

Environmental Project No. 589 2001  
Miljøprojekt

## Children and the unborn child

Exposure and susceptibility to chemical substances - an evaluation

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# Preface

Concern has been raised that infants and children are at higher risk than adults from exposure to environmental chemicals. In particular, this important discussion has focused on the potential higher exposure of infants and children and increased vulnerability to the detrimental effects of chemicals. In 1998, the Danish government initiated a cross-ministerial overview concerning “protection of children and pregnant women against dangerous substances” and a conference was held in October 1998 in order to pay more attention to this topic and to discuss and identify possible needs for further development. The overall impression from the discussions at the conference was that children and pregnant women should generally be recognised as special risk groups with respect to effects from exposure to chemical substances. As a consequence, the present project was initiated by the Danish EPA. The purpose of the project was to elaborate a detailed review and update the knowledge on the exposure and vulnerability of humans to chemical substances during the embryonic, foetal and postnatal periods. The report is intended to form the scientific basis for future regulatory work of the Danish EPA in the protection of children and the unborn child to environmental chemical substances.

The report has been prepared by the Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration as a contract work for the Danish Environmental Protection Agency.

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The report reflects the views and opinions of the authors as well as of the members of the Steering Committee although consensus has not been reached on every aspect discussed in the report. It should also be noted that the report does not necessarily represent the views and opinions of the involved institutions.

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# 1 Introduction

Patterns of illness in children have changed considerably in this century with increased incidences of e.g. asthma, birth defects, childhood cancers, and learning disabilities being reported. The aetiologies of many diseases of childhood are due to a combination of factors, including genetic susceptibility, socio-economic factors and environmental exposures during vulnerable periods of development. (US-EPA 1998, EHP 1998a, Routt Reigart 1998, Gee 1999, Suk 1999).

Children are at risk of exposure to a lot of high-production-volume synthetic chemical substances which have been introduced into the market within the past 50 years; these chemical substances are used widely in consumer products and are dispersed in the environment.

Children as well as the unborn child have in some cases appeared to be uniquely vulnerable to chemical substances because of their biological growth and development. Furthermore, children may be more heavily exposed than adults to certain chemicals and pollutants in the environment as children, on a body weight basis, breathe more air, drink more water, and eat more food than adults; additionally, their behaviour patterns, such as play close to the ground and hand-to-mouth activities, can increase their exposure.

Recently concern about the probable vulnerability of infants and children to environmental chemicals has been an important issue for discussion. Initially, the USA identified this as an area of concern and more recently the EU has been active in this area. Also international organisations as e.g., OECD and Non Governmental Organisations (NGOs) have considered this area of importance.

## *US-EPA / ILSI conference*

In 1990, the U.S. Environmental Protection Agency (US-EPA) and the International Life Sciences Institute (ILSI) sponsored a conference on *Similarities and differences between children and adults: implications for risk assessment*; the conference papers are published in ILSI (1992). The conference came out with a number of conclusions:

- Children are not simply small adults but rather are a unique population for health risk assessment.
- Differences in sensitivity between children and adults are chemical specific and must be studied and evaluated on a case-by-case basis.
- Because of their physical vulnerability and diminutive stature, infants and children are often assumed to be more susceptible to the potential toxic effects of the chemicals found in the environment.
- An analysis based on available data, however, suggests that susceptibility clearly depends on the substance and the exposure situation. In some cases, there may be no difference at all in the responses of children and adults. In other cases, different physiological and metabolic factors, pharmacokinetics, and diet and behav-

our patterns, among other influences, demonstrably can render children more or less susceptible than adults.

#### *National Research Council*

In 1993, the National Research Council (NRC) in USA published a report *Pesticides in the diets of infants and children* (NRC 1993). NRC pointed out that

- Age-related variability in the susceptibility to chemical substances may occur and children can be either more or less susceptible than adults depending on the chemical in question.
- New-borns are considered to differ most in comparison to adults.
- Quantitative differences in toxicity between children and adults are generally less than a factor of 10.
- Children's intake of pesticide residues from foodstuffs are different when compare to adults.
- Differences in exposure are generally the more important source contributing to a higher risk than the age-related differences in susceptibility to toxicants.

#### *Symposia in USA*

Recently, two important symposia have been held in the USA: *The Conference on Children's Environmental Health: Research, Practice, Prevention, and Policy, 21-23 February 1997 in Washington, DC* and *The U.S. EPA Conference on Preventable Causes of Cancer in Children, 15-16 September 1997 in Arlington, VA*. The papers from these symposia are published in EHP (1998a).

Both of these symposia identified as cause for concern the rise in the prevalence of many disease processes involving children, such as the rise in incidence of certain childhood cancers including brain cancers, acute lymphoblastic leukaemia, Wilm's tumour, and testicular cancer. Neither of these symposia alleged that environmental agents are directly responsible for these rises in incidence, but both provided indications as to the interaction of environmental exposure with other factors to produce greater risks of cancer in children.

