1 and type 2 diabetes and high blood pressure.

The meta-analyses and systematic reviews included in the benefit assessment did not allow for conclusions regarding the optimal length of exclusive and partial breastfeeding which secures the positive health effects.

Contaminants in breastmilk and infant formula

- Persistent contaminants have accumulated in the environment and are biomagnified in the food chain and therefore present in our bodies. The concentrations in breastmilk reflect the amounts accumulated in the mother.
- A prolonged national and international effort has been made to reduce emissions of environmental contaminants, and breastmilk levels of most contaminants discussed in this assessment have been steadily declining for the past thirty years, in some cases by as much as 90%. Concentrations of contaminants in Norwegian breastmilk have been lowered substantially as a result.
- The contaminants examined in this assessment are those which have actually been studied in human breastmilk in relation to infant and child health. These are PCBs, dioxins, DDT/DDE, HCB and mercury. In the statement on human breastmilk below, the term "contaminants" refers to these substances. Current levels of environmental contaminants in Norwegian breastmilk are considerably lower than in most of the cohorts used to characterise the potential negative impact on infant health of these contaminants.
- *Infant formula* also contains contaminants, although these have a somewhat different profile. Infant formula contains lower concentrations of the classical environmental contaminants than breastmilk, but higher concentrations of other unwanted substances. Infants are exposed to contaminants irrespective of feeding path, whether breastmilk or formula.
- Microbial contamination of infant formula may be of concern as this may cause diarrhoea and, in severe cases, bacteraemia and meningitis. *Cronobacter* spp. (formerly *Enterobacter sakazakii*), is a rare cause of invasive infection with a high death rate among newborn infants. Possible outbreaks due to microbiological hazards in infant formula or contaminated water are issues in developing countries, but no such outbreaks have been registered in Norway.

Negative health effects from contaminants in breastmilk

- The studies on potential negative health effects of contaminants in breastmilk in this report are based on 24 papers from 10 cohorts conducted in seven different countries (USA, Canada, Faroe Isles, Spain, Germany, the Netherlands and Slovakia). No such data on impact on infant and child health of exposure to contaminants via breastmilk was available from Norway.
- In four out of seven cohorts, no associations were found between neurodevelopment and postnatal exposure to PCB, dioxins, DDT/DDE and HCB in breastmilk. Associations with contaminants were found in three cohorts, although they were not found to persist over time. VKM concludes that there is *limited suggestive* evidence for a negative effect from contaminants in breastmilk (at the level in these cohorts) on neurodevelopment.
- There were too few papers on postnatal exposure to contaminants and growth or immune response-associated diseases to allow conclusions to be drawn.

Exposure to contaminants in breastmilk in Norway compared with exposure in the cohorts included in the risk part of the present report

- The mere presence of environmental contaminants in breastmilk does not necessarily imply that breastfed infant run a significant risk of adverse health effects; the degree of accumulation in the infant's body must be taken into consideration.

The estimated cumulative amounts of contaminants received by infants (per kg of body weight) are an approximation of the accumulated level. In the included cohorts these were generally substantially higher than the corresponding amounts currently obtained in Norway, whether seen prenatally, early postnatally or at 1 year of age. Even with a breastfeeding duration of 2 years, the cumulative amounts of contaminants in present-day Norwegian infants do not reach similar peak levels as infants in the included cohorts. Further, the highest cumulative amount per kg body weight in present-day Norwegian infants is reached later in infancy than in infants in the cohorts used for characterisation of possible negative health impact.

Benefit and risk assessment

Taking the present-day levels of contaminants in breastmilk and the long duration of breastfeeding (12 months) in Norway into account, VKM concludes that:

- The benefits of breastmilk clearly outweigh the possible risk of impaired neurodevelopment from contaminants in breastmilk.
- The benefits of breastmilk in terms of defence against infections clearly outweigh the possible risk of reduced resistance to infections from contaminants in breastmilk, at least as long as the child is breastfed.
- The reduced risk of overweight and obesity associated with breastfeeding clearly outweighs the possible risk presented by contaminants in breastmilk.
- As regards the beneficial effects of breastmilk on risk of type 1 and type 2 diabetes and high blood pressure, the evidence suggests a *probable* beneficial effect later in life. There are no studies investigating these health outcomes in relation to contaminants in breastmilk.
- No conclusion can be drawn on other immune response-associated diseases due to inconclusive results on the benefit side and few and disperse studies on the risk side.

Following a comprehensive assessment of scientific literature on the positive health effects of breastmilk and concentrations in breastmilk of compounds representing possible health hazards, and given current knowledge about concentrations of contaminants in Norwegian breastmilk and breastfeeding duration in Norway, VKM concludes that the benefits associated with breastmilk clearly outweigh the risk presented by current levels of contaminants in breastmilk. This conclusion is not affected by whether a child is exclusively or partially breastfed up to the age of 6 months and partially breastfed up to 12 months of age.

12 Data gaps

The basis for being able to use observational epidemiological studies to reach convincing conclusions is that there is evidence from at least two independent and well-designed studies. In general, a major challenge in the present benefit risk assessment was a lack of comparable exposures and health outcomes.

- There is a need for studies which describe breastfeeding extent (exclusive or partial) and duration in detail and in similar manners.
- There is a need for a research program which includes several cohorts and measure health outcomes in the same manner.
- There is a need for long-term studies, i.e. studies investigating benefits of breastmilk and possible risks of contaminants in breastmilk in children breastfed for up till 12 months or more.
- Especially if negative effects are found, there is a need for follow-up studies of the children to investigate if the effects are transient.
- Hitherto, there are scarce data on breastmilk concentrations of POPs other than dioxins, PCBs, DDT/DDE and HCB. There is a need to know more about the concentrations of other persistent pollutants, especially those encompassed by the Stockholm convention, like PFOS/PFOA and brominated compounds.
- There is a need for more knowledge about unwanted substances with a short half-life, like acrylamide and phenols, both in breastmilk and in infant formula.
- The methodology of PBPK modelling should be refined further. There are knowledge gaps as to sensitive windows of exposure after birth where PBPK modelling would bring the field further.
- There is a lack of good data on infant and child body composition, not least fat mass. Such data are needed for advanced PBPK modelling.
- With better understanding of the toxicokinetics of contaminants in infants and children, TWI or similar tolerable intakes could be refined.
- Some contaminants may have effects which become apparent decades after exposure. Long-term studies are therefore warranted.
- Methodology for separating prenatal versus postnatal exposure needs to be further refined.

Appendix 1: Tables of excluded studies

Articles were excluded if they did not allow for a clear separation between prenatal and postnatal exposure or if they included premature which were not separated in the statistical analyses or if they did not seem relevant for Nordic background levels.

Appendix 1, Table 1: Excluded studies, PCBs, dioxins and PBDEs

	Reference
1	Blanck HM, Marcus M, Rubin C, Tolbert PE, Hertzberg VS, Henderson AK, Zhang RH. (2002). Growth in girls exposed in utero and postnatally to polybrominated biphenyls and polychlorinated biphenyls. Epidemiology, 13(2), 205-210.
2	Chao HR, Tsou TC, Huang HL, Chang-Chien GP. (2011). Levels of breast milk PBDEs from southern Taiwan and their potential impact on neurodevelopment. Pediatr Res, 70(6), 596-600.
3	Eggesbo M, Thomsen C, Jorgensen JV, Becher G, Odland JO, Longnecker MP. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates. Environ Res, 111(6), 737-743.
4	Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jorgensen E. (2006). Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med, 3(8), e311.
5	Hertz-Picciotto I, Bergman A, Fangstrom B, Rose M, Krakowiak P, Pessah I, Hansen R, Bennett DH. (2011). Polybrominated diphenyl ethers in relation to autism and developmental delay: a case-control study. Environ Health, 10(1), 1.
6	Holdke B, Karmaus W, Kruse H. (1998). [Body burden of polychlorinated biphenyl compounds in whole blood of 7-10-year-old children in the area of a hazardous waste incineration facility]. Gesundheitswesen, 60(8-9), 505-512.
7	Horvathova M, Jahnova E, Palkovicova L, Trnovec T, Hertz-Picciotto I. (2011). The kinetics of cell surface receptor expression in children perinatally exposed to polychlorinated biphenyls. J Immunotoxicol, 8(4), 367-380.
8	Jackson LW, Lynch CD, Kostyniak PJ, McGuinness BM, Louis GM. (2010). Prenatal and postnatal exposure to polychlorinated biphenyls and child size at 24 months of age. Reprod Toxicol, 29(1), 25-31.
9	Jacobson JL, Jacobson SW. (2002). Breast-feeding and gender as moderators of teratogenic effects on cognitive development. Neurotoxicol Teratol, 24(3), 349-358.
10	Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, Kim S, Park S, Hwang I, Jeon J, Yang H, Giesy JP. (2011). Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol, 45(17), 7465-7472.
11	Leijs, M. M., Koppe, J. G., Olie, K., van Aalderen, W. M., de, V. P., ten Tusscher, G. W. (2009) Effects of dioxins, PCBs, and PBDEs on immunology and hematology in adolescents. Environ.Sci.Technol., 43, (20) 7946-7951.
12	Madia, F., Giordano, G., Fattori, V., Vitalone, A., Branchi, I., Capone, F. et al. (2004) Differential in vitro neurotoxicity of the flame retardant PBDE-99 and of the PCB Aroclor 1254 in human astrocytoma cells. Toxicol.Lett., 154, (1-2) 11-21.
13	Nishijo M, Tawara K, Nakagawa H, Honda R, Kido T, Nishijo H, Saito S. (2008). 2,3,7,8-Tetrachlorodibenzo-p-dioxin in maternal breast milk and newborn head circumference. J Expo Sci Environ Epidemiol, 18(3), 246-251.
14	Park JS, She J, Holden A, Sharp M, Gephart R, Souders-Mason G, Zhang V, Chow J, Leslie B, Hooper K. (2011). High postnatal exposures to polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) via breast milk in California: does BDE-209 transfer to breast milk? Environ Sci Technol, 45(10), 4579-4585.
15	Patandin S, Weisglas-Kuperus N, de Ridder MA, Koopman-Esseboom C, van Staveren WA, van der Paauw CG, Sauer PJ. (1997). Plasma polychlorinated biphenyl levels in Dutch preschool children either breast-fed or formula-fed during infancy. Am J Public Health, 87(10), 1711-1714.
16	Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, van der Slikke JW, Koppe JG. (1993). Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ Health Perspect, 101(6), 504-508.
17	Pluim HJ, de Vijlder JJ, Olie K et al. (1993) Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ Health Perspect 101, 504-508.
18	Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, van der Slikke JW, Koppe JG. (1993). Effects of

	Reference
	pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. Environ Health Perspect, 101(6), 504-508.
19	Sandberg DE, Vena JE, Meyer-Bahlburg HFL, Beehler G, Swanson M, Weiner J (2001), Endocrine disruptors and children's sex-dimorphic behaviour. APMIS, 109: S506–S507.
20	Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de CR, Anto JM, Torrent M. (2010). Early life environment, neurodevelopment and the interrelation with atopy. Environ Res, 110(7), 733-738.
21	Teufel M (1992) [PCB exposure of children in East and West Germany]. Klin Padiatr 204, 348-354.
22	Winneke G, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, Wiener JA, Steingruber HJ. (1998). Developmental neurotoxicity of polychlorinated biphenyls (PCBS): cognitive and psychomotor functions in 7-month old children. Toxicol Lett, 102-103, 423-428.
23	Winneke G, Walkowiak J, Lilienthal H. (2002). PCB-induced neurodevelopmental toxicity in human infants and its potential mediation by endocrine dysfunction. Toxicology, 181-182, 161-165.

Appendix 1, Table 2: Excluded studies, heavy metals (Hg and Pb)

	Reference
1	Afeiche M, Peterson KE, Sanchez BN, Cantonwine D, Lamadrid-Figueroa H, Schnaas L, Ettinger AS, Hernandez-Avila M, Hu H, Tellez-Rojo MM. (2011). Prenatal lead exposure and weight of 0- to 5-year-old children in Mexico city. Environ Health Perspect, 119(10), 1436-1441.
2	Dorea JG, Bezerra VL, Fajon V, Horvat M. (2011). Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. Clin Chim Acta, 412(17-18), 1563-1566.
3	Dorea JG, Marques RC, Isejima C. (2012). Neurodevelopment of Amazonian infants: antenatal and postnatal exposure to methyl- and ethylmercury. J Biomed Biotechnol, 2012, 132876.
4	Grandjean P, Budtz-Jorgensen E, Steuerwald U, Heinzow B, Needham LL, Jorgensen PJ, Weihe P. (2003). Attenuated growth of breast-fed children exposed to increased concentrations of methylmercury and polychlorinated biphenyls. FASEB J, 17(6), 699-701.
5	Baghurst PA, McMichael AJ, Wigg NR, Vimpani GV, Robertson EF, Roberts RJ, Tong SL. (1992). Environmental exposure to lead and children's intelligence at the age of seven years. The Port Pirie Cohort Study. N Engl J Med, 327(18), 1279-1284.
6	Counter SA, Buchanan LH, Ortega F, Amarasiriwardena C, Hu H. (2000). Environmental lead contamination and pediatric lead intoxication in an Andean Ecuadorian village. Int J Occup Environ Health, 6(3), 169-176.
7	Jedrychowski W, Perera F, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, Edwards S, Skarupa A, Lisowska-Miszczyk I. (2009). Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: the prospective cohort study in three-year olds. Early Hum Dev, 85(8), 503-510.
8	Miyake Y, Tanaka K, Yasutake A, Sasaki S, Hirota Y. (2011). Lack of association of mercury with risk of wheeze and eczema in Japanese children: the Osaka Maternal and Child Health Study. Environ Res, 111(8), 1180-1184.
9	Sanin LH, Gonzalez-Cossio T, Romieu I, Peterson KE, Ruiz S, Palazuelos E, Hernandez-Avila M, Hu H. (2001). Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants. Pediatrics, 107(5), 1016-1023.
10	Weitzman M, Kursmark M. (2009). Breast-feeding and child lead exposure: a cause for concern. J Pediatr, 155(5), 610-611.

Appendix 1, Table 3: Excluded studies, pesticides

	Reference
1	Carrizo D, Grimalt JO, Ribas-Fito N, Sunyer J, Torrent M. (2006). Physical-chemical and maternal determinants of the accumulation of organochlorine compounds in four-year-old children. Environ Sci Technol, 40(5), 1420-1426.
2	Carrizo D, Grimalt JO, Ribas-Fito N, Sunyer J, Torrent M. (2007). Influence of breastfeeding in the accumulation of polybromodiphenyl ethers during the first years of child growth. Environ Sci Technol, 41(14), 4907-4912.
3	Eriksson P, Talts U. (2000). Neonatal exposure to neurotoxic pesticides increases adult susceptibility: a review of current findings. Neurotoxicology, 21(1-2), 37-47.
4	Grimalt JO, Carrizo D, Gari M, Font-Ribera L, Ribas-Fito N, Torrent M, Sunyer J. (2010). An evaluation of the sexual differences in the accumulation of organochlorine compounds in children at birth and at the age of 4 years. Environ Res, 110(3), 244-250.
5	Hardell L, Lindstrom G, van BB. (2002). Is DDT exposure during fetal period and breast-feeding associated with neurological impairment? Environ Res, 88(3), 141-144.
6	Lackmann GM, Schaller KH, Angerer J. (2004). Organochlorine compounds in breast-fed vs. bottle-fed infants: preliminary results at six weeks of age. Sci Total Environ, 329(1-3), 289-293.
7	Link B, Gabrio T, Zollner I, Piechotowski I, Kouros B. (2007). Sentinel health department project in Baden-Wuerttemberg (Germany)--a useful tool for monitoring children's health and environment. Int J Hyg Environ Health, 210(3-4), 351-355.
8	Mariussen E, Fonnum F. (2006). Neurochemical targets and behavioral effects of organohalogen compounds: an update. Crit Rev Toxicol, 36(3), 253-289.
9	Miyake Y, Tanaka K, Yasutake A, Sasaki S, Hirota Y. (2011). Lack of association of mercury with risk of wheeze and eczema in Japanese children: the Osaka Maternal and Child Health Study. Environ Res, 111(8), 1180-1184.
10	Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, Yanagawa T, Fukushige J, Watanabe T. (2007). Immunologic effects of perinatal exposure to dioxins, PCBs and organochlorine pesticides in Japanese infants. Chemosphere, 67(9), S393-S398
11	Noakes PS, Taylor P, Wilkinson S, Prescott SL. (2006). The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study. Chemosphere, 63(8), 1304-1311.
12	Pohl HR, Tylenda CA. (2000). Breast-feeding exposure of infants to selected pesticides: a public health viewpoint. Toxicol Ind Health, 16(2), 65-77.
13	Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de CR, Anto JM, Torrent M. (2010). Early life environment, neurodevelopment and the interrelation with atopy. Environ Res, 110(7), 733-738.

Appendix 2: Quality Assessment Tool Tables

Reference					Requires Yes for level		
					A	B	C
1. General questions and study design							
a) Research question clearly formulated?	Yes	No	Can´t tell	NA	x	x	
b) Endpoint/outcome clearly defined?	Yes	No	Can´t tell	NA	x	x	
c) Was the study design suited to test the research hypothesis?	Yes	No	Can´t tell	NA	x	x	
2. Sampling (*Ascertainment of cases and non-cases)*							
a) Source population/study base well defined? Recruitment done in an acceptable way?	Yes	No	Can´t tell	NA	x	x	
b) Criteria for inclusion/exclusion clearly formulated and sufficient?	Yes	No	Can´t tell	NA	x	x	
c) Time points of data collection clearly described (year of sampling, month or year of follow-up, child's age etc.)	Yes	No	Can´t tell	NA	x		
d) Health outcome/endpoint clearly ascertained and assessed?	Yes	No	Can´t tell	NA	x	x	
3. Breastmilk consumption/contaminant exposure							
a) Type of contaminant exposure reported in sufficient detail?	Yes	No	Can´t tell	NA	x	x	
b) Is length of breastfeeding clearly defined?	Yes	No	Can´t tell	NA	x	x	
c) Is it clearly defined whether the infants have been fully breastfed (=exclusive+predominantly) or partly breastfed (all other breastfeeding)?	Yes	No	Can´t tell	NA	x		
d) Time period between contaminant assessment and diagnosis acceptable?	Yes	No	Can´t tell	NA	x if relevant		
e) Is prenatal **contaminant** exposure assessed appropriately?	Yes	No	Can´t tell	NA	x	x	
f) Is postnatal **contaminant** exposure assessed appropriately?	Yes	No	Can´t tell	NA	x	x	
4. Relevance for the present purpose	Yes	No	Requires Yes for further questions below				
5. Gestational length							
a) Are premature infants separated in statistical analysis?	Yes	No	Can´t tell	NA	x		
6. Confounding (pairity, mothers age etc.)							
a) Were important confounders identified/ascertained and considered by authors?	Yes	No	Can´t tell	NA	x	x	
b) The distribution of confounders similar in cases and non-cases adequately described	Yes	No	Can´t tell	NA	x		

Reference					Requires Yes for level A	B	C
and taken into consideration?							
7. Statistical power (key studies)							
a) Was the study power considered and sample size and power calculations reported?	Yes	No	Can´t tell	NA	x		
b) In view of multiple tests, were by chance findings considered?	Yes	No	Can´t tell	NA	x		
c) Sufficient size of study population and no. of outcomes/cases?	Yes	No	Can´t tell	NA	x		
8. Statistical analysis							
a) Follow-up period clearly identified?	Yes	No	Can´t tell	NA	x		
b) Loss to follow-up described?	Yes	No	Can´t tell	NA	x		
c) Statistical analysis ppropriately handled?	Yes	No	Can´t tell	NA	x		
d) Relevant confounders adequately handled: Restriction, Stratified analysis, Multivariate modelling, Interaction tested?	Yes	No	Can´t tell	NA	x	x	
e) Ascertainment/detection bias considered (eg. cases detected due to screening)?	Yes	No	Can´t tell	NA	x		
9. Summary of the study quality	A	or	B	or C			

A The results from studies that have an acceptably low level of bias are considered valid. These studies adhere mostly to the commonly held concepts of high quality including the following: a comprehensive study design; clear description of the participants, setting, interventions, and control group(s); appropriate measurement of outcomes; appropriate statistical and analytical methods and reporting; no reporting errors; less than 30% percent dropout (depending on the length of the study see the QAT for clinical studies) or over 50% participation rate for prospective cohort studies; clear reporting of dropouts; and no obvious bias. Where appropriate, studies must provide a valid estimation of contaminant exposure, from assessments and/or biomarkers with a reasonable range of measurement error, and justification for approaches to control for confounding in the design and analyses.

B Studies may have some bias, but not sufficient to invalidate the results. They do not meet all the criteria in category “A”, they have some deficiencies but none likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems.

C Studies have significant bias that may invalidate the results. These studies have serious errors in design, analysis, or reporting; there are large amounts of missing information, or discrepancies in reporting.

Appendix 3: Tables of included studies

Appendix 3, Table 1: Included studies with PCBs, dioxins

	References	Quality assessment
1	Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect, 117(6), 1014-1020.	B
2	Forns J, Torrent M, Garcia-Esteban R, Grellier J, Gascon M, Julvez J, Guxens M, Grimalt JO, Sunyer J. (2012). Prenatal exposure to polychlorinated biphenyls and child neuropsychological development in 4-year-olds: an analysis per congener and specific cognitive domain. Sci Total Environ, 432, 338-343.	B
3	Gascon M, Verner MA, Guxens M, Grimalt JO, Forns J, Ibarluzea J, Lertxundi N, Ballester F, Llop S, Haddad S, Sunyer J, Vrijheid M. (2013). Evaluating the neurotoxic effects of lactational exposure to persistent organic pollutants (POPs) in Spanish children. Neurotoxicology, 34, 9-15.	B+
4	Gladen BC, Ragan NB, Rogan WJ. (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr, 136(4), 490-496.	B
5	Gladen BC, Rogan WJ. (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr, 119(1 Pt 1), 58-63.	B
6	Grandjean P, Poulsen LK, Heilmann C, Steuerwald U, Weihe P. (2010). Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environ Health Perspect, 118(10), 1429-1433.	B
7	Grandjean P, Budtz-Jorgensen E, Steuerwald U, Heinzow B, Needham LL, Jorgensen PJ, Weihe P. (2003). Attenuated growth of breast-fed children exposed to increased concentrations of methylmercury and polychlorinated biphenyls. FASEB J, 17(6), 699-701.	B
8	Heilmann C, Budtz-Jorgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. (2010). Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect, 118(10), 1434-1438.	B
9	Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1995). Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev, 43(2), 165-176.	B
10	Jacobson JL, Jacobson SW, Humphrey HE. (1990). Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol, 12(4), 319-326.	B
11	Jusko TA, Sonneborn D, Palkovicova L, Kocan A, Drobna B, Trnovec T, Hertz-Picciotto I.(2012). Pre- and postnatal polychlorinated biphenyl concentrations and longitudinal measures of thymus volume in infants. Environ Health Perspect, 120(4), 595-600.	B
12	Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, van der Paauw CG, Tuinstra LG, Sauer PJ. (1996). Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics, 97(5), 700-706.	B+
13	Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1998). Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev, 50(3), 283-292.	B+
14	Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr, 134(1), 33-41.	B+
15	Rogan WJ, Gladen BC. (1991). PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol, 1(5), 407-413.	B
16	Verner MA, Plusquellec P, Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL, Charbonneau M, Haddad S. (2010). Alteration of infant attention and activity by polychlorinated biphenyls: unravelling critical windows of susceptibility using physiologically based pharmacokinetic modeling. Neurotoxicology, 31(5), 424-431.	B
17	Vreugdenhil HJ, Slijper FM, Mulder PG, Weisglas-Kuperus N. (2002). Effects of perinatal	B+

	References	Quality assessment
	exposure to PCBs and dioxins on play behavior in Dutch children at school age. Environ Health Perspect, 110(10), A593-A598.	
18	Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. (2002). Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr, 140(1), 48-56.	B+
19	Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, Steingruber HJ, Wundram S, Winneke G. (2001). Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet, 358(9293), 1602-1607.	B
20	Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H. (2000). Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect, 108(12), 1203-1207.	B+
21	Winneke G, Kramer U, Sucker K, Walkowiak J, Fastabend A, Heinzow B, Steingruber HJ. (2005). PCB-related neurodevelopmental deficit may be transient: follow-up of a cohort at 6 years of age. Environ Toxicol Pharmacol, 19(3), 701-706.	B

Appendix 3, Table 2: Included studies with heavy metals (only Hg)

	References, Hg	Quality assessment
1	Grandjean P, Weihe P, White RF. (1995). Milestone development in infants exposed to methylmercury from human milk. Neurotoxicology, 16(1), 27-33.	B
2	Jensen TK, Grandjean P, Jorgensen EB, White RF, Debes F, Weihe P. (2005). Effects of breast feeding on neuropsychological development in a community with methylmercury exposure from seafood. J Expo Anal Environ Epidemiol, 15(5), 423-430.	B

Appendix 3, Table 3: Included studies with pesticides

	Reference	Quality assessment
1	Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect, 117(6), 1014-1020.	B
2	Gascon M, Verner MA, Guxens M, Grimalt JO, Forns J, Ibarluzea J, Lertxundi N, Ballester F, Llop S, Haddad S, Sunyer J, Vrijheid M. (2013). Evaluating the neurotoxic effects of lactational exposure to persistent organic pollutants (POPs) in Spanish children. Neurotoxicology, 34, 9-15.	B+
3	Gladen BC, Ragan NB, Rogan WJ. (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr, 136(4), 490-496.	B
4	Gladen BC, Rogan WJ. (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr, 119(1 Pt 1), 58-63.	B
5	Rogan WJ, Gladen BC. (1991). PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol, 1(5), 407-413.	B
6	Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. (2006). Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clin Exp Allergy, 36(10), 1236-1241.	B

Appendix 4: List of studies categorised as C

	Reference	Reason for C
1	Chen Z, Robison L, Giller R, Krailo M, Davis M, Davies S, Shu XO. (2006). Environmental exposure to residential pesticides, chemicals, dusts, fumes, and metals, and risk of childhood germ cell tumors. Int J Hyg Environ Health, 209(1), 31-40.	The exposure is only anamnestic, not measured levels of contaminants in blood or milk.
2	Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. (2010). POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. Environ Int, 36(2), 180-187.	Not possible to discern pre- and postnatal exposure.
3	Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. (2000). Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ Health Perspect, 108(3), 205-211.	Missing relevant breastfeeding data. No stratification full/partial breastfeeding.
4	Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S. (2008). Immune cell counts and risks of respiratory infections among infants exposed pre- and postnatally to organochlorine compounds: a prospective study. Environ Health, 7, 62.	Not possible to discern pre- and postnatal exposure.
5	Kaneko H, Matsui E, Shinoda S, Kawamoto N, Nakamura Y, Uehara R, Matsuura N, Morita M, Tada H, Kondo N. (2006). Effects of dioxins on the quantitative levels of immune components in infants. Toxicol Ind Health, 22(3), 131-136.	Low relevance, due to inadequacies in the descriptions of groups, and no exposure characteristics of the formula fed group.
6	Karmaus W, Davis S, Chen Q, Kuehr J, Kruse H. (2003). Atopic manifestations, breast-feeding protection and the adverse effect of DDE. Paediatr Perinat Epidemiol, 17(2), 212-220.	Too scarce breastfeeding history.
7	Karmaus W, Kuehr J, Kruse H. (2001). Infections and atopic disorders in childhood and organochlorine exposure. Arch Environ Health, 56(6), 485-492.	Breastfeeding anamnesis insufficiently described.
8	Marques RC, Dorea JG, Bernardi JV, Bastos WR, Malm O. (2009). Prenatal and postnatal mercury exposure, breastfeeding and neurodevelopment during the first 5 years. Cogn Behav Neurol, 22(2), 134-141.	Not possible to separate pre- and postnatal effects. Hg in vaccines was the focus of the article. No control group
9	Marques RC, Dorea JG, Bernardi JV, Bastos WR, Malm O. (2008). Maternal fish consumption in the nutrition transition of the Amazon Basin: growth of exclusively breastfed infants during the first 5 years. Ann Hum Biol, 35(4), 363-377.	Not possible to discern pre- and postnatal exposure. Hg from fish the focus of attention. No control group.
10	Marques RC, Garrofe DJ, Rodrigues BW, de Freitas RM, de Freitas FM, Malm O. (2007). Maternal mercury exposure and neuro-motor development in breastfed infants from Porto Velho (Amazon), Brazil. Int J Hyg Environ Health, 210(1), 51-60.	All were exclusively breastfed. Not possible to separate pre- and postnatal effects. No control group.
11	Matsuura N, Uchiyama T, Tada H, Nakamura Y, Kondo N, Morita M, Fukushi M. (2001). Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan--the second report from research on environmental health. Chemosphere, 45(8), 1167-1171.	Low relevance, due to inadequacies in the descriptions of groups, and no exposure characteristics of the formula fed group.
12	Matsuura N, Uchiyama T, Tada H, Nakamura Y, Kondo N, Morita M, Fukushi M. (2001). Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan--the second report from research on environmental health. Chemosphere, 45(8), 1167-1171.	Low relevance, due to inadequacies in the descriptions of groups, and no exposure characteristics of the formula fed group.
13	Nagayama J, Okamura K, Iida T, Hirakawa H, Matsueda T, Tsuji H, Hasegawa M, Sato K, Ma HY, Yanagawa T, Igarashi H, Fukushige J, Watanabe T. (1998). Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. Chemosphere, 37(9-12), 1789-1793.	Of low relevance, since N is low. Insufficient description of the study group.

	Reference	Reason for C
14	Pan IJ, Daniels JL, Herring AH, Rogan WJ, Siega-Riz AM, Goldman BD, Sjodin A. (2010). Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: an analysis of the Pregnancy, Infection, and Nutrition Babies Study. Paediatr Perinat Epidemiol, 24(3), 262-271.	Does not try to discern pre and postnatal effects. Little variation in exposure, all breastfed long.
15	Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. (2009). Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: an analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect, 117(3), 488-494.	Relevant, but study designed for other purpose. Does not try to discern pre and postnatal effects, and does not include non-breastfed.
16	Ribas-Fito N, Julvez J, Torrent M, Grimalt JO, Sunyer J. (2007). Beneficial effects of breastfeeding on cognition regardless of DDT concentrations at birth. Am J Epidemiol, 166(10), 1198-1202.	Lacks important cofounders.
17	Ribas-Fito N, Cardo E, Sala M, Eulalia de MM, Mazon C, Verdu A, Kogevinas M, Grimalt JO, Sunyer J. (2003). Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants. Pediatrics, 111(5 Pt 1), e580-e585.	Postnatal results not assessed.
18	Schell LM, Gallo MV, Ravenscroft J, DeCaprio AP. (2009). Persistent organic pollutants and anti-thyroid peroxidase levels in Akwesasne Mohawk young adults. Environ Res, 109(1), 86-92.	Cannot be used for assessing effects of postnatal exposure.
19	Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. (2008). Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'- DDE, and other toxicants in Akwesasne Mohawk youth. Environ Health Perspect, 116(6), 806-813.	Cannot be used for assessing effects of postnatal exposure.
20	Torres-Sanchez L, Schnaas L, Cebrian ME, Hernandez MC, Valencia EO, Garcia Hernandez RM, Lopez-Carrillo L. (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. Neurotoxicology, 30(6), 1162-1165.	Too scarce breastfeeding data. No information about breastfeeding length or exclusive or partial breastfeeding.

Appendix 5: Summary Tables

Summary Table 1-24, Table 1.

Reference	**Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect, 117(6), 1014-1020.**
Study design and type	Prospective cohort study, Nuanavik (1995-1998).
Objective	Examine relationship between PCBs and OH-PCBs, PCP and HCB in maternal plasma at delivery, umbilical cord plasma and infant plasma at 7 months and thyroid hormone status.
Number of participants and country	204 infants at birth, 130 at 7 months, Canada.
Baseline characteristics of study subjects	Pregnant Inuit women in Nunavik, 1995-2001.
Exposure	PCBs and OH-PCBs, PCP and HCB.
Measurement of exposure (Biomarker, internal validation)	Biomarkers: PCBs and OH-PCBs, PCP and HCB in maternal plasma at delivery (n=120), cord plasma (n=95) and in infant plasma at 7 months (n=130).
Follow-up period, drop-outs	7 months, 17.8% drop out.
Length of breastfeeding	0-7 months.
Degree of breastfeeding (fully/partly)	Not given.
Outcome	Changes in thyroid hormone levels in maternal plasma, cord plasma or infant plasma.
Measurement of outcome	TSH, fT4, T3, TBG.
Statistical analysis	Simple regressions between potential confounding variables and thyroid parameters. Covariates associated at a p-value ≤0.10 were included in multiple regression models to assess their confounding influence. Covariates modifying the regression coefficient of the contaminants by >10% with any of the THs were included in adjusted models for all TH parameters. The following covariates were evaluated in pregnant women: age at delivery, pre-pregnancy body weight, socioeconomic status, alcohol and cigarette consumption, fish consumption during pregnancy, and plasma Se concentration, age at delivery and at 7 months of age: The primary caregiver's score for socioeconomic status, maternal alcohol and cigarette consumption, maternal

Reference	**Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. (2009). Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect, 117(6), 1014-1020.**
	fish consumption during pregnancy, sex, breastfeeding status, as well as Se level in cord blood, gestational age. Because breastfeeding is an important source of postnatal exposure, an interaction term between OC concentrations and breastfeeding status at 7 months of age was also tested in multiple regression models. Statistical models assessing the relationship between the lipophilic contaminants PCB-153 or HCB and thyroid parameters were further adjusted for total plasma lipid concentrations.
Results	No association was observed between contaminants and thyroid hormones at 7 months of age. In pregnant women, a positive association between HO-PCBs and T3 concentrations (β=0.57, p=0.02) was found. In umbilical cord blood, PCB-153 concentrations were negatively associated with thyroid binding globulin (TBG) levels (β= -0.26, p=0.01).
Conclusion	There is little evidence that the environmental contaminants analysed in this study affect thyroid hormone status in Inuit mothers and their infants.
Confounders adjusted for	Covariates modifying the regression coefficient of the contaminants by >10% with any of the TH.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	The study is relevant, but shows no effect of postnatal exposure.

Summary Table 1-24, Table 2.