#### *US-EPA initiatives*

In September 1996, the US-EPA published a National Agenda which outlined an approach to the protection of children from environmental risk and suggested a research strategy to develop the scientific information necessary to improve protection of children (US-EPA 1996). In the same year the Food Quality Protection Act (FQPA 1996) was approved which requires the US-EPA to upgrade risk assessment procedures for setting residue tolerances in foods; this act gave special emphasis to the protection of infants and children from pesticides. In April 1997, President Bill Clinton issued an Executive Order that demanded increased emphasis on the protection of children from environmental health risks and safety risks (Clinton 1997). In May 1997 the Administrator of the US-EPA established the Office of children's Health Protection to support the Agency as it implements the President's Executive Order as well as the National Agenda. The mission of the Office is to make the protection of children's health a fundamental goal of public health and environ-



mental protection in the United States. The US-EPA activities are published in the *Children's Environmental Health Yearbook* (US-EPA 1998).

#### *Initiatives in Europe food additives*

In 1997, ILSI Europe organised a workshop on the “Applicability of the ADI to infants and children”; the workshop papers are published in Clayton et al. (1998). In answering the question *how big are the differences between infants or children and adults from a susceptibility point of view?*, the workshop concluded that the issue of age-related differences in susceptibility to food additives and other compounds should be addressed on a case-by-case basis. To another question addressed *Are differences in food intake (between infants or children and adults) a point of concern?*, the answer was that the fact that infants and children have a higher intake of some food items than adults is not part of the ADI (acceptable daily intake) but should clearly be taken into careful consideration when the ADI is used to establish the use levels of food additives in such foods. To a third question *Are special safety factors or regulatory principles required for infants and children?*, the workshop strongly recommended that special safety factors for infants and children should not be used, and consequently special ADIs should not be established. In case that studies indicate that infants and children are most sensitive to a particular chemical, these studies should drive the derivation of the ADI. (Larsen & Pascal 1998).

#### *pesticides*

The applicability of the ADI for permitted pesticides to infants and young children has been discussed in a report (Schnipper & Larsen 1997) prepared for an ad hoc SCF (Scientific Committee for Food) meeting on pesticides in baby food. Similarly to food additives, it was not recommendable that special safety factors should be used for infants and children and no special ADIs should be established for this age group. The ADI should, however, not be considered applicable to infants below 12 weeks of age, since the usual toxicity test regimen does not cover this situation. This means that special considerations will have to be applied to pesticide residues in infant formulas and drinking water consumed by infants below the age of 12 to 16 weeks. Furthermore, the higher intake, on a per kg of body weight basis, of some food items and beverages for infants and children should be taken into careful consideration when the ADI is used in the establishment of the MRLs (Maximum Residue Level) for pesticides in such foods and beverages.

#### *endocrine disrupters*

In the recent years there has been growing concern regarding possible harmful consequences of exposure to chemical substances which may modulate/ disrupt the endocrine system. Endocrine disrupters are believed to interfere with the complex function of hormones and signalling molecules. The activity may be by mimicking the action of naturally-produced hormone such as oestrogen or testosterone, by blocking receptors in cells receiving the hormones or by affecting the synthesis, transport, metabolism and excretion of hormones. During developing the activity of the endocrine system is high and the unborn child may therefore be specifically vulnerable to exposure of endocrine disrupting chemicals

Recently, a working group under the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) finished a report in which the

evidence for effects in humans and in environment were discussed together with the urgent demand for proper test systems for identifying chemicals causing endocrine disrupting effects. With regard to evidence the CSTE came to the opinion that “although there are associations between endocrine disrupting chemicals, so far investigated, and human health disturbances, a causative role of these chemicals in diseases and abnormalities possibly related to an endocrine disturbance has not been verified”. (CSTEE 1999).

The European Commission has elaborated an EU-strategy on hormone disrupters which has been adopted in the Council in March 2000. The Commission stresses the need for an EU-strategy on this field as very serious adverse effects on human health and the environment may be the follow of exposure to endocrine disrupting chemicals (EU-Commission 1999). The aim of the strategy is to co-ordinate and strengthen the efforts in relation to a) identification of specific substances of concern (based on existing knowledge), b) development of new test methods for the detection of endocrine effects, c) research to uncover the mechanisms, the dose-response and the consequences on environment and human health from exposure to these substances, d) regulation as the endocrine disrupting effects are to be incorporated in the regulative framework, and e) information to the public.

#### *toys*

Toys may be a significant pathway of exposure for unintended chemical exposure to especially small children. According to the EU Council Directive 88/378/EEC, toys may generally not contain dangerous chemical substances in amounts giving rise to development of adverse effects in children and specific migration limits have been set for eight metals. However, a general concern on harmful substances in toys has been expressed. Based on the EU Council Directive 88/378/EEC and an opinion of the CSTE (CSTE 1988), the European Commission gave a mandate to CEN (the European Committee on Standardisation) to develop a harmonised standard for organic chemical compounds in toys. Regulations are required for the following groups of substances: solvents, preservatives, wood preservatives, plasticisers, flame retardants, monomers, and colorants.