Reference	**Forns J, Torrent M, Garcia-Esteban R, Grellier J, Gascon M, Julvez J, Guxens M, Grimalt JO, Sunyer J. (2012). Prenatal exposure to polychlorinated biphenyls and child neuropsychological development in 4-year-olds: an analysis per congener and specific cognitive domain. Sci Total Environ, 432, 338-343.**
Study design and type	Prospective birth cohort, Menorca (1997-99).
Objective	To assess any potential detrimental effects of prenatal and postnatal exposure to current levels of PCBs on general neuropsychological development and specific cognitive domains.
Number of participants and country	482 mother-child pairs at inclusion (6% not eligible). At 4 years of age, at 4 years of age, 98% of 470 mother-child pairs remained in the follow-up. 405 with cord blood samples, 278 with blood sample at age 4 years, Spain.
Baseline characteristics of study subjects	Women in Menorca presenting for antennal care during 12 months from mid-1997.
Exposure	PCB-28, -52, -101, -118, 138, -153, -180, DDE, HCB.
Measurement of exposure (Biomarker, internal validation)	POPs in cord blood and in children at age 4 years.
Follow-up period, drop-outs	4 years, 2% drop out.
Length of breastfeeding	Recorded, not shown.
Degree of breastfeeding (fully/partly)	Recorded, not shown.
Outcome	General neuropsychological development (418 children) (355 mother-child pairs with complete info on neuropsychological development assessment and OC levels in cord serum).
Measurement of outcome	McCarthy's scale of children's abilities (MCSA) adapted to the Spanish population at 4 years. The general cognitive scale and the five subscales (verbal, perceptive-performance, memory, quantitative and motor) were examined. In addition MCSA subtests were reorganised into new sub-area scores (executive functions, working memory, visual and verbal span, verbal memory, gross and fine motor skills and cognitive functions of posterior cortex).
Statistical analysis	Multivariate regression model for general cognitive scale considering a priori selected covariates using a backwards selection procedure, for both prenatal and postnatal PCB-exposure. Covariates retained in the model had associations with MCSA general cognitive scale with p-values of <0.05 or resulted in a change in the regression coefficient of the sum of PCBs ≥10%. Potential modification of effects by duration of breastfeeding and child's

Reference	**Forns J, Torrent M, Garcia-Esteban R, Grellier J, Gascon M, Julvez J, Guxens M, Grimalt JO, Sunyer J. (2012). Prenatal exposure to polychlorinated biphenyls and child neuropsychological development in 4-year-olds: an analysis per congener and specific cognitive domain. Sci Total Environ, 432, 338-343.**
	fish intake were evaluated.
Results	No statistically significant effects of the sum of prenatal PCBs on MCSA scores were seen. Individual congener analyses yielded significant detrimental effects of prenatal PCB153 on the majority of MCSA scores, while no effects were reported for other congeners. The levels of PCBs at 4 years of age were not associated with neuropsychological development. PCB levels in serum were not lipid adjusted and therefore not easily comparable with other regions.
Conclusion	None of the associations observed were modified by either duration of breastfeeding or fish intake at 4 years of age. Prenatal (but not postnatal) exposure PCB153 was associated with reduced scores on neuropsychological development at 4 years of age, including executive function, verbal functions and visuospatial abilities, but not on motor development.
Confounders adjusted for	Prenatal models were adjusted for psychologist, child's age, maternal social class, folic acid supplementation during pregnancy, maternal cigarettes during pregnancy, paternal education, child's sex, and duration of breastfeeding. Postnatal exposure models were adjusted for psychologist, child's age, duration of breastfeeding, maternal education and child sex.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Highly relevant because the results can separate between pre-and postnatal exposure. However, whereas measurement at 4 years of age reflects the child’s total exposure, it provides no information on variation in exposure during the postnatal period. The study had no measure of HOME Score

Summary Table 1-24, Table 3.

Reference	**Gascon M, Verner MA, Guxens M, Grimalt JO, Forns J, Ibarluzea J, Lertxundi N, Ballester F, Llop S, Haddad S, Sunyer J, Vrijheid M. (2013). Evaluating the neurotoxic effects of lactational exposure to persistent organic pollutants (POPs) in Spanish children. Neurotoxicology, 34, 9-15.**
Study design and type	Prospective birth cohort, subsample of the INMA cohort (2003-2008).
Objective	To assess whether lactational exposure to PCB-153, DDE, or HCB as estimated with a PBPK model, is associated with decrements in mental and psychomotor development scores of the Bayley Scales of Infant Development (BSID) test in children aged around 14-months, of a subsample of the Spanish INMA birth cohort, and to compare this with the effects of prenatal exposure.
Number of participants and country	2150 infants at birth, 1175 at follow-up, Spain mainland.
Baseline characteristics of study subjects	Children of women (>16 years age, single pregnancy, planned deliver at reference hospital, no problems with communication) enrolled during the first pregnancy trimester from three regions (Gipuzkoa, Sabadell, Valencia) (sampled 2003-2008). 10 children later excluded due to pathologies, 75 due to difficulties and suboptimal cooperation with psychologist, 9 were excluded because of lacking information on co-variables in adjusted models.
Exposure	PCB153, DDE, HCB.
Measurement of exposure (Biomarker, internal validation)	PCB153, DDE, HCB in maternal serum (geometric mean (GM) for PCB153: 28.2, DDE: 132.5, HCB: 42.7 ng/g lipid) at pregnancy week 7-26 (median 12.9 weeks). Child's exposure estimated by PBPK (Verner et al., 2009). Length of breastfeeding (exclusive and partly) assessed at testing at 14 months.
Follow-up period, drop-outs	14 months (range 11-21 months).
Length of breastfeeding	Recorded, mean duration excusive breastfeeding 3.8 months and partly breastfeeding 6.1 months.
Degree of breastfeeding (fully/partly)	Recorded. Milk consumption during partial breastfeeding was calculated as a constant decrease from 71 to 55% of daily intake in exclusively breastfed children over the period of partial breastfeeding (consumption based on data from 382 mother-child pairs from Sabadell).
Outcome	Child neuropsychological assessment.
Measurement of outcome	Bayley Scales of Infant Development, BSID-I test (giving MDI and PDI test scores) in children aged around 14 months of age (range 11-21 months).
Statistical analysis	Association between POP concentration (log transformed) and BSID's mental and psychomotor scores were assessed by linear regressions (Covariates considered by backward selection procedure; p-value <0.05 with test scores or resulted in a change in estimate of >10% were retained). Monthly postnatal exposure was analysed separately and also joined into four periods of three months. Linearity of the association between POPs and BSID scores was assessed by using Generalised Additive Models; associations between DDE and test scores were not linear. DDE levels were therefore included in the models as a binary variable, where the median was used as a cut-off.

Reference	**Gascon M, Verner MA, Guxens M, Grimalt JO, Forns J, Ibarluzea J, Lertxundi N, Ballester F, Llop S, Haddad S, Sunyer J, Vrijheid M. (2013). Evaluating the neurotoxic effects of lactational exposure to persistent organic pollutants (POPs) in Spanish children. Neurotoxicology, 34, 9-15.**
Results	Mean estimated POP level in child's blood increased after birth due to breastfeeding (Maximal PCB-153: 60.85 in the 2nd three month period, DDE: 199.17 in the 1st three months period, HCB: 60.49 in the 2nd three months period). Correlation between prenatal and estimated postnatal concentrations decreased with months of life. Correlation between PCB-153, DDE and HCB was low in maternal blood, but correlation increased with postnatal life because breastmilk was the common exposure source in children. Increasing prenatal PCB-153 exposure was associated with lower PDI scores, but was not associated with MDI scores (as reported separately before from this cohort), also when postnatal PCB-153 exposure was included in the model. Postnatal PCB-153 exposure was not associated with any of the outcomes. Pre- or postnatal DDE or HCB exposure was not associated with any of the outcomes.
Conclusion	Breastfeeding increases children's blood persistent organic pollutants (POPs) levels during postnatal life. No associations were found between different periods of postnatal exposure to these POPs and mental and psychomotor scores. Deleterious effects of PCB-153 on neuropsychological development are mainly attributable to prenatal exposure.
Confounders adjusted for	Mental scale model: sex, study region, gestational age, day-care attendance, birth weight, maternal social class, maternal region of birth. For prenatal exposure, model was also adjusted for predominant breastfeeding. Psychomotor scale model: Region of study, gestational age, paternal social class.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	This study is of high relevance because it predicts postnatal exposure to three different POPs based on PBPK modelling, and investigates associations between PDI and MDI and children's concentration of POPs in blood at different periods during the first year of life. Furthermore, the study population is large compared to other studies on this topic. Measure of HOME score was lacking in the study

Summary Table 1-24, Table 4.

Reference	**Gladen BC, Ragan NB, Rogan WJ. (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr, 136(4), 490-496.**
Study design and type	Prospective cohort study, North Carolina (1978-82).
Objective	Studied whether prenatal or lactational exposures to background levels of PCBs or DDE were associated with altered pubertal growth and development in humans.
Number of participants and country	858 infants at birth, 594 at puberty follow-up, USA.
Baseline characteristics of study subjects	No eligibility criteria, random cohort.
Exposure	PCBs and DDT/DDE.
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure: Composite average of all measurement available in milk, maternal serum, cord blood and/or placenta. Scaled to one measure (comparable to milk). Average taken of all. Postnatal exposure: LEM: average concentration * duration of breastfeeding.
Follow-up period, drop-outs	10-15years, 856-262=594 (316 girls, 278 boys)
Length of breastfeeding	88% initiates breastfeeding, median total duration not given. Divided into categories: bottle feeding, short, medium, long, very long. Short: 0-9 weeks (0-4 mostly breastfed), medium: 10-19 weeks (0-4 mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks
Degree of breastfeeding (fully/partly)	Fully and partly breastfed assessed separately.
Outcome	Weight and height at 14 years, age at menses, breast stage 3-5, pubes 3-5.
Measurement of outcome	Self-reported height and weight (annual questionnaires). Tanner stages of puberty, self reported based on line drawings, respondendts indicated which stage was closest to their current development.
Statistical analysis	Fitted regression models (SAS procedure Mixed), mixed effect models with random term to account for repeat measurements on the same child. Stratified on gender.
Results	No statistically significant association between hight and weight and exposure to DDE or PCB in boys and girls, but prenatal exposure to DDE was associated with a tendency (not statistically significant) to higher weight in boys at 14 years, but not girls. No significant association between exposure and pubertal maturation, but some tendency (not statistically significant) that girls with higher prenatal exposure maured earlier. This was significant

Reference	**Gladen BC, Ragan NB, Rogan WJ. (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr, 136(4), 490-496.**
	when 25 white girls were excluded from the analyses. Bottle-fed girls tended to mature later (not significantly).
Conclusion	No association between PCB postnatal exposure and growth or puberty signs (but tendency to later maturation bottle-fed girls). Prenatal exposure to PCBs positively associated with weight in girls.
Confounders adjusted for	Prenatal model: adjusted for breastfeeding, maternal weight (not height, not paternal) for weight height included cubic. Postnatal model: Either PCB or DDE, not breastfeeding (because of high correlation). Maternal BMI not adjusted for.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	The study is relevant, but shows no effect of postnatal exposure

Summary Table 1-24, Table 5.

Reference	**Gladen BC, Rogan WJ. (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr, 119(1 Pt 1), 58-63.**
Study design and type	Prospective cohort study, North Carolina (1978-82).
Objective	Determining whether early developmental effects of perinatal exposure to PCBs or DDE persist. Study association between DDT/DDE and PCB and neuropsychological development.
Number of participants and country	859 infants at birth, 712 at follow-up at ages 5.5 to 10.5 years.
Baseline characteristics of study subjects	No eligibility criteria, random cohort.
Exposure	PCBs and DDT/DDE.
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure: Composite average of all measurement available in either milk, maternal serum, cord blood and/or placenta. Scaled to one measure (comparable to milk). Average taken of all. Postnatal exposure: LEM: average concentration * duration of breastfeeding.
Follow-up period, drop-outs	3, 4, and 5 years. 83% had outcome data from at least at one follow-up.
Length of breastfeeding	88% initiates breastfeeding, median total duration not given. Divided into categories: bottle feeding, short, medium, long, very long. Short: 0-9 weeks (0-4 mostly breastfed), medium: 10-19 weeks (0-4 mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks
Degree of breastfeeding (fully/partly)	Mostly or partly.
Outcome	Neuropsychological development.
Measurement of outcome	McCarty scores 3, 4, 5 years. Information on school grades in English and Maths obtained as well.
Statistical analysis	Categorised pre- and postnatal exposure. Uncertain whether postnatal models adjusted for prenatal exposure. Adjusted means given. Confounders: maternal age, race, occupation, smoke, alcohol, sex, number of older siblings, breastfeeding in 5 categories. No information on HOME score, maternal IQ, paternal occupation/education. Included for uncertain reasons only breastfed children in study of postnatal exposure. Adjustment for breastfeeding probably inappropriate, although in categories. Effect of breastfeeding not shown.

Reference	**Gladen BC, Rogan WJ. (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr, 119(1 Pt 1), 58-63.**
Results	Prenatal effects: 1) No association PCB and McCarthy at 3, 4 and 5 years. 2) For DDE borderline significance with McCarthy, but no dose response. 3) Neither PCB nor DDE prenatally showed association with school grades. Postnatal effects: 1) Lower scores McCarthy associated with middle category of PCB exposure (at 3 years, not 4 and 5years). 2) Higher scores with middle category (same pattern all years). Higher levels of postnatal DDE, but not PCB, were associated with poorer grades.
Conclusion	Although some significant and consistent patterns were seen, due to inconsistent dose-response patterns authors conclude that no effect of pre- and postnatal PCB and DDE can be observed at 3, 4 and 5 years on neuropsychological development, as assessed by McCarthy and school grades.
Confounders adjusted for	Lacks important confounders (HOME score, maternal IQ).
Quality (A, B or C)	B Due to lack of confounders and inaccurate description of breastfeeding and how statistical analysis was performed.
Relevance for our risk assessment purpose	Relevant.

Summary Table 1-24, Table 6.

Reference	**Grandjean P, Poulsen LK, Heilmann C, Steuerwald U, Weihe P. (2010). Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environ Health Perspect, 118(10), 1429-1433.**
Study design and type	Prospective birth cohort study, Faroe isles cohort 3 (1999-2001).
Objective	The objective was to assess whether sensitisation and development of allergic disease is associated with duration of breastfeeding and prenatal or postnatal exposures to PCBs and methyl mercury.
Number of participants and country	656 infants at birth, 464 children at 7 year follow-up, Faroe Island.
Baseline characteristics of study subjects	Consecutive singleton births during 1999-2001. Obstetric variables, including birth date, birth weight, gestational age, parity, and maternal age, maternal smoking and alcohol use during pregnancy were obtained; dietary history during pregnancy was obtained from approximately half of the mothers.
Exposure	PCBs and mercury. PCB (sum PCB/g lipid=PCBs 138, 153 and 180 multiplied by 2) as indicator of exposure to lipid-soluble contaminants and Hg.
Measurement of exposure (Biomarker, internal validation)	Blood sample at 5 and 7 years, blood was not obtained from 67 participants. In 49 cases insufficient serum was available for PCB analysis. IgE and clinical data were available for 464. Length of breastfeeding was obtained by questionnaire at 5 or 7 years (not clear). PCB (sum PCB/g lipid=PCBs 138, 153 and 180 multiplied by 2) as indicator of exposure to lipid-soluble contaminants. PCB analysed in child serum at 5/7 years and in maternal serum (34th week) for prenatal exposure (GC with electron capture detection). Milk sampled 5-7 days after parturition. Sum PCB was calculated as the sum the sum of PCBs 138, 153, and 180 multiplied by 2, expressed on lipid weight. Missing maternal serum data were estimated based on milk, with an average ratio of 1.13. Mercury measured (atomic absorption) in maternal hair at parturition and in cord blood, and in child's blood at 5 and 7 years. Missing cord blood measurements were converted from maternal blood using average ratio between the two. Mercury concentration: Maternal hair 2.21 (1.3-4.1) µg/g Cord blood 11.3 (7.4-21.0) g/L Child 5 year of age, blood 2.65 (1.35-5.4) µg/L Child 7 year of age, blood 2.01 (1.01-4.3) µg/L
Follow-up period, drop-outs	5 and 7 years, 29% missing IgE results from original cohort at 7 years of different reasons, 76 of originally 656 did not participate in the follow up (12% drop out).
Length of breastfeeding	Exclusive breastfeeding 4.6 +/- 2.0 months. Total duration of any breastfeeding 9.8 +/- 6.6 months.
Degree of breastfeeding (fully/partly)	Exclusive breastfeeding, any breastfeeding.

Reference	**Grandjean P, Poulsen LK, Heilmann C, Steuerwald U, Weihe P. (2010). Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environ Health Perspect, 118(10), 1429-1433.**
Outcome	Change in level of immunoglobulin E (IgE), grass-specific IgE, and occurrence of allergic disease.
Measurement of outcome	Maternal interview on the child's current health and past medical history, Occurrence of asthma and atopic dermatitis at the follow-up examination was determined by a single paediatrician, who examined all the cohort children and interviewed the mother. Total level of immunoglobulin E (IgE), grass-specific IgE, and occurrence of allergic disease.
Statistical analysis	Exposure parameters and total IgE were log-transformed for linearity. Comparisons were done with independent samples t-test and correlation coefficients. Associations explored by linear regression. Adjustment for covariates; i.e., sex, age, season of birth, preterm birth (34th through 36th week, n=11), low birth weight (<2500 g, n=4), maternal age, parity, maternal fish intake and smoking during pregnancy, parental smoking at home, day care attendance, and the child's body mass index, was then included in the model to ascertain whether they materially affected (>10%) estimated effects of immunotoxicant exposure. For grass-specific IgE concentration and the duration of breastfeeding, which deviated from normal distribution also after transformations. Spearman's nonparametric correlation coefficients and logistic regressions were applied. Statistical significance was assumed when $p<0.05$ (two-sided).
Results	Serum PCB concentration at 7 years was positively associated with total IgE concentration. Longer duration of breastfeeding also appeared to predict a higher IgE concentration at 7 years, but the association was not significant after adjustment for PCB. Duration of breastfeeding was positively associated with grass specific IgE, but with no association with PCB. Postnatal mercury exposure did not affect the outcomes. An inverse association (protective) between prenatal methylmercury concentrations and grass-specific serum IgE concentrations was seen. A history of asthma or atopic dermatitis was not associated with the duration of breastfeeding. Children with a history of atopic dermatitis (n=60) had lower PCB levels (particularly prenatal) than those without atopic dermatitis. Children with asthma (n=35) did not have statistically significant higher PCB levels than children without asthma. Hg exposure was not associated with a history of asthma or atopic dermatitis
Conclusion	These findings suggest that developmental exposure to immunotoxicants may both increase and decrease the risk of allergic disease and that associations between breastfeeding and subsequent allergic disease in children may, at least in part, reflect lactational exposure to immunotoxic food contaminants.
Confounders adjusted for	Adjustment for covariates, i.e., sex, age, season of birth, preterm birth (34th through 36th week, n=11), low birth weight (<2500 g, n=4), maternal age, parity, maternal fish intake and smoking during pregnancy, parental smoking at home, day care attendance, and the child's body mass index was included in the model to ascertain whether they materially affected (>10%) estimated effects of immunotoxicant exposure.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	The study is of relevance for the risk assessment purpose. The results might indicate that PCB (or other contaminants) in breastmilk might cause the IgE increase in children and thus interact with beneficial effects from breastfeeding. However, prenatal PCB-exposure was not attempted to be adjusted for, nor were the individual's PCB-exposure via breastmilk (concentration in breastmilk multiplied with duration of breastfeeding) taken into consideration.

Summary Table 1-24, Table 7.

Reference	**Grandjean P, Budtz-Jorgensen E, Steuerwald U, Heinzow B, Needham LL, Jorgensen PJ, Weihe P. (2003). Attenuated growth of breast-fed children exposed to increased concentrations of methylmercury and polychlorinated biphenyls. FASEB J, 17(6), 699-701.**
Study design and type	Prospective longitudinal cohort study, Faroe isles cohort 2 (1994-1995).
Objective	Relationship between pre and postnatal exposure to mercury and PCB on growth.
Number of participants and country	182 infants at birth, 171 at 18 month follow-up, 121 at 42 month follow-up, Faroe Islands.
Baseline characteristics of study subjects	Infants born 1994-95, from the central and north western villages with easy access to fish and whale. Includes singleton consecutive spontaneous births at term, birth before the 36th week and congenital neurological disease were exclusion criteria.
Exposure	Prenatal Hg exposure, pre- and postnatal PCB exposure.
Measurement of exposure (Biomarker, internal validation)	Biomarkers: Hg in cord blood, total PCB/g lipid in breastmilk (sum of PCB 138, 153, 180) x 2 in transition milk). Lactational exposure was estimated by multiplying PCB concentration by duration of exclusive breastfeeding. Cumulated PCB after weaning was estimated by PCB concentration in 121 of the children at age 54 months.
Follow-up period, drop-outs	18, 42 and 54 months, 6 and 15% drop out.
Length of breastfeeding	Total breastfeeding 7.8 +/- 5.7 months. Exclusive breastfeeding 3.5 +/- 2.0 months. 18.2% exclusively breastfed for 6 months. 7.2% did not breastfeed at all.
Degree of breastfeeding (fully/partly)	Exclusive breastfeeding.
Outcome	Height and weight at 18 and 42 months.
Measurement of outcome	Weight (kg) and height (cm) at 18 and 42 months measured by paediatrician.
Statistical analysis	Multiple regressions. Three models.
Results	At 18 months, children who had been exclusively breastfed for at least 6 months weighed 0.59 kg less (95% CI=0.03, 1.16 kg) and were 1.50 cm (95% CI=0.52, 2.47 cm) shorter than those not breastfed. At 42 months the association was no longer significant if body size at 18 months was included as a covariate. The negative effect of breastfeeding as such was abolished by adjustment for lactational or prenatal mercury exposure. Association with mercury exposure remained statistically significant after adjustment for PCB. The PCB concentration level was generally a less important predictor and inclusion of PCB changed the mercury associations only negligibly. Prenatal and lactational exposure was correlated and not independent. Therefore,

Reference	**Grandjean P, Budtz-Jorgensen E, Steuerwald U, Heinzow B, Needham LL, Jorgensen PJ, Weihe P. (2003). Attenuated growth of breast-fed children exposed to increased concentrations of methylmercury and polychlorinated biphenyls. FASEB J, 17(6), 699-701.**
	the relative impact of pre- and postnatal PCB and Hg exposure could not be fully determined.
Conclusion	Duration of breastfeeding was negatively associated with body weight. The authors suggest that the previously observed reduced growth in breastfed infants could be at least partially explained by environmental contaminants in breastmilk.
Confounders adjusted for	Birth weight, sex, maternal height, smoking, age at 42 months examination, ponderal index (for effect of PCB on body weight at age 54 months). Several more investigated, covariates were kept in the model if $p<0.2$ (backward elimination).
Quality (A, B or C)	B
Relevance for our risk assessment purpose	This is of relevance for the risk assessment. However, it must be taken into consideration that the study cannot fully distinguish between pre- and postnatal PCB exposure.

Summary Table 1-24, Table 8.

Reference	**Grandjean P, Weihe P, White RF. (1995). Milestone development in infants exposed to methylmercury from human milk. Neurotoxicology, 16(1), 27-33.**
Study design and type	Prospective cohort study, Faroe isles cohort 1 (1986-1987).
Objective	Look at significance of methyl mercury exposure from breastmilk on milestone development in Faroese infants at age 1.
Number of participants and country	1022 singleton births, hair samples from 583 children at 1 year follow-up, Faroe Islands.
Baseline characteristics of study subjects	Infants born 1986-1987 at the hospitals in Torshavn, Klaksvik and Suderoy, Faroe Islands.
Exposure	Mercury.
Measurement of exposure (Biomarker, internal validation)	Maternal hair at time of delivery, child hair at 1 years. Mother's frequency of fish and pilot whale consumption during pregnancy was recorded at delivery.
Follow-up period, drop-outs	583 children at 1 year (57.1 % of the cohort).
Length of breastfeeding	0-12 months, divided into short (≤1 months), medium (1-4 months), long (up to 5 months) and very long (≥5 months).
Degree of breastfeeding (fully/partly)	Duration of nursing without supplement and the age of weaning was recorded by district health nurses. Reported (in months) as "mostly breastfed" and "age of weaning".
Outcome	Child milestone development reported by district nurses, partly based on observation by the nurse, partly based on interview with the mother.
Measurement of outcome	1) sits without support 2) creeps 3) gets up into standing position without support.
Statistical analysis	Nonparametric statistics, correlations with Spearman's, differences between groups with Mann-Whitney U-test.
Results	R=0.48 for relationship between length of breastfeeding and Hg concentrations in hair at age 12 months. Milestone development associated with child hair Hg at 12 months. Developmental milestone achievements was not associated with indices of prenatal exposure
Conclusion	The beneficial effects of breastfeeding seem to overrule or compensate for neurotoxic effects on milestone development that could be due to contaminants in human milk.

Reference	**Grandjean P, Weihe P, White RF. (1995). Milestone development in infants exposed to methylmercury from human milk. Neurotoxicology, 16(1), 27-33.**
Confounders adjusted for	Smoking, maternal age, marital status related to length of breastfeeding. Seafood consumption, alcohol and parity not related to length of breastfeeding. Low birth weight (n=9), preterm birth and complications during parturition associated with shorter nursing periods, but not caesarean sections or pregnancy complications.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Relevant.

Summary Table 1-24, Table 9.

Reference	**Heilmann C, Budtz-Jorgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. (2010). Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect, 118(10), 1434-1438.**
Study design and type	Prospective cohort study, Faroe isles cohort 3 (1999-2001).
Objective	The main objective was to assess the possible dependence of antibody concentrations against diphtheria and tetanus toxoids in children with regard to prenatal and postnatal PCB exposures.
Number of participants and country	656 infants at birth, 587 children at 5 and 7 year follow-up, Faroe Islands. A subgroup of these cohort members first came for a follow-up study at 12 and 18 months of age. 116 children had sufficient serum for analysis of PCBs at 18 months. Most of these children also participated in the subsequent follow-up. 587 children participated in the examinations at ages 5 (n=532) and/or 7 years (n=464).
Baseline characteristics of study subjects	Consecutive spontaneous singleton births at term 1999-2001. All followed the national vaccination program.
Exposure	PCB (p,p-DDE, hexachlorobenzene, and β-hexachlorocyclohexane, mercury)
Measurement of exposure (Biomarker, internal validation)	Biological samples obtained from the mother at the last antenatal examination at week 32 of pregnancy, transitional milk samples at post parturition days 4-5, and serum samples from the child at successive clinical examinations. ΣPCB concentration was calculated as the sum of congeners CB-138, CB-153, and CB-180 multiplied by 2 (lipid weight). In addition to the ΣPCB concentration, the weighted sum of the three main mono-ortho-substituted congeners CB-105, CB-118, and CB-156 was calculated using toxicity equivalency factors to obtain the dioxin equivalent concentration. The analyses also provided the concentrations of the pesticide metabolite p,p-dichlorodiphenyldichloroethene (p,p-DDE), hexachlorobenzene, and β-hexachlorocyclohexane, which occurred at lower levels but were detectable in almost all samples. However, because of close correlations with ΣPCB, these additional substances were not examined further. Due to a large number of missing values for the PCB concentration at 18 months, levels were imputed based on the known association with the observed PCB concentrations at birth and at 5 years, as well as the length of the breastfeeding period. As a measure of methylmercury exposure, total mercury concentrations were measured in cord blood, maternal hair at parturition, and hair and blood from the children at the clinical examinations.
Follow-up period, drop-outs	81% participated at 5 years, 71% participated at 7 years. 43 of those participating at 7 years did not participate at 5 years.
Length of breastfeeding	Exclusive breastfeeding: 4.6 +/- 2 months. Total duration of any breastfeeding: 9.8 +/- 6.6 months.
Degree of breastfeeding (fully/partly)	Exclusive breastfeeding.

Reference	**Heilmann C, Budtz-Jorgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. (2010). Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect, 118(10), 1434-1438.**
Outcome	Vaccine response to diphtheria and tetanus toxoids.
Measurement of outcome	Serum concentrations of antibodies against tetanus toxoids were measured by SSI using enzyme-linked immunosorbent assay. Antibodies against diphtheria toxoids were measured using a standard Vero cell-based neutralisation assay employing 2-fold dilutions of serum samples. For both assays, calibration was performed using international and local standard antitoxins.
Statistical analysis	Associations of PCB exposure with log transformed antibody concentrations were determined using standard regression techniques. Covariates: sex and age. Both antibody outcomes obtained after the 5-year booster were adjusted for the booster status (i.e., with and without other vaccines), and the analysis of the immediate post-booster response also included the time interval since booster inoculation. Odds ratios (ORs) were calculated for the effect of PCB exposure on the probability of having an antibody concentration >0.1 IU/mL. PCB exposure parameters were entered into the regression models, one at a time, after logarithmic transformation. Residual plots were used to assess the model fit, and the possible significance of second- and third-order terms was determined. Additional covariates (birth weight, maternal smoking during pregnancy, and duration of breastfeeding) were then included to determine the possible influence on the PCB regression coefficients. Separate regressions were also made to estimate the possible impact of methylmercury exposure. All two-tailed p-values <0.05 were considered statistically significant. For children without exposure measure in serum at 18 months (425 of 532 children at 5 years), the PCB concentration was imputed based on a known association with the observed PCB concentrations at birth and at 5 years, as well as the length of the breastfeeding period by use of the multiple imputation method in SAS version 9.1.
Results	At age 5 years (before booster vaccination) the antidiphtheria antibody concentration was inversely associated with PCB concentrations in milk and 18-month serum. At 7 years an inverse association of concentrations of antibodies against both diphtheria and tetanus toxoids with PCB concentrations at 18 months of age was seen. The strongest associations suggested a decrease in the antibody concentration by about 20% for each doubling in PCB exposure. At age 5 years, the odds of an antidiphtheria antibody concentration below a clinically protective level of 0.1 IU/L increased by about 30% for a doubling in PCB in milk and 18-month serum. Maternal pregnancy serum concentration, showed only a small and not statistically significant association with antibody concentrations. The same was true for serum PCB concentrations determined at the same time as antibody assessments, at ages 5 and 7 years (cross-sectional analyses). The average PCB concentration at 18 months was slightly higher than the average maternal level during pregnancy.
Conclusion	Breastmilk PCB exposure associated with reduced serum concentration of antibodies against diphtheria and tetanus vaccinations. No significant association was observed with prenatal PCB exposure.
Confounders adjusted for	As described in statistical analysis.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	The paper is relevant. Serum PCB is measured in only 1/5 of the children at 18 months and imputed in the rest. The basis for this imputation is not clearly described and is a weakness of the study

Summary Table 1-24, Table 10.

Reference	**Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1995). Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev, 43(2), 165-176.**
Study design and type	Prospective cohort study, Dutch cohort (1990-1992).
Objective	Examine relationship between prenatal exposure to PCBs and lactational exposure to PCBs and dioxins and the neurological condition at 18 months.
Number of participants and country	418 infants at birth, 209 breastfed (BF) and 209 bottle fed (BF). 373 at 18 month follow-up, The Netherlands.
Baseline characteristics of study subjects	Pregnant women from Groningen and Rotterdam, June 1990-June 1992. Selection criteria: parity (first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used. All newborns underwent a neurological examination according to Precthl.
Exposure	Dioxins and PCBs.
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure in the total study population**:** PCBs sum (118, 138, 153, 180) (gc-ec) in mother`s plasma (last month of pregnancy, 36-40th week) and umbilical cord blood. The breastmilk samples from the BF group (24 hour sample within 2 weeks postpartum) were used as indirect measures for prenatal exposures to PCBs (20 congeners plus 6 dioxin-like PCBs and dioxins (17) (gc-ms). Postnatal exposure: PCBs and dioxins levels in milk and formula, respectively. Information was given on missing samples.
Follow-up period, drop-outs	18 month, drop-outs described.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding. Duration in weeks of exclusive breastfeeding taken into consideration.
Degree of breastfeeding	Fully breastfed in at least 6 weeks.
Outcome	Age specific neurological examination with focus on motor functions. Qualitative appraisal of brain integrity, in which developmental age levels are unimportant. On the basis of examination each toddler was classified as normal, mildly normal, or abnormal. 408 of 418 were classified as neurological normal. Neurological findings were also evaluated in terms of optimality. Criteria for the 57 items of the neurological optimality score at 18 months are given. Quality of movements in terms of fluency
Measurement of outcome	Neurological optimality score (NOS) at 18 months, normal, mildly abnormal, abnormal, fluency cluster score
Statistical analysis	Chi-square, Student's t-test, Mann-Witney U-test used to compare groups. Effect of PCBs and dioxin exposure on the neurological condition investigated by multiple linear regression analysis. Dependant variable were neurological optimality score and the fluency cluster score at 18 months. Independent variables in the regression analysis were the logarithmically transformed PCB and dioxin levels, social, perinatal and obstetrical variables

Reference	**Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1995). Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Hum Dev, 43(2), 165-176.**
	from the obstetric optimality list, and the study centre. P value of .05 or less was considered significant. The final model included education of the father, parity, study centre, and smoking of the father during pregnancy, and sum PCBs measured in cord blood or mothers blood.
Results	No association between lactational exposure to PCBs and dioxins and neurological conditions were found. In contrast, breastfed children had a higher fluency cluster score compared to formula fed children. Neither PCB nor dioxin exposure via breastmilk were associated with the neurological optimality score (regression analysis for the neurological optimality score). After adjusting for co-variates, transplacental PCB exposure was negatively related to the neurological condition at 18 months.
Conclusion	Although greater amounts of PCBs and dioxins are transferred via nursing than via placental transfer, lactational exposure of PCBs was not found to negatively affect the neurological conditions studied. A small beneficial effect of breastfeeding on fluency cluster score was found. Transplacental PCB passage has a small negative effect on the neurological condition at 18 months.
Confounders adjusted for	Education of father, parity, study centre, smoking of the father during pregnancy, sum $PCB_{cord.}$
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Of relevance, but with limitations. Breastfeeding is only included as a (-1)/1 variable while breastfeeding duration appears not to be taken into consideration. However, the study is suitable for assessing the difference between formula feeding and breastfeeding for the outcomes studied.

Summary Table 1-24, Table 11.

Reference	**Jacobson JL, Jacobson SW, Humphrey HE. (1990). Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol, 12(4), 319-326.**
Study design and type	Prospective longitudinal cohort study, Michigan (1980-1981).
Objective	Relationship between PCB exposure and growth and activity in children at 4 years.
Number of participants and country	236 "fish exposure cohort" (children of mothers consuming fish from Lake Michigan) + 87 farm exposure cohort" (children of mothers exposed to polybrominated biphenyl (PBB) contaminated farm products). 231 children (growth outcome) and 265 children (activity outcome) at 4 year follow-up.
Baseline characteristics of study subjects	Children of Lake Michigan fish consumers (women eating more than 11.8 kg trout or salmon from L. Michigan during over a 6 years period; fish eaters constituted 77.3% of participants), randomly selected women not eating such fish (4.6% of participants) and PBB exposed farmers in the Michigan PBB incidence exposed to PBB 1973-76 (18.1 of participants). All were born in 1980-81.
Exposure	PCB, PBB (DDT, HCB betaHCH, oxychlordane, heptachlorepoxide, trans-nonachlor, mirex in 4 years sample. Only HCB (3 cases) and DDT, none of the organochlorine pesticides were detected. Lead at age 4 measured.
Measurement of exposure (Biomarker, internal validation)	Biomarkers (PCB) in cord blood, breastmilk and child serum at 4 years. Total PCB; Packed column electron capture GC, Aroclors 1016 and 1260 used as standards. Prenatal exposure based on cord blood PCB concentration. Postnatal lactation exposure based on breastmilk PCB level and duration of breastfeeding.
Follow-up period, drop-outs	4 years, 25% drop out.
Length of breastfeeding	Mean 29.6 weeks, SD 29.0.
Degree of breastfeeding (fully/partly)	Not given.
Outcome	Growth and activity in children.
Measurement of outcome	Child's weight, height, head circumference at 4 years. McCarthy Scales of Children's abilities at 4 years.
Statistical analysis	Stepwise multiple regression analyses. Toxic effect inferred if effect of exposure was significant ($p<0.05$) after adjusting for confounders. Dose dependence tested by division into tertiles.
Results	Prenatal PCB exposure level was associated with lower weight at age 4 years, but not to height or head circumference. Activity was negatively related to 4-year serum PCB level. Growth was unaltered by postnatal PCB exposure and activity level decreased with increasing postnatal PCB exposure. This was examined by a multivariate analysis of variance based on two PCB levels in breastmilk and three durations of breastfeeding. Effect was infant

Reference	**Jacobson JL, Jacobson SW, Humphrey HE. (1990). Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol, 12(4), 319-326.**
	was breastfed for more than 1 year (15 children).
Conclusion	Effects of *in utero* exposure to PCBs on physical growth persist beyond the foetal and infant period. Growth was unaltered by postnatal PCB exposure and activity level decreased with increasing postnatal PCB exposure.
Confounders adjusted for	25 variables assessed as potential confounders, encompassing 5 domains: socio-environmentals (SES, HOME Inventory), other demographic, perinatal risk, other environmental exposures (PBB, DDT, lead), and situational. Variables that was related to exposure measure at $p<0.10$ was analysed for effect. In addition, maternal and paternal height and weight, and the children's gender were covariates.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	The relevance of this study for our risk assessment is questionable. The number of children that were breastfed more than 12 months was low (n=15). The significance of the negative effects on activity is thus questionable. The PCB exposure level is higher than the present in Europe. The concomitant PBB exposure is treated as a confounder; it is not taken into consideration that the mechanism of action of different PBBs is similar as PCBs (both dl-PCBs and ndl-PCBs).

Summary Table 1-24, Table 12.

Reference	Jensen TK, Grandjean P, Jorgensen EB, White RF, Debes F, Weihe P. (2005). Effects of breast feeding on neuropsychological development in a community with methylmercury exposure from seafood. J Expo Anal Environ Epidemiol, 15(5), 423-430.
Study design and type	Prospective cohort study, Faroe isles cohort 1 (1986-1987).
Objective	Look at significance of methyl mercury exposure from breastmilk in Faroese infants at age 7.
Number of participants and country	1022 infants at birth, 910 children at 7 year follow-up, Faroe Islands.
Baseline characteristics of study subjects	Mothers' frequency of fish and pilot whale consumption, alcohol, smoking during pregnancy. Birth weight and length. Parity, expected term.
Exposure	Mercury.
Measurement of exposure (Biomarker, internal validation)	Mothers' hair Hg at birth of child, umbilical cord Hg, child hair Hg at 1 years. Cord blood PCB concentrations in 435 children examined at age 7.
Follow-up period, drop-outs	7 years. 910 children included in statistical analysis.
Length of breastfeeding	Short: 0-9 weeks (0-4 mostly breastfed), medium: 10-19 weeks (0-4 mostly breastfed), long: mostly breastfed for 20 weeks, very long: weaning after 49 weeks.
Degree of breastfeeding (fully/partly)	Exclusive breastfeeding and any breastfeeding, reported retrospectively.
Outcome	A number of neuropsychological tests; NES finger tapping test, NES Hand Eye Coordination Test, NES Continuous Performance Test, Wechsler Intelligence Scale for Children (WISC-R), Bender visual motor gestalt test, Boston naming test, California verbal learning test.
Measurement of outcome	Neuropsychological tests at age 7 years.
Statistical analysis	Exclusive breastfeeding was entered as a dichotomous variable (0-4 months; 44 months) and total number of months of breastfeeding was entered as a continuous variable in the regression analyses. Multiple linear regression equations were then developed taking into account the effect of potential confounders.
Results	Children who were breastfed longer (both exclusively and in total) performed slightly better on most neuropsychological tests before confounder adjustment. After adjustment with the uniform set of confounders, the positive effect of breastfeeding was reduced, although breastfeed children still

Reference	**Jensen TK, Grandjean P, Jorgensen EB, White RF, Debes F, Weihe P. (2005). Effects of breast feeding on neuropsychological development in a community with methylmercury exposure from seafood. J Expo Anal Environ Epidemiol, 15(5), 423-430.**
	performed slightly better on most tests. Children who were breastfed longer had a significantly higher hair Hg concentration at age 1 year.
Conclusion	Breastfeeding was not associated with reduction in neuropsychological performance at age 7. On the other hand, breastfed children did not perform significantly better, as observed in previous studies in populations less exposed to Hg contamination.
Confounders adjusted for	A uniform set of confounders previously identified (Grandjean *et al*., 1997) was used in all analyses to ensure that related test outcomes were adjusted for the same covariates. These were age (continuous variable) and sex of the child (boy; girl), child in daycare (yes; no), medical risk factors of the child for neurobehavioral dysfunction eg. '(yes; no), maternal Raven score (continuous variable), professional training of each parent (yes; no), paternal employment at time of examination (yes; no), and for the computer-assisted NES tests familiarity with computers (yes; some and none).
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Relevant.

Summary Table 1-24, Table 13.

Reference	**Jusko TA, Sonneborn D, Palkovicova L, Kocan A, Drobna B, Trnovec T, Hertz-Picciotto I. (2012). Pre- and postnatal polychlorinated biphenyl concentrations and longitudinal measures of thymus volume in infants. Environ Health Perspect, 120(4), 595-600.**
Study design and type	Prospective cohort study (2002-2004).
Objective	Assess whether thymus volume at later ages is influenced by prenatal and early postnatal PCB exposure.
Number of participants and country	1134 women enrolled, 971 infants at 6 months follow-up, and 887 infants at 16 months, Slovakia.
Baseline characteristics of study subjects	811 women recruited in Michalovce, region with substantial environmental contamination, and 323 mothers in Svidnik, region with less environmental contamination and lower serum PCBs than in Michalovce. Exclusion criteria described. Characteristics of infant and mothers well described.
Exposure	PCBs.
Measurement of exposure (Biomarker, internal validation)	15 PCB congeners determined in maternal, and 6- and 16 month serum samples. Gc-ec analysis of CB-28, 52, 101, 105, 114, 118, 123+149, 138+163, 153, 156+171, 157, 167, 170, 180 and 189. Serum PCBs determined in 1104 mothers. At age 6 months, 249 infant samples were selected for analysis based on their corresponding maternal PCB concentrations. 6 months infant samples were randomly sampled within strata defined by maternal PCB concentrations: a) <75 percentile, b) between 75 and 85 percentiles, c) between 85 and 95 percentiles, and d) >95 percentile. At 16 months of age, 831 infant serum specimens were analysed.
Follow-up period, drop-outs	Follow-up of children at 6 and 16 months of age. 971 (86%) mother-infant pairs still participating at age 6 months, and 887 (78%) were still participating at 16 months of age.
Length of breastfeeding	Duration of exclusive breastfeeding values at birth set to zero, and values at 6 and 16 months of age corresponded to the duration of exclusive breastfeeding up until that point in time.
Degree of breastfeeding (fully/partly)	Months of exclusive breastfeeding 0 to <3 months (n=495, 47%), 3-6 months (n=543, 51%) and >6 months (n=18, 2%). Partial breastfeeding was not included.
Outcome	Thymus volume.
Measurement of outcome	Thymus volume measured using a sonographic scanner by radiologists unaware of maternal and infant PCB concentrations. A trans sternal approach used to measure the maximal transverse diameter (width) of thymus, and in the plane perpendicular to this width, the largest sagittal area (longitudinal scan plan) was also measured. These 2 measurements were multiplied to obtain a “thymic index”, a proxy for thymus volume. Thymus volume measured in 1047 newborns, 940 children 6 months of age, and 820 children 16 months of age, for whom 1020 (95%), 241 (26%), and 806 (98%) had PCB measurements.
Statistical analysis	Maternal and infant PCB congeners selected for inclusion in the statistical method if at least 80% of measurements were above LOD. When individual

Reference	**Jusko TA, Sonneborn D, Palkovicova L, Kocan A, Drobna B, Trnovec T, Hertz-Picciotto I. (2012). Pre- and postnatal polychlorinated biphenyl concentrations and longitudinal measures of thymus volume in infants. Environ Health Perspect, 120(4), 595-600.**
	congener value below LOD, LOD was divided by the square root of 2. The sum PCB variable was the arithmetic sum of 138+163, 153, 170 and 180. A mixed multivariate method was used. Repeated measure models were fitted including a repeated measure of standardised thymus volume at birth, 6- and 16 months of age as dependent variable. Several sets of models were used. One set of models focused on maternal PCB concentrations in relation to all three thymus measurements; the other examined time-varying PCB exposure based on maternal and 6- and 16 month infant PCB concentrations as exposures of interest. Continuous PCB concentrations were modelled as natural log values to reduce influence of extreme values. Linear term adequately modelled PCB-thymus volume association. To allow for age to modify the association between PCB and thymus volume, PCB x age, where age was categorical variable (0, 6, 16 months, corresponding to the month of assessment) was included. Acyclic graphs used to select covariates for the model. Covariates included ethnicity (Romani/others), infant sex, district of residence, infant weight, and duration of exclusive breastfeeding (months). Duration of exclusive breastfeeding was parameterised as a time-varying covariate and included an interaction with categorical time so that separate effects could be measured at 6 and 16 months of age. Since only a portion (n=249) of 6-month infant PCB samples were analysed, and the samples analysed were selected based on maternal PCB concentration, sampling weight were added for the 6-monthtime point. Two sensitivity analyses were performed to evaluate the role of exclusive breastfeeding on PCB concentrations and thymus development. The statistics are well described.
Results	Higher maternal PCB concentration was associated with reduced thymus volume at birth (a 0.21 SD reduction in thymus volume for an increase in total maternal PCB concentration from the 10 to the 90 percentile; 95% confidence interval (CI: -0.37, -0.05), whereas maternal concentration was not predictive of 6- and 16 month thymus volume. Six- month infant PCB concentration was associated with a 0.40 SD decrease in 6-month thymus volume (95%; CI: -0.76, -0.04), and the association was weaker when exclusive breastfeeding was not adjusted for. An indication of positive association between 16 month infant PCB concentration and thymus volume was also seen. Since thymus volume peaks at 6-8 months age and then shrinks, the authors speculate that this could be related to delayed thymus maturation.
Conclusion	The potential adverse effect of *in utero* PCB exposure on thymic development may extend beyond the neonatal period. Postnatal PCB exposure was influential, however, limited statistical power at 6 month of age. The cohort was highly exposed to PCBs.
Confounders adjusted for	Models were adjusted for ethnicity (Romani/others), sex, district of residence, infant weight, and duration of exclusive breastfeeding (months). 6- and 16 month PCB models were fitted without adjustment for breastfeeding to evaluate the role of confounding. Potential heterogeneity by exclusive breastfeeding duration at the 6- and 16 month time points was also examined.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Relevant.

Summary Table 1-24, Table 14.

Reference	**Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, van der Paauw CG, Tuinstra LG, Sauer PJ. (1996). Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics, 97(5), 700-706.**
Study design and type	Prospective longitudinal cohort study, Dutch cohort (1990-1992).
Objective	Effects of prenatal and postnatal exposure to PCBs and dioxins, and effects of breastfeeding vs. formula feeding on mental and psychomotor development.
Number of participants and country	207 infants at birth (105 breastfed and 102 formula fed), 182 at 7 month follow-up, The Netherlands.
Baseline characteristics of study subjects	Pregnancy women from the Rotterdam area; selection criteria. Parity (first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used.
Exposure	PCBs and dioxins.
Measurement of exposure (Biomarker, internal validation)	Measure of prenatal PCB exposure in the total study population**:** PCB plasma sum (118, 138, 153, 180) (gc-ec) in mothers blood (last month of pregnancy, 36-40th week) and umbilical cord blood. The breastmilk samples from the breastfeeding group (24 hour sample within 2 weeks postpartum) were used as indirect measures for prenatal exposures to PCBs (20 congeners plus 6 dioxin-like PCBs and dioxins (17) (gc-ms). Measure of postnatal exposure to PCBs and dioxins through lactation was estimated in the breastfeeding group: multiplication of the PCB-milk-sum, respectively, the total PCB-dioxin TEQ level in milk, and the duration of breastfeeding. Same measurements (118, 138, 153, 180) (gc-ec) from formula fed group. Information was given on missing samples.
Follow-up period, drop-outs	3, 7 and 18 months. Number of drop-out given; less than 4% at 3 months, appr. 5% at 18 months.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding.
Degree of breastfeeding (fully/partly)	Fully breastfed in at least 6 weeks.
Outcome	Neurodevelopment, mental scores and psychomotor scores.
Measurement of outcome	The Dutch standardised version of Bayley Scales of infant development (MDI and PDI), the BOS 2-30, at 3, 7 and 18 months of age. All Bayley tests were performed at infant's home by one examiner unaware of PCB and dioxin exposure of infant, and one parent present.
Statistical analysis	Multiple regression analysis, Statistical package for the social sciences (SPSS/PC, Cary, NC), used to study effects of prenatal and postnatal exposure to PCBs and dioxins separately and combined. Since it is assumed that that breastfeeding per se has positive effects on development of children, the amount of breastfeeding received was studied separately. This variable "duration of breastfeeding in weeks" was divided into 3 categories (zero

Reference	**Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, van der Paauw CG, Tuinstra LG, Sauer PJ. (1996). Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics, 97(5), 700-706.**
	(formula fed, short (6-10 weeks) and long 11-12 weeks) at 3 months, and zero, 6-16 weeks, and >16 weeks at 7 and 18 months, respectively). In the multiple regression analysis this categorical variable was entered as a continuous independent variable with a value 0, 1 and 2.
Results	Higher *in utero* PCB exposure was related to lower psychomotor scores at 3 months of age: a doubling of PCB exposure resulted in a decrease of 3 points. Breastfed infants scored significantly higher on the psychomotor score at 7 months of age compared with formula fed. However, when corrected for confounders, the psychomotor score for the 66% highest exposed breastfed children (>756 pg total PCB-dioxin TEQs) was negatively influenced by this postnatal exposure of PCBs and dioxins, and was comparable with formula fed infants. Breastfed infants also scored higher on the mental scale at 7 months in a dose dependent way. No significant influence of the perinatal PCB and dioxin exposure on mental outcome at 3 and 7 months. At 18 month neither the mental nor the psychomotor score was related to perinatal PCB or dioxin exposure, nor to the duration of breastfeeding.
Conclusion	Prenatal PCB exposure had a small negative effect on psychomotor score at 3 months. PCB and dioxin through breastfeeding had adverse effect on psychomotor outcome at 7 months. The mental outcome at 7 months positively influenced by breastmilk per se; the perinatal exposure to PCBs and dioxins does not influence this outcome. At 18 months development is affected neither by PCB nor dioxin exposure nor by feeding type.
Confounders adjusted for	The socioeconomic, obstetric, and neonatal conditions were assessed by means of obstetrical optimality scale. The following confounders were identified by univariate analyses: level of education of parents, (high 1) at least secondary education completed; or low (0), profession categorised into 3, maternal smoking, alcohol use (yes or no), parity, duration of gestation, birth weight, Apgar score after 1 and 5 min, sex of infant, and duration of breastfeeding (in weeks). Thyroid hormones measured in maternal serum during last months of pregnancy, in cord plasma and in infants\ plasma in the second week. HOME inventory examined at the age of 18 months. Confounders adjusted for included gestational age, parity, HOME score (at 18 months), maternal education, which were correlated at a level $p<0.10$, with at least one of the dependent variables, and which were entered into the multiple regression analysis as independent variables. The other possible confounders were not significantly related to the exposure levels.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Good. The study differentiated between pre- and postnatal exposure and both breastfeeding duration and calculated exposure were included as variables. HOME score and other relevant confounders were adjusted for.

Summary Table 1-24, Table 15.

Reference	**Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1998). Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev, 50(3), 283-292.**
Study design and type	Prospective follow-up cohort study, Dutch cohort (1990-1992).
Objective	Relationship between prenatal exposure to PCBs and lactational exposure to PCBs and dioxins and the neurological condition at 42 months.
Number of participants and country	418 infants at birth (half of the infants were breastfed and half formula fed), 394 children at 42 month follow-up, The Netherlands.
Baseline characteristics of study subjects	Pregnancy women from Groningen and Rotterdam, June 1990-June 1992. Selection criteria: parity(first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used.
Exposure	Dioxins and PCBs.
Measurement of exposure (Biomarker, internal validation)	Measure of prenatal PCB exposure in the total study population**:** sum PCB (118, 138, 153, 180) (gc-ec) in mothers blood and umbilical cord blood. In order to assess the lactational exposure, breastmilk (24 hr sample) was collected in the 6th week postpartum. The breastmilk samples were analysed for PCBs (23 congeners plus 3 dioxin-like PCBs) and dioxins (17) (gc-ms). In addition child`s blood sampled at 42 months. Info given on missing samples.
Follow-up period, drop-outs	42 month, drop-outs described.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding
Degree of breastfeeding (fully/partly)	Fully breastfed in at least in 6 weeks.
Outcome	Neurological examination with focus on motor functions at 42 months
Measurement of outcome	All children underwent a neurological examination 2nd week after birth according to Prechtl. At 42 months, the children were neurologically examined according to Touwen/Hempel. In contrast to other developmental tests (e.g. cognitive tests) which are quantitative measures for the child`s neurological abilities, the neurological exam is used for the qualitative appraisal of brain function. Neurological findings were also evaluated in terms of optimality. Criteria for the 57 items of the neurological optimality score at 42 months are described. The neurological examinations were performed by 2 researchers, one at each centre. The study centre was included as an explanatory variable. The obstetrical, socioeconomic, pre-, intra-, and immediate postpartum conditions recorded by means of a 72-items questionnaire (obstetrical optimality score).
Statistical analysis	Chi-square, Student's t-test, Mann-Witney U-test used to compare groups. Effect of PCBs and dioxin exposure on the neurological condition was investigated by multiple linear regression analysis. Dependant variable were neurological optimality score (NOS) and the fluency cluster score at 18

Reference	**Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC. (1998). Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Hum Dev, 50(3), 283-292.**
	months. Independent variables in the regression analysis were the logarithmically transformed PCB and dioxin levels, the type of feeding in early life, duration of breastfeeding, social, perinatal and obstetricial variables from the obstetric optimality list, and the study centre. P value of .05 or less considered significant. The final model consisted of the study centre and the obstetrical optimality score. The statistical analyses are well described.
Results	After adjustment for covariates, neither prenatal PCB exposure nor postnatal exposure to PCBs and dioxins was found to be related to the neurological condition at 42 months of age.
Conclusion	Neither prenatal PCB exposure, nor postnatal exposure to PCBs and dioxins were found to be related to the neurological condition (monofunctions) at 42 months of age.
Confounders adjusted for	A number of relevant possible confounders were taken into consideration.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Good. The independent variables included PCB and dioxin levels in plasma and milk, breastfeeding or not breastfeeding during early life, duration of breastfeeding as well as socioeconomic-, obstetrical-, and perinatal- conditions, and the obstetrical optimality score. $p<0.05$.

Summary Table 1-24, Table 16.

Reference	**Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr, 134(1), 33-41.**
Study design and type	Prospective follow-up cohort study, Dutch cohort (1990-1992).
Objective	To study possible adverse effects of environmental exposure to PCBs and dioxins on cognitive functioning in young children.
Number of participants and country	418 infants at birth (half of the infants were breastfed and half formula fed), 395 children at 42 month follow-up (appr. 9% dropout since recruitment), The Netherlands.
Baseline characteristics of study subjects	Pregnancy women from Groningen and Rotterdam, June 1990-June 1992. Selection criteria: parity (first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used. The child`s home environment was assessed by a Dutch version of HOME score. The verbal IQ of parent, assessed by 2 described subtests.
Exposure	Dioxins and PCBs.
Measurement of exposure (Biomarker, internal validation)	Prenatal PCB exposure in total study population**:** sum PCB (118, 138, 153, 180) (gc-ec) in mothers plasma and umbilical cord blood. Lactational exposure: PCB and dioxin levels (23 congeners plus 3 dioxin-like PCBs) and dioxins (17) (gc-ms) in breastmilk (24 hr sample collected in the 6th week postpartum) multiplied by number of weeks of breastfeeding. Current PCB body burden was estimated from PCBs in 43-months-old plasma samples. Info given on missing samples. PCB and dioxin levels (TEQs) were indirect measure of prenatal exposure.
Follow-up period, drop-outs	42 month, drop-outs described.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding.
Degree of breastfeeding (fully/partly)	Fully breastfed in at least 6 weeks.
Outcome	Neurodevelopment; cognitive abilities at 42 months.
Measurement of outcome	Cognitive abilities assessed with Kaufmann Assessment battery for Children in 395 42 month old children. In a subgroup (n=193) verbal comprehension assessed with Reynell Language Development Scales.
Statistical analysis	Chi-square, Student's t-test, Mann-Witney U-test used to compare groups. Effect of prenatal, lactational, and current exposure to PCBs and dioxins on cognitive abilities at 42 months of age was investigated by multiple linear regression adjusted for covariables. Dependent variables were scores on the overall cognitive scale, the sequential and simultaneous processing scale of the K-ABC, and the verbal comprehension scale of the RDLS. Each

Reference	**Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr, 134(1), 33-41.**
	outcome variable was analysed with each exposure variable separately and in a regression analysis. Covariables entered in the final regression analyses were selected from variables known from literature and clinical knowledge to have an effect on developmental outcome.
Results	After adjustment for covariates, maternal PCBs was associated with lower scores on the overall cognitive and sequential and simultaneous processing scales of the Kaufmann Assessment Battery for children (all p<.05). The highest exposed group (PCBs >3 µg/l) scored 4 points lower on all 3 scales of the K-ABC when compared with the lowest exposed group (PCBs<1.5 µg/l). Neither lactational exposure nor current exposure to PCBs and dioxin were related to 42-months cognitive performance.
Conclusion	No associations between lactational exposure to PCBs and dioxins nor current PCB body burden and cognitive abilities at 42 months of age were found. *In utero* exposure to "background" PCB levels was associated with poorer cognitive functioning in preschool children. Children of mothers at the upper end of exposure especially at risk.
Confounders adjusted for	Covariables entered in the final regression model were: maternal age at birth, parity, sex, feeding type during infancy, breastfeeding period in weeks, HOME score, paternal and maternal education, parental verbal IQ score, smoking and alcohol use during pregnancy, and the study centre/examiner (Groeningen and Rotterdam). The effect of prenatal exposure was measured in the whole group, the formula fed and the breastfed groups. In addition the effect of prenatal exposure to TEQs and non-DL PCBs from breastmilk was studied in the breastfeeding group. The effect of lactational exposure to PCB- and dioxin TEQs, PCBs and non-DL-PCBs was also studied in the breastfeeding group. The effect of 42 months PCB body burden on cognitive abilities was investigated in the whole group and both feeding groups separately. Significant results p<0.05.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Good. The study differentiates between possible effects of prenatal, postnatal- (through milk exposure) and current PCB exposure on studied outcome. Relevant confounders were adjusted for.

Summary Table 1-24, Table 17.

Reference	Rogan WJ, Gladen BC. (1991). PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol, 1(5), 407-413.
Study design and type	Prospective cohort study, North Carolina (1978-82).
Objective	To determine whether prenatal and postnatal exposure to "background" levels of PCBs and DDE affected child development through the age of 2 years..
Number of participants and country	858 infants (later referred to as 856) at birth, 676 at 18 month follow-up and 670 children at 24 month follow-up, USA.
Baseline characteristics of study subjects	No eligibility criteria, random cohort.
Exposure	PCBs and DDT/DDE
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure: Composite average of all measurement available in either milk, maternal serum, cord blood and/or placenta. Scaled to one measure (comparable to milk). Average taken of all. Postnatal exposure: LEM: average concentration * duration of breastfeeding.
Follow-up period, drop-outs	18 and 24 months.
Length of breastfeeding	For examining transplacental effects 712 children (with Bayley score) were grouped into bottle-fed (10.8%) and breastfed, according to duration. Short duration (10.5%): 0-4 weeks mostly breastfed, weaning at 9 weeks; medium duration (19.7%): 0-4 weeks mostly breastfed, weaning after 9 weeks or mostly breastfed for 5-19 weeks and weaned at 19 weeks; long duration (35.8%): mostly breastfed for 5-19 weeks and weaned after 19 weeks or mostly breastfed for 20 weeks or longer and weaned by 49 weeks, and very long duration (23.2%): those mostly breastfed for 20 weeks or longer and weaned after 49 weeks. , very long: weaning after 49 weeks.
Degree of breastfeeding (fully/partly)	Mostly and partly breastfed.
Outcome	Neurodevelopment; mental and psychomotor
Measurement of outcome	Bayley two subscales: the psychomotor development index, PDI and the mental development index, MDI at 18 months and 24 months.
Statistical analysis	Linear regression.
Results	At 18 and 24 months, adjusted scores on PDI were 4-9 points lower among children in the top fifth percentile of transplacental PCB exposure, significant only at 24 months. Thus, psychomotor delay up to 2 years of age associated with the highest 5% of transplancental exposure to PCBs. No

Reference	**Rogan WJ, Gladen BC. (1991). PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol, 1(5), 407-413.**
	effect on MDI. No consistent association between exposure to PCBs or DDE through breastmilk and outcomes (MDI and PDI).
Conclusion	Prenatal: Effects on psycho motoric development, stronger with age, only after threshold >3.4ppm. Postnatal: Effects small and non-significant and not shown.
Confounders adjusted for	Prenatal effects: Breastfeeding in 4 categories, sex, race, actual age, older sibs, maternal age, education, occupation, smoking, alcohol, examinator. Postnatal effects; adjusted for breastfeeding in weeks breastfed (and breastfeeding is included in postnatal exposure assessment). Not adjusted for HOME score or maternal IQ. HOME score probably less relevant at such a young age.
Quality (A, B or C)	B (because of lack of HOME score and IQ).
Relevance for our risk assessment purpose	Relevant.

Summary Table 1-24, Table 18.

Reference	**Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. (2006). Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clin Exp Allergy, 36(10), 1236-1241.**
Study design and type	Mother-child pregnancy cohort study, Menorca (1997-1999).
Objective	Study association between DDE and asthma and effect modification by breastfeeding.
Number of participants and country	482 infants at birth, 462 children at 6 year follow-up, Spain.
Baseline characteristics of study subjects	No eligibility criteria, all recruited. Participation rate not given (probably not 100%), e.g. characteristics associated with participation not given either. 202 were breastfed, 43 not breastfed, 40 missing information on breastfeeding (among those with DDE measured at 4 years).
Exposure	DDE and DDT.
Measurement of exposure (Biomarker, internal validation)	Prenatal: Cord blood. Postnatal: Child serum at 4 years.
Follow-up period, drop-outs	4 and 6 years DDE in cord of 402 (83%) and in blood of 285 children at 4 years.
Length of breastfeeding	Zero, 0-20 weeks and >20 weeks. 0=70, 0-20 166, >20 166, i.e. median duration 20 weeks=5 months exclusive breastfed. 17% never breastfed.
Degree of breastfeeding (fully/partly)	Repeatedly interviewed with regard to breastfeeding, 6 and 14 months, 2 years of age and reproducible results. Only asked for duration of exclusive breastfeeding.
Outcome	Asthma and wheezing.
Measurement of outcome	Parentally reported wheeze in interviews: a) at any age or b) “persistent wheeze” if present at 6.5 years + one preceding year or c) doctor-diagnosed asthma. Defined atopy by SPT at 6 years.
Statistical analysis	Multivariable, logistic regression. Generalised estimating equations (GEE) with unstructured correlation matrix to assess effect of DDE on wheezing each year adjusted for wheezing previous years. GAM modelling for dose-response. Interaction terms: year and DDE, Stratification by child atopy and interaction with duration of breastfeeding studied.
Results	Prenatal exposure DDE: Association with asthma. 2-fold increased risk of asthma or persistent wheeze at 6.5 years (Per IQR 1.91). Prenatal DDT: No association. Breastfeeding protected against diagnosed asthma (OR=0.33, 95% CI=0.08-0.87) and wheezing (OR=0.53, 95% CI=0.34-0.82) in children with low

Reference	**Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. (2006). Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clin Exp Allergy, 36(10), 1236-1241.**
	and high DDE levels at birth. Postnatal exposure as assessed by child levels at 4: No association with asthma/wheeze. No effect modification by breastfeeding. No effect modification by atopy (if anything, more among non-atopic). Breastfeeding had a protective effect on asthma.
Conclusion	Prenatal effects of DDE on risk of asthma and wheeze at 6 years. No postnatal effects. Postnatal exposure to DDE/DDT as assessed by child levels at 4: No association with asthma/wheeze. No modification by breastfeeding. No modification by atopy (if anything, more among non-atopic). Breastfeeding had a protective association with asthma. Diagnosed asthma and wheezing at 6 years were associated with DDE at birth indicating an impact of prenatal effect.
Confounders adjusted for	Maternal asthma, atopy, smoking, education, BMI, family size, birth weight, gestational age, child BMI, parity, child gender.
Quality (A, B or C)	B (Lacking information on total duration of breastfeeding, but since levels are measured directly in the children not important). Outcome not objectively assessed, but report bias not likely. Advanced statistics.
Relevance for our risk assessment purpose	Yes

Summary Table 1-24, Table 19.

Reference	**Verner MA, Plusquellec P, Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL, Charbonneau M, Haddad S. (2010). Alteration of infant attention and activity by polychlorinated biphenyls: unravelling critical windows of susceptibility using physiologically based pharmacokinetic modeling. Neurotoxicology, 31(5), 424-431.**
Study design and type	Prospective longitudinal birth cohort, Nuanavik (1995-98).
Objective	To examine time-specific associations between simulated PCB-153 levels and indicators of infant behavioural function in an Inuit population.
Number of participants and country	Canada. 333 infants at birth, 168 infants at 11 month follow-up, Canada.
Baseline characteristics of study subjects	333 Inuit women from three villages on the Hudson Bay, enrolled at first or second prenatal medical examination between 1995 and 2002. Exclusion criteria were participation with previous child, loss to follow-up, refusal to participate, neonatal death, failure to obtain biological sample, and relocation to another village.
Exposure	PCB-153 as surrogate for environmental mixture.
Measurement of exposure (Biomarker, internal validation)	PCB-153 in maternal blood drawn at delivery or within a few weeks postpartum. PCB 153, DDE, lead, Hg and DHA/AA in cord blood in subset of 83-85 children. Infant blood PCB-153 profiles were estimated using a previously validated (at 6 months) PBPK modeling framework (Verner et al., 2009) based on the mothers' pre-pregnancy weight and height, age at delivery, blood PCB-153 level, date of blood sampling, duration of exclusive breastfeeding, as well as infants' weight and height at delivery and 6 and 11 months. Area under the curve (AUC) of infant blood PCB-153 level was calculated for each month of life.
Follow-up period, drop-outs	11 months.
Length of breastfeeding	Length of exclusive breastfeeding was recorded, mean 155.9 days (range 0-466).
Degree of breastfeeding (fully/partly)	Not clear if partly breastfeeding was recorded, exclusive breastfeeding was included in the PBPK.
Outcome	Infant behaviour.
Measurement of outcome	Behaviour rating scales (BRS) of Bayley Scales of infants, BSID-II at 11 months of age. Video coding of inattention and activity measured during the administration of mental developmental subscale of BSID-II.
Statistical analysis	Association between log estimated month-by-month area under the curve PCB 153 (month 1-11) concentration in blood or estimates of cord blood PCB-153 levels (month 0) and behavioural measures at 11 months were assessed by multiple linear regression models. Potential confounders that were related to the dependent variable at a p value <0.20 were entered stepwise. At the first step each estimate of infant PCB-153 level for a given period was entered and the following order was determined by the strength of the correlation. Control variables were retained in the model if their inclusion

Reference	**Verner MA, Plusquellec P, Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL, Charbonneau M, Haddad S. (2010). Alteration of infant attention and activity by polychlorinated biphenyls: unravelling critical windows of susceptibility using physiologically based pharmacokinetic modeling. Neurotoxicology, 31(5), 424-431.**
	altered the exposure-effect association at step of entry by >10%.
Results	The median maximal predicted concentration in child's blood was 241 (range 25-2142) ng/g lipids. Cord blood PCB-153 level was simulated in the subset which was missing this information to provide a surrogate measure of prenatal exposure. Median simulated cord blood PCB-153 was 103 (range 15-706) ng/g lipids whereas median measured cord blood PCB-153 was 76 (range 16-551) ng/g lipids. The correlation between cord blood PCB-153 and postnatal blood PCB-153 decreased by age. Whereas inattention was related to prenatal exposure, activity level, measured by non-elicited activity, was best predicted by postnatal exposure, with the strongest association obtained for simulated PCB levels during the 4th month of life.
Conclusion	Inattention was related to prenatal PCB-153 exposure. Increased non-elicited activity was associated with postnatal exposure, with the strongest association obtained for simulated PCB levels during the 4th month of life.
Confounders adjusted for	Numerous tested, and included as described in statistical analysis. Different control variables were included for different time periods.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Highly relevant, because postnatal PCB-153 exposure has been predicted by a PBPK and associations between outcomes and prenatal and postnatal exposure can be separated.

Summary Table 1-24, Table 20.

Reference	**Vreugdenhil HJ, Slijper FM, Mulder PG, Weisglas-Kuperus N. (2002). Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age. Environ Health Perspect, 110(10), A593-A598.**
Study design and type	Prospective follow-up cohort study, Dutch cohort (1990-1992).
Objective	Evaluate whether effects of prenatal and postnatal exposure to environmental levels of PCBs and dioxins on play behaviour of school children
Number of participants and country	207 infants at birth (105 breastfed and 102 formula fed), 158 children at 7 year follow-up). The Netherlands.
Baseline characteristics of study subjects	Pregnancy women from the Rotterdam area 1990-1992; selection criteria: parity (first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used.
Exposure	PCBs and dioxins.
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure in the total study population: PCBs sum (118, 138, 153, 180) (gc-ec) in mother`s plasma (last month of pregnancy, 36-40th week) and umbilical cord blood. The breastmilk samples from the breastfeeding group (24 hour sample within 2 weeks postpartum) were used as indirect measures for prenatal exposures to PCBs (20 congeners plus 6 dioxin-like PCBs and dioxins (17) TEQs) (gc-ms). Postnatal exposure estimated in the breastfeeding group: multiplying, respectively, breastmilk levels of TEQs, and the PCB sums ((sum of 118, 138, 153 and 180) and the 20 non-dioxin-like PCBs with the number of weeks of breastfeeding. Same measurements (118, 138, 153, 180) (gc-ec) from formula fed group. Information was given on missing samples.
Follow-up period, drop-outs	7.5 years.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding.
Degree of breastfeeding (fully/partly)	Fully breastfed in at least 6 weeks.
Outcome	Assessment of play behaviour (neurodevelopment) scored on 3 subscales: masculine, feminine and composite.
Measurement of outcome	Parents asked to fill in the Dutch version of Pre-school activities inventory (PSAI) (PSAI discriminate play behaviour between and within sexes) and a questionnaire on problem behaviour and a questionnaire on health at school age.
Statistical analysis	To compare groups for a single variable, Student`s t test (for continuous variables), the chi-square test (for categorical variables), or the Mann.Witney U test was used. Multiple regression analysis (Statistical package for the social sciences SPSS version 9, Chicago, IL.) was used to study effects of prenatal and postnatal exposure to PCBs and dioxins on play behaviour scales. Variables likely to affect play behaviour were included in the regression model as a fixed set of variables. These variables were: sex, highest education of either parent, parental verbal IQ, type of feeding during infancy

Reference	**Vreugdenhil HJ, Slijper FM, Mulder PG, Weisglas-Kuperus N. (2002). Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age. Environ Health Perspect, 110(10), A593-A598.**
	(breastfed or formula fed), duration of breastfeeding, HOME score, and assessment age. Statistic model is well described.
Results	Difference between boys and girls $p<0.05$. In boys, higher prenatal PCB exposure was related with less masculinised play, assessed by the masculine scale. In girls higher prenatal PCB levels were associated with more masculinised play, assessed by the composite scale. Higher prenatal dioxin levels associated with more feminised play, assessed by the feminine scale, by both boys and girls. These effects were not measureable in children raised in more optimal conditions. No association of postnatal exposure with play or problem behaviour was shown at school age.
Conclusion	Neurotoxic effects of prenatal PCB and dioxin exposure may persist into school age, resulting in a subtle cognitive and motor development. Postnatal exposure through lactation was not related to play behaviour.
Confounders adjusted for	Candidate confounders were: alcohol and smoking during pregnancy, duration of gestation, birth weight, maternal age at birth, and parity. Confounders adjusted for were: sex, parental education level, parental verbal IQ, feeding type, duration of breastfeeding, HOME score, age at assessment, parity.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Good. The study differentiates between pre-and postnatal exposure and both breastfeeding duration and calculated exposure were included as variables. HOME score and other variable confounders were adjusted for.

Summary Table 1-24, Table 21.

Reference	**Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. (2002). Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. J Pediatr, 140(1), 48-56.**
Study design and type	Prospective follow-up cohort study, Dutch cohort (1990-1992).
Objective	Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school; follow-up study of the Dutch cohort (earlier studies Huisman et al. 1995, Huisman et al. 1995, Koopman-Esseboom 1996, Patandin et al. 1999).
Number of participants and country	418 infants at birth, 372 at 6 year follow-up, The Netherlands.(187 from Groningen and l from Rotterdam;
Baseline characteristics of study subjects	Healthy mother-infant pairs from the Rotterdam and Groningen area 1990-1992; study design described elsewhere : selection criteria: parity(first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, full breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used. Characteristics of participating and non-participating children at school age described.
Exposure	PCBs and dioxins.
Measurement of exposure (Biomarker, internal validation)	Prenatal exposure in the total study population: PCBs sum (118, 138, 153, 180) (gc-ec) in mother`s plasma (last month of pregnancy, 36-40th week) and umbilical cord blood. The breastmilk samples from the breastfeeding group (24 hour sample within 2 weeks postpartum) were used as indirect measures for prenatal exposures to PCBs (20 congeners plus 6 dioxin-like PCBs and dioxins (17) TEQs) (gc-ms). Postnatal exposure estimated in the breastfeeding group: multiplying, respectively, breastmilk levels of TEQs, and the PCB sums ((sum of 118, 138, 153 and 180) and the 20 non-dioxin-like PCBs with the number of weeks of breastfeeding. Same measurements (118, 138, 153, 180) (gc-ec) from formula fed group. Information was given on missing samples.
Follow-up period, drop-outs	6.5 years. Dropout 10%. Reason for drop-out described.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding.
Degree of breastfeeding (fully/partly)	Fully breastfed in at least in 6 weeks.
Outcome	Neurodevelopment; cognitive and motor abilities.
Measurement of outcome	The Dutch version of McCarthy's Scales of children`s abilities (GCI, memory and motor) was used to assess cognitive and motor abilities at 6.5 years. Questionnaire addressing obstetric, social, economic and perinatal conditions, HOME test to assess the intellectual support and stimulation provided by the child`s home environment, verbal IQ of parents measured by two subtests.

Statistical analysis	Multiple regression, Statistical package for the social sciences (SPSS version 9, Chicago, IL.) to study effects of prenatal and postnatal exposure to PCBs and dioxins separately and combined. Variables likely to affect neurodevelopment included in the regression model included birth wt, duration of breastfeeding, foetal exposure to alcohol, and cigarette smoking, maternal age at birth, parental education level and parity (items on a questionnaire), type of feeding during infancy, duration of breastfeeding and infant sex (see confounders). Statistic model well described.
Results	Prenatal PCB exposure was related to poorer neurological condition at birth and at 18 months of age, lower psychomotor abilities at 3 month and lower cognitive abilities at 42 months of age (earlier studies). Prenatal PCB levels were not related to General cognitive index (GCI), memory and motor skills after adjustment for co- variables at 6.5 years of age. Effects of prenatal exposure on the GCI, memory and motor scores were not significantly different for breastfed and formula fed children. In the 2 feeding groups separately, prenatal PCB exposure was not related to GCI or memory skills. In formula fed children, however, higher maternal PCB levels tended to be related to lower motor scores. Formula fed group parental and home characteristics were less optimal compared to breastfed group. Present results give evidence for effect modifications by parental and home environmental conditions in the total cohort. Postnatal exposure to PCBs/dioxins through lactation not significantly related to GCI, memory, and motor scores and effects of postnatal exposure were not significantly modified by parental and home environmental characteristics.
Conclusion	Neurotoxic effects of prenatal exposure to PCB and dioxins persist into school age, resulting in subtle cognitive and motor developmental delays. More optimal intellectual stimulation provided by a more advantageous parental and home environment may counteract these effects of prenatal exposure to PCBs and dioxins. Postnatal exposure to PCBs and dioxins through lactation was not related to cognitive and motor abilities at school age.
Confounders adjusted for	Candidate confounders were among others: level of education of parents, (high 1) at least secondary education completed; or low (0), profession categorised into 3, maternal smoking, alcohol use (yes or no), parity, duration of gestation, birth weight, Apgar score after 1 and 5 min, sex of infant, duration of breastfeeding (in weeks), verbal IQ of parent that spend the most time with the child. Thyroid hormones measured in maternal serum during last months of pregnancy, in cord plasma and in infants\ plasma in the second week. HOME inventory examined at the age of 7 years. Confounders adjusted for were: sex, parental education level, parental verbal IQ, feeding type, duration of breastfeeding, HOME score, age at assessment, parity.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Good. The study differentiates between pre- and postnatal exposure and include breastfeeding as covariate. HOME score and other relevant confounders have been adjusted for.

Summary Table 1-24, Table 22.

Reference	**Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, Steingruber HJ, Wundram S, Winneke G. (2001). Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet, 358(9293), 1602-1607.**
Study design and type	Prospective cohort study (1993-1995).
Objective	Establish whether adverse effects of PCBs on mental and motor development in early childhood is of only prenatal or additional postnatal origin, and if a favourable home environment can counteract this effect.
Number of participants and country	171 healthy mother-infant pairs at birth, 91 children at 42 month follow-up, Germany.
Baseline characteristics of study subjects	Mother-infant pairs recruited from Düsseldorf hospitals 1993-1995. Selection criteria: parity (first or second born), 37-42 weeks gestation, an Apgar score at 5 min of at least 7-8, native German families, no serious illnesses or complications during pregnancy and delivery. 70% of those fulfilling these criteria agreed to participate until their babies were 7 months. The HOME score by Caldwell and Bradley, infant version adapted to fit German cultural context at 18 month of age, was used for assessment of the home environment both as a developmental determinant and an influential confounder. Infant version used consisted of 45 items.
Exposure	PCBs.
Measurement of exposure (Biomarker, internal validation)	Sum of PCB 138, 153 and 180 marker for PCB exposure in cord blood, venous blood at 42 months, and spot milk at around 2 weeks postpartum. PCB analyses done on a high resolution gc-ec. Lipid content determined photometrically. Cord blood levels defined prenatal exposure and PCB levels in early milk defined neonatal exposure. A rough estimate of dose, (early milk PCB x months of breastfeeding adjusted for prenatal and perinatal exposure), and PCB serum level at 42 months, were used to test possible effect of postnatal exposure on psychomotor and cognitive development. Breastfeeding length (</=2 weeks; >2 weeks-4 months; >4 months).
Follow-up period, drop-outs	91 remained at 42 month follow-up. Sample structure changed only marginally.
Length of breastfeeding	Breastfeeding length (</=2 weeks; >2 weeks-4 months; >4 months).
Degree of breastfeeding (fully/partly)	Not given.
Outcome	Psychomotor and cognitive development assessed at 7, 18, 30 and 42 months.
Measurement of outcome	Testing done at home by the same examiner. Of the Bayley Scales of Infant Development, the mental and motor scales were used at 7, 18 and 30 months of age. Mental scale assesses the child`s level of cognitive functioning, language development, and social/personal development. The motor scale assesses fine, and gross motor functioning. At 42 months intelligence were measured with the German version of the Kaufmann Assessment Battery for children. Scores from the Sequential Processing and Simultaneous Processing subscales were combined to yield the Mental Processing

Reference	**Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, Steingruber HJ, Wundram S, Winneke G. (2001). Environmental exposure to polychlorinated biphenyls and quality of the home environment: effects on psychodevelopment in early childhood. Lancet, 358(9293), 1602-1607.**
	Composite-Index which were standardised to a mean of 100 and SD 15. Possibility of additional developmental impact of postnatal PCB intake was tested by two models. First model used a rough estimate of dose, (early milk PCB x months of breastfeeding) adjusted for prenatal and perinatal exposure. This model was applied to the repeated measurements approach of Bayley Scales of Infant Development (motor and mental) and to the Kaufmann Assessment Battery for Children. Second model was applicable to the Kaufmann Assessment Battery for Children as the outcome variable only, and used PCB in 42 month serum adjusted for prenatal and perinatal exposure.
Statistical analysis	Data were treated both descriptively and inferentially with SAS. Multiple linear regression was used to analyse association of HOME and PCBs with target variables. Model building involved two steps; in the first step variables were selected based on theoretical knowledge of their effect on mental or motor development, or on empirical evidence from previous studies on the relation of either PCBs or the HOME to the respective outcome variables. In the second step, selection of variables was data driven; only those variables which had correlations at $p<0.20$ with both the exposure index and at least two of the outcome variables after inclusion of the first step variables. The variables in the first step were: parental education, sex, maternal intelligence quotient, as well as HOME and PCB simultaneously. Those in the second step were: parity, smoking in pregnancy, and body mass index. All outcome variables were uniformly adjusted for these variables.
Results	No significant or borderline association with calculated postnatal PCB exposure was found for both Bayley Scales of Infant Development measures. However, a significant negative effect of postnatal exposure was noted in both models as applied to Kaufmann Assessment Battery for Children at 42 months ($t=-1.89$, $p=0.031$ for dose and $t=-2.01$, $p=0.025$ for PCB at 42 months). Negative associations between early milk PCB and mental/motor development were reported at all ages becoming significant from 30 months onwards. Thus, increasing PCB levels in early milk predicted decreasing Kaufmann scores. Lean matrix of cord blood with associated analytical difficulties could explain lack of associations of cord PCB with later psycho developmental endpoints. Over 30 months, for a PCB increase from 173 (5 percentile) to 679 (95 percentile) ng/g lipids in milk there was a decrease of 8.3 points in the Bayley Scales of infant Development scores, and a 9.1 decrease in the Bayley Scales of infant Motor scores. Also negative effect of postnatal PCB exposure via breastfeeding at 42 months. Home environment had a positive effect from 30 months onwards.
Conclusion	Negative associations between early milk PCBs and mental/motor development at all ages, significant from 30 months onwards. Intelligence at 42 months negatively assoc. with early breastmilk PCB levels. Prenatal PCB exposure was not associated with child development. A favourable home environment had the opposite effect and supported mental and motor development until 42 months of age.
Confounders adjusted for	Age at examination, gestational age, alcohol/smoking during pregnancy, Apgar score, neonatal illness/jaundice, spontaneous delivery, parity, lead in cord blood, chronic diseases during past year, duration of breastfeeding, parental education, parental occupation, body-mass index of mother, maternal intelligence verbal quotient.
Quality (A, B or C)	B
Relevance for our risk assessment purpose	Focus is on effect of prenatal PCB exposure on psychomotor and cognitive development. Possibility of additional developmental impact of postnatal PCB intake was tested. A rough measure of postnatal exposure was used and pre- and postnatal exposure was not clearly distinguished. Thus, relevance is lower.

Summary Table 1-24, Table 23.

Reference	**Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H. (2000). Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect, 108(12), 1203-1207.**
Study design and type	Prospective longitudinal follow-up cohort study, Dutch cohort (1990-1992).
Objective	To investigate whether exposure to PCBs and dioxins is associated with the prevalence of infectious or allergic diseases, changes in antibody responses or changes in lymphocyte phenotype in Dutch preschool children.
Number of participants and country	207 infants at birth (105 breastfed and 102 formula fed), 175 children at 42 month follow-up, The Netherlands.
Baseline characteristics of study subjects	Pregnancy women from the Rotterdam area; selection criteria. Parity (first or second born), 37-42 weeks gestation, no congenital abnormalities, no diseases, normal birth, all white, breastfeeding at least 6 weeks, formula feeding during 7 months from one batch formula food. Obstetrical optimality scale used.
Exposure	PCBs and dioxins.
Measurement of exposure (Biomarker, internal validation)	Measure of prenatal PCB exposure in the total study population**:** PCB plasma sum (118, 138, 153, 180) (gc-ec) in mothers blood (last month of pregnancy, 36-40th week) and umbilical cord blood. The breastmilk samples from the breastfeeding group (24 hour sample within 2 weeks postpartum) were used as indirect measures for prenatal exposures to PCBs (20 congeners plus 6 dioxin-like PCBs and dioxins (17) (gc-ms) expressed as TEQs (WHO1998). Same measurements (118, 138, 153, 180) (gc-ec) from formula fed group. Current PCB body burden defined as the sum of the 4 PCB congeners in plasma from the 42 month old children. Information was given on missing samples.
Follow-up period, drop-outs	42 month follow up study. 7% drop out.
Length of breastfeeding	Categorised into zero (0), short (6-16 weeks) and long (>16 weeks) breastfeeding.
Degree of breastfeeding (fully/partly)	Fully breastfed in at least 6 weeks.
Outcome	Immunologic effects.
Measurement of outcome	Prevalence of infectious and allergic diseases was assessed by parental report of doctors' diagnosis via questionnaire, and humoral immunity was measured by antibody levels for mumps, measles and rubella after primary vaccination. Immunologic marker analyses of lymphocytes were performed in a subgroup of 85 children.
Statistical analysis	Multiple regression analysis, Statistical package for the social sciences (SPSS/PC, Cary, NC), used to study effects of prenatal and postnatal exposure to PCBs and dioxins separately and combined. Since it is assumed that that breastfeeding per se has positive effects on development of children, the amount of breastfeeding received was studied separately. This variable "duration of breastfeeding in weeks" was divided into 3 categories (zero

Reference	**Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H. (2000). Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect, 108(12), 1203-1207.**
	(formula fed, short (6-10 weeks) and long 11-12 weeks) at 3 months, and zero, 6-16 weeks, and >16 weeks at 7 and 18 months, respectively). In the multiple regression analysis this categorical variable was entered as a continuous independent variable with a value 0, 1 and 2.
Results	Prenatal PCB exposure was associated with an increased number of lymphocytes, T-cells, and CD3CD8+ (cytotoxic), CD4+CD45RO+ (memory), T-cells receptor (TcR) αβ+, and CD3+HLA-DR+ (activated) T cells and lower antibody levels to mumps and measles at school age. Adjusted for confounders, prenatal PCB exposure was associated with less shortness of breath with wheeze, and current PCB body burden was associated with higher prevalence of recurrent middle-ear infections and of chicken pox and a lower prevalence of allergic reactions. A higher dioxin TEQ was associated with a higher prevalence of coughing, chest congestion, and phlegm.
Conclusion	In Dutch preschool children the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may prevent development of allergy, so PCB exposure might be associated with a lower prevalence of allergic diseases. The negative effect of a higher postnatal PCB exposure was counteracted by the positive effect of longer duration of breastfeeding in infancy. The study did not provide data to discourage breastfeeding at present background PCB levels.
Confounders adjusted for	Level of education of parents, (high 1) at least secondary education. Completed; or low (0), profession categorised into 3, maternal smoking, alcohol use (yes or no), parity, duration of gestation, birth weight, sex of infant, duration of breastfeeding (in weeks less or more than 16 weeks), family history of atopy in one or more parents, and day or nursery school attendance for the child. $p<.05$.
Quality (A, B or C)	B+
Relevance for our risk assessment purpose	Relevant. Interpretation of the study is complex. Breastfeeding duration was found to counteract the increased OR for middle ear infection associated with postnatal exposure to PCBs via breastfeeding. If common infections acquired early in life prevent the development of allergy, this might explain the lower prevalence of allergy associated with postnatal PCBs exposure.

Summary Table 1-24, Table 24.

Reference	**Winneke G, Kramer U, Sucker K, Walkowiak J, Fastabend A, Heinzow B, Steingruber HJ. (2005). PCB-related neurodevelopmental deficit may be transient: follow-up of a cohort at 6 years of age. Environ Toxicol Pharmacol, 19(3), 701-706.**
Study design and type	Prospective cohort study (1993-1995).
Objective	The purpose of this follow-up study was to determine if PCB-related cognitive impairment observed until 42 months of age persists up to 72 months (6 years).
Number of participants and country	171 healthy mother-infant pairs at birth, 91 children at 42 month follow-up, 70 children at 6 years (72 months) follow-up Germany.
Baseline characteristics of study subjects	Mother-infant pairs recruited from Düsseldorf hospitals 1993-1995. Selection criteria: parity (first or second born), 37-42 weeks gestation, an Apgar score at 5 min of at least 7-8, native German families, no serious illnesses or complications during pregnancy and delivery.
Exposure	PCBs.
Measurement of exposure (Biomarker, internal validation)	Sum of PCB 138, 153 and 180 marker for PCB exposure in cord blood, venous blood at 42 months, and spot milk at around 2 weeks postpartum. PCB analyses done on a high resolution gc-ec. Lipid content determined photometrically. Cord blood levels defined prenatal exposure and PCB levels in early milk defined neonatal exposure. PCB serum level at 42 months, were used to test possible effect of postnatal exposure on psychomotor and cognitive development. PCBs was determined in blood samples from 61 children at 4 years (87 participants in total), 50 of the children with PCB results at 4 years participated at follow up at 6 years (70 participants in total).
Follow-up period, drop-outs	91 remained at 42 month follow-up. 70 remained at 72 months follow up. Sample structure changed only marginally.
Length of breastfeeding	Not given in paper, from Walkowiak J 2001: Breastfeeding length (</=2 weeks; >2 weeks-4 months; >4 months).
Degree of breastfeeding (fully/partly)	Not given.
Outcome	Psychomotor and cognitive development assessed at 42 and 72 months. HOME assessed at 18 months and 72 months
Measurement of outcome	At 72 months intelligence were measured with the German version of the Kaufmann Assessment Battery for children (same test as 42-months, but age-adapted). Scores from the Sequential Processing and Simultaneous Processing subscales were combined to yield the Mental Processing Composite-Index which were standardised to a mean of 100 and SD 15. HOME-scale administered during a semi-structured interview with the mother in the presence of the child.
Statistical analysis	Data were treated both descriptively and inferentially with SAS. Multiple linear regressions were used to analyse association of HOME and PCBs with target variables. Sum PCBs in milk, and PCB in serum at 42 months (adjusted for prenatal exposure), served as two independent variables. Briefly, a pre-specified set of variables (parental education, maternal IQ, and HOME (72 months) or Sum PCBs alternatively) based on theoretical knowledge or

Reference	**Winneke G, Kramer U, Sucker K, Walkowiak J, Fastabend A, Heinzow B, Steingruber HJ. (2005). PCB-related neurodevelopmental deficit may be transient: follow-up of a cohort at 6 years of age. Environ Toxicol Pharmacol, 19(3), 701-706.**
	on empirical evidence from previous neurodevelopmental studies, was included in the first step, and – following a data-driven procedure based on correlations with both exposure and outcome at $p \leq 0.20$ – other variables (sex, birth weight, duration of breastfeeding, HOME (18 months)) were added to the final model in the second step. Gender, parental education, maternal IQ, birth weight, breastfeeding (duration), HOME (18 months) were included in the final model, plus HOME (72 months) and Sum PCBs in this or reverse sequence.
Results	Whereas, at 42 months of age, significant negative associations with PCBs in milk (prenatal exposure) and in 42-months-serum (postnatal exposure) were found, this was no longer significant at 72 months, although associations still remained negative. The positive effect of the home environment became even more pronounced
Conclusion	The authors concluded that early PCB-exposure at levels in this cohort (background levels) possibly induces transient delay in cognitive development rather than irreversible deficit.
Confounders adjusted for	Gender, parental education, maternal IQ, birth weight, breastfeeding (duration), HOME (18 months)
Quality (A, B or C)	B
Relevance for our risk assessment purpose	This is a follow-up at 6 years in a cohort in which association between prenatal and postnatal PCB-exposure via breastmilk and reduced neurodevelopment has been reported previously at 4 years. The same test has been administered at 4 years and 6 years. Thus, relevance is high.

Appendix 6: Literature search on contaminants in infant formula

Database: Embase <1980 to 2012 Week 04>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

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1 infant formula*.ti,ab,sh. (7860)
2 baby formula*.ti,ab,sh. (93)
3 infant fed*.ti,ab,sh. (121)
4 milk based formula*.ti,ab,sh. (599)
5 milkbased formula*.ti,ab,sh. (3)
6 artificial formula*.ti,ab,sh. (143)
7 artificial milk.ti,sh,ab. (7675)
8 bottle milk.ti,ab,sh. (35)
9 bottlefed.ti,ab,sh. (83)
10 bottle fed.ti,ab,sh. (1584)
11 breast milk substitut*.ti,ab,sh. (429)
12 breastmilk substitut*.ti,ab,sh. (112)
13 formula feeding.ti,ab,sh. (1733)
14 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 (16549)
15 pesticide*.ti,ab,sh. (76504)
16 persistant organic pollutant*.ti,ab,sh. (11)
17 persistent organic pollutant*.ti,ab,sh. (3283)
18 environmental pollutant*.ti,ab,sh. (27769)
19 environmental exposur*.ti,ab,sh. (120777)
20 environmental contaminant*.ti,ab,sh. (6449)
21 dioxin*.ti,ab,sh. (26360)
22 polychlorinated biphenyl*.ti,ab,sh. (31688)
23 pcb*.ti,ab,sh. (27899)
24 pollutant*.ti,ab,sh. (70642)
25 metals, heavy.sh. (11548)
26 heavy metal*.ti,ab,sh. (53545)
27 mercury.ti,ab,sh. (70506)
28 lead.sh. (65775)
29 cadmium*.ti,ab,sh. (80445)
30 pfos.ti,ab,sh. (1920)
31 perfluorooctane sulfonate*.ti,ab,sh. (1000)
32 perfluorooctanesulfonic acid*.ti,ab,sh. (703)
33 pfoa.ti,ab,sh. (1751)
34 perfluorooctanoic acid*.ti,ab,sh. (1599)
35 pfc.ti,ab,sh. (12802)
36 perfluoro compound*.ti,ab,sh. (1828)
37 perfluorinated acid*.ti,ab,sh. (170)
38 brominated flame retardant*.ti,ab,sh. (1223)
39 flame retardant*.ti,ab,sh. (4121)
40 ddt.ti,ab,sh. (17850)
41 chlorphenotane*.ti,ab,sh. (10341)
42 "1,1 dichloro 2,2 bis(4 chlorophenyl)ethylene".sh. (3205)
43 dde.ti,ab,sh. (7137)
44 dichlorodiphenyl dichloroethylene.ti,ab,sh. (1645)
45 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 (528118)
46 child growth.ti,ab,sh. (8367)
47 child development.ti,ab,sh. (67095)
48 infant growth.ti,ab,sh. (1410)
49 infant development.ti,ab,sh. (3050)
50 language development.ti,ab,sh. (19032)
51 child* language.ti,ab,sh. (3925)
52 preschool child*.ti,ab,sh. (446941)
53 child, preschool.sh. (682538)
54 cognitive development*.ti,ab,sh. (8434)
55 cognition.ti,ab,sh. (201496)
56 cognitive defect*.ti,ab,sh. (72656)
57 cognitive disorder*.ti,ab,sh. (4570)
58 child* intelligence.ti,ab,sh. (449)
59 nerve cell differentiation.ti,ab,sh. (19521)
60 neurodevelopment*.ti,ab,sh. (23036)

61 immune system*.ti,ab,sh. (153455)
62 allerg*.ti,ab,sh. (333017)
63 sensiti?ation.ti,ab,sh. (88052)
64 hypersensitiv*.ti,ab,sh. (177785)
65 child behavio?r.ti,ab,sh. (47063)
66 behavio?r* disorder*.ti,ab,sh. (46573)
67 sexual* matur*.ti,ab,sh. (24713)
68 thyroid.ti,ab,sh. (251098)
69 infection*.ti,ab,sh. (1908429)
70 autism*.ti,ab,sh. (38834)
71 infantile autism*.ti,ab,sh. (3129)
72 autistic disorder*.ti,ab,sh. (15380)
73 attention deficit disorder*.ti,ab,sh. (45891)
74 adhd*.ti,ab,sh. (24161)
75 childhood mental disorder*.ti,ab,sh. (62)
76 adolescen* mental disorder*.ti,ab,sh. (164)
77 intellectual impair*.ti,ab,sh. (7210)
78 intellectual disabilit*.ti,ab,sh. (50815)
79 cognitive impair*.ti,ab,sh. (52266)
80 cognitive disabilit*.ti,ab,sh. (1066)
81 obesity*.ti,ab,sh. (359732)
82 obese*.ti,ab,sh. (133795)
83 overweight*.ti,ab,sh. (63788)
84 weight gain.ti,ab,sh. (110098)
85 cancer*.ti,ab,sh. (2391609)
86 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 (6843057)
87 14 and 45 and 86 (154)
88 prematurity.ti,ab,sh. (75988)
89 premature infant*.ti,ab,sh. (29230)
90 premature child*.ti,ab,sh. (1133)
91 infant, premature.sh. (36498)
92 88 or 89 or 90 or 91 (117783)
93 87 not 92 (152)
94 limit 93 to humans (132)
95 limit 94 to yr="1990 -Current" (130)
96 remove duplicates from 95 (104)
97 limit 96 to (danish or english or german or norwegian or swedish) (99)

Appendix 7: Tests for neurodevelopment and home environment

Bayley Scales of Infant Development

The Bayley Scales of Infant Development Second edition (BSID-II) is a standard series of measurements originally developed by psychologist Nancy Bayley and used primarily to assess the mental, motor and behavioural development of infant and preschool children. The BSID-II is the most important and most widely used developmental assessment test. One of its primary uses is to evaluate infants suspected of delay or atypical development to determine eligibility for services and to track progress over time. Children at risk for developmental disability are those born prematurely or with low birth weight or with major congenital anomaly, delayed milestones, or other risk factors. The Bayley Scales are also preferred as a tool in developmental research.

The BSID-II is a *developmental* test, not an intelligence test, and thus assesses different abiliteis present at different ages. US norms have been developed for 17 age groups between the ages of one month and 3.5 years (42 months), with 50 boys and 50 girls in each age group. There are three scales: mental scale, motor scale and a 30-item behavior rating scale (BRS). The mental scale provides an index score of general cognitive development (i.e., the Mental Development Index, MDI). The motor scale provides an index of overall motor development (i.e., Psychomotor Development Index, PDI). From these two scales, four "facet" scores can be derived: Cognitive, Language, Motor and Personal/Social. These are intended for identifying relative strengths and weaknesses. The BRS provides a qualitative assessment of behavior reflecting three main factors: Orientation/Engagement, Emotional Regulation, and Motor Quality. The BRS is considered a critical aspect of the BSID-II because an infant's state (including engagement, arousal level, and motivation at the time of testing) may substantially influence the Mental and Motor scores.

The BSID-II takes between 45 - 60 minutes to administer. Raw scores of successfully completed items are converted to scale scores and to composite scores. These scores are used to determine the child's performance compared with norms taken from typically developing children of their age (in months). The assessment is often used in conjunction with the Social-Emotional Adaptive Behavior Questionnaire. Completed by the parent or caregiver, this questionnaire establishes the range of adaptive behaviors that the child can currently achieve and enables comparison with age norms.

Reference: Strauss, E., Sherman, E.M.S., & Spreen, O. 2006. A compendium of neuropsychological tests. Administration, norms, and commentary. 3rd ed. New York: Oxford University Press.

Home Inventory and the Bradley scale

The Home Observation for Measurement of the Environment (HOME) Inventory is designed to measure the quality and quantity of stimulation and support available to a child in the home environment. It is a descriptive profile which yields a systematic assessment of the caring environment in which the child is reared. The focus is on the child in the home environment, the child as an active recipient of inputs from objects, events, and transactions occurring in connection with the family surroundings. It is intended to be used by practitioners, as well as researchers, and ideally it should be combined with information from individual assessments of the child in a context of a multimodal assessment procedure.

The HOME involves an hour long semi-structured interview in the home with the main caregiver and child to collect information about the nature and variety of the child's day-to-day experiences and the parenting capacity of the caregivers and to explore a range of other

aspects of the child's world and the life of the family. The HOME has been shown to be a good predictor of outcomes for children

The *HOME Inventory* was developed in the 1960's and has been used with large numbers of children in a variety of different contexts around the world. It is therefore a well-established and tested tool for undertaking a standardised assessment of a child's home environment.

There are four *HOME* inventories for children aged 0-3, 3-6, 6-10 and 10-14 years. There are additional disability adapted inventories for a range of disabled children and for child care contexts. Each inventory contains a group of scales to assess different aspects of the child's environment. Each scale contains a number of items that are scored according to a glossary. The *HOME* therefore provides a framework for practitioners to assess all aspects of the home environment, including parenting, which directly impact on the child.

The HOME Inventory has been used successfully in research and in practice. It is easy to administer and score and has sound psychometric properties. Even though it requires special training, it is straightforward to complete and to score and at the same time the whole procedure is not threatening to the family. The combination of interview and direct observation allows for an assessment of the caring environment along with a more detailed assessment of individual children. However one of the most serious restrictions of this inventory is the lack of a standardised procedure for administration. Another limitation comes from the measurement scale itself. The choice of a binary scale makes it easier for the interviewer to score but it deprives the researcher or the practitioner of more subtle information needed to make informed judgements.

Reference: Totsika, V. & Sylva, K., 2004. Child and Adolescent Mental Health Volume 9, No. 1, pp. 25-35 http://www.familieschildrenchildcare.org/fccc_static_PDFs/Sylva_2004.pdf.

McCarthy Scales of Children's Abilities (MSCA)

The McCarthy Scales of Children's Abilities (MSCA) is a psychological test given to young children. The MSCA was created by Dorothea McCarthy in 1972. The MSCA was intended to measure children from ages 2 to 8. It includes 18 subtests that yield a General Cognitive Index and scores for verbal, perceptual-performance, quantitative, motor, and memory scales. The test materials are appealing to children, but considerably older normative data set makes this a limited instrument in comparison to more currently normed measures that were developed for, or extend into, the same age range. Thus, it is no longer advisable to administer the MSCA.

Reference: Baron, I. S. (2004). Neuropsychological evaluation of the child. New York: Oxford University Press.

Kaufman Assessment Battery for Children (K-ABC)

The Kaufman Assessment Battery for Children (K-ABC) is an instrument for assessing intellectual skills and cognitive development in children aged from 2 years 6 months to 12 years 5 months. The test was developed by Alan S. Kaufman and Nadeen L. Kaufman in 1983 and revised in 2004. The battery has been normed on 2000 children in the US with equal representation of boys and girls for each of 20 age groups. The K-ABC utilizes two component processes according to principal factor analysis, simultaneous and sequential processing. It is based on Luria's theory of simultaneous and successive information processing. These define intelligence in terms of the child's problem solving and processing styles. Seven subtests are used to measure simultaneous processing, while three subtests are included in assessment of sequential processing. There are also six achievement subtests, and

a nonverbal scale with six subtests to aid in assessment of cultural or linguistic minorities and handicapped children.

A short form of the K-ABC includes the subtests Hand Movements (reproducing hand-movement sequences), Word Order (serial recall of words), Triangles (puzzle assembly), and Matrix Analogies (identification of principles of similarity). This form gives an estimated mental processing composite (MPC), with good to excellent reliabilities with the full version and has been used extensively.

Thus, the K-ABC gives special attention to certain emerging testing needs, such as use with handicapped groups, application to problems of learning disabilities, and appropriateness for cultural and linguistic minorities. The authors rightly caution, however, that success in meeting these special needs must be judged through practical use over time. They also point out that the K-ABC should not be regarded as “the complete test battery”; like any other test, it should be supplemented and corroborated by other instruments to meet individual needs, such as the Stanford-Binet, Wechsler Intelligence Scale for Children, or other neuropsychological tests. The K-ABC has been translated, adapted and standardized in more than 15 countries.

Reference: Baron, I. S. (2004). Neuropsychological evaluation of the child. New York: Oxford University Press.

Appendix 8: Information on materials used in infant feeding (baby) bottles and teats after the BPA ban, including possible migration of such chemicals, received from EU countries

VKM asked various EU countries for information about the materials used in, and possible migration of, chemicals from infant feeding (baby) bottles and teats after the BPA ban. Information about chemical contaminants in infant formula was also requested. The information received are summarised here.

Answers were received from Cyprus, Finland, Germany, Iceland, The Slovak Republic, Sweden, and United Kingdom, and are very much appreciated.

According to information from Germany, polycarbonates based on other bisphenols are not used for baby bottles. UK reported that they did not have any information on the use or intent to use bisphenol F (BPF), bisphenol B (BPB) or bisphenol S (BPS) as substitutes for BPA, but that they were aware that such substitutes are being/were investigated by British industry. The Slovak republic reported that of 36 baby bottles controlled in 2009-2011 the content of BPA was not detectable in any samples (detection limit was 0.02 mg/kg), and that they plan to monitor also BPF and BFS within their official control of baby bottles in 2012.

The results of a study on the analytical identification and quantification of migration of chemicals from plastics in baby bottles found in the European Union market made of materials that are now present as substitutes for polycarbonate (PC) were reported by (Simoneau et al., 2012). A total of 449 baby bottles with a focus on first age or sets of bottles were purchased from 26 European Union countries, Canada, Switzerland and the USA. From this collection, which contained several duplicates, a total of 277 baby bottles were analysed. The materials included different types of plastic such as polycarbonate (PC), polyamide (PA), polyethersulphone (PES), polypropylene (PP), but also silicone, and from the United States a co-polyester marketed under the trade name Tritan^{TM}. The bottles were subjected to the conventional migration test for hot fill conditions, i.e. 2 h at 70ºC. The simulant used was that specified in European Union legislation (2007/19/EC) for milk, i.e. 50% ethanol. The migration solutions in general showed a low release of substances. Results showed that bottles made of PP and silicones showed a greater number of substances in the migration solutions and in greater quantity. Chemicals from PP included alkanes, which could be found in >65% of the bottles at levels up to 3500 mg/kg; and benzene derivatives in 17% of the baby bottles and found at levels up to 113 mg/kg. Some substances were found on a regular basis such as plasticisers, esters and antioxidants (e.g. tris(2,4-di-tert-butylphenyl)phosphate, known as Irgafos 168. Some substances found were not included in the Community positive list, which means that those should not be found even in the first migration. Such substances included 2,6-di-isopropylnaphthalene (DIPN), found in 4% of the bottles at levels up to 25 mg/kg, 2,4-di-tert-butyl phenol (in 90% of the bottles at levels up 400 mg/kg). Moreover, BPA was detected and quantified in baby bottles made of PA, but limited to one brand and model specific (but labelled BPA free). Results for baby bottles made of silicone also indicated the presence of components, e.g. potentially coming from inks (benzophenone, DIPN), which could come for example from the presence of instruction leaflets in the bottles). In the case of silicone, phthalates were also found in relevant concentrations, with levels for diisobutyl phthalate (DiBP) and dibutyl phthalate (DBP) from the first migration test of 50-150 mg/kg and diethylhexyl phthalate (DEHP) at levels 25-50 mg/kg.

Other countries also reported the same materials found in baby bottles. United Kingdom reported that a market leading UK brand of baby bottles (manufactured in England) were made of either PES or PP. Cyprus reported that from samples of baby bottles analysed in

2011, the materials were mainly found to be PC and PP, while one sample was made of PES. The Slovak republic reported that in 2011 PC, PP and a few bottles made of Tritan™ coplyester or polyester were found on their market.

The teats used on the feeding bottles are also a potential source of contaminants to the infants.

Germany reported that to their knowledge teats for baby bottles are made of natural rubber latex or silicone, and that in both cases plasticizers are not used. Bundesinstitut für Risikobewertung (BfR) in Germany gives recommendations for silicone elastomers (silicone rubbers) as well as natural and synthetic rubber used in food contact. Cyprus also reported that latex (kaoutsouk) and silicone were used in teats, and that they had no information regarding potential use of plasticizers in these materials. They also stated that there are possible migrations of *N*-nitrosamines and *N*-nitrosatable compounds (Directive 93/11/EEC) from teats made of kaoutsouk. UK reported that commonly teats produced in UK are made of silicone, and are advertised as "BPA free" and "100% silicone" or "food grade silicone", and that they are the preferred teat for wide-necked baby bottles, whereas latex teats are also available in UK for traditional style bottles. They further reported that they did not have any information on any plastic materials used for teats, however, if used they would be covered by the requirements of Regulation 1935/2004 and Regulation 10/2011. The restrictions on the use of certain plasticizers and epoxidised soybean oil (ESBO) would preclude their use in the UK for such items. They further stated that with established silicone and latex alternatives it appears unlikely that plastic teats would be available even in small numbers.

Commission Directive 93/11/EEC of 15 March 1993 concerning the release of the N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers.

Regarding other contaminants than BPA in baby bottles and teats, UK stated that advertising materials in the UK occasionally mention that baby care materials are free from phthalates and polyvinyl chloride (PVC) as well as PBA, however, the use of either is not a recognised issue in UK. No concerns have been raised by the public or industry as to other materials in baby bottles than BPA.

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