Following notification by Denmark, a targeted risk assessment (opinion paper) has been conducted by the CSTEE on phthalates in children's toys and child care articles (CSTEE 1998). Subsequent to this, risk reduction steps have been developed. Recently, EU has adopted measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of 6 specific phthalates (DINP, DEHP, DBP, DIDP, DNOP, BBP) (EU 1999).

#### *Amsterdam Conference*

In 1998, the “First International Conference on Children's Health & Environment” took place in Amsterdam 12-13 August. At this conference, the susceptibility of children including the unborn child to exposure to chemical substances was discussed. No proceedings are at present available from the conference.

#### *London Conference*

In 1999, the “Third Ministerial Conference Environment and Health” was held in London, 16-18 June.

One of the reports presented at this conference *Children in their environment: vulnerable, valuable and at risk* (Gee 1999) provides a summary of what we know, and don't know, about children's vulnerability to a selection of key environmental hazards. The report points out that "Children are not "little adults" but are particularly vulnerable to pollutants because of their immature biological development, behaviour, metabolism, greater exposure to pollutants relative to body weight, and longer life at risk than adults".

The conference concluded that "Children are more susceptible than adults to the effects of environmental health threats and therefore require special protection" and proposed specific actions to address the public health problems of injuries, environmental tobacco smoke, asthma and emerging threats.

#### *Initiatives in Denmark*

In Denmark, the US-EPA National Agenda gave rise to a number of questions to the Minister of Health regarding the responsibility for protection of children and pregnant women against exposure to environmental contaminants and later the Prime Minister was asked to investigate the needs for a national agenda. In June 1998, the Prime Minister requested a common review by the Ministry of Environment and Energy, the Ministry of Food, Agriculture and Fisheries, and the Ministry of Health. In order to put more focus on and discuss these concerns and to form the basis for the common review, a conference on "Protection of children and pregnant women against dangerous substances" was held on 28 October 1998 and planned jointly by the Ministry of Health, the Ministry of Environment and Energy, the Ministry of Food, Agriculture and Fisheries, The Ministry of Labour, the Ministry of Housing and Urban Affairs, the Ministry of Social Affairs and the Ministry of Education. The ministerial report (in Danish) was published in September 1999 (Danish Ministerial Report 1999).

Another initiative taken in Denmark was a "Seminar on Environment and Child Health" in Copenhagen 19 March 1998 organised by the European Environment Agency, Child Watch International, Danish Children's Council, Danish Ecological Council, and WHO.

#### *Test guidelines for reproduction and developmental toxicity*

##### *OECD*

A number of chemicals are known to produce developmental neurotoxic effects in humans and other species. These effects may arise in the offspring from exposure of the mother during pregnancy and lactation and can occur at lower dose levels than in adults. In Copenhagen in June 1995, an OECD Working Group on Reproduction and Developmental Toxicity discussed the need to update existing OECD Test Guidelines for Reproduction and Developmental Toxicity, and the development of new Guidelines for end points not yet covered.

The working group recommended that a guideline for developmental neurotoxicity should be written, based on a US guideline. In June 1996, a consultation Meeting was held in Copenhagen to provide the OECD Secretariat with guidance on the outline of a new guideline on developmental neurotoxicity. In October 1999, a proposal for a new OECD Guideline 426 Developmental Neurotoxicity Study was developed based on comments from the scientific community.

Concurrently with the revision of the existing guideline on 2-generation reproductive toxicity (TG 416), the work on testing and assessment of endocrine disrupters began in December 1996 and in February 1998, a

Working Group on Endocrine Disrupter Testing and Assessment (EDTA) was established. A revised TG 416 was circulated for comments in October 1998 and it was asked whether there is a chance to build in endocrine disruption endpoints into the current revision of the TG 416.

*EU*

For the evaluation of new industrial chemicals (i.e. introduced after 18 September 1981), Annex V of Council Directive 67/548/EEC (1967) includes a series of test methods. These include among others a teratogenicity test in rodents and non-rodents (B.31) and one-generation (B.34) and two-generation (B.35) reproduction toxicity tests. The test methods in Annex V are based on OECD guidelines and are normally updated concurrently with updating of the OECD guidelines.

*US-EPA*

A guideline for developmental neurotoxicity study was issued by US-EPA in 1991 (US-EPA 1991) and a revised US guideline was proposed in 1995.

The guideline provides an evaluation of the potential functional and morphological effects on the developing nervous system that may arise in offspring following exposure of the maternal animal during gestation and lactation. The guideline was designed to test a wide variety of classes of chemicals, e.g., pesticides and industrial chemicals; however, it was never intended that every chemical should be required to undergo such testing. Testing is required on a case-by-case basis taking into consideration what other toxicity information is available on each chemical or class of chemicals. (Francis 1992).

*Aim of the project*

The present project has been initiated by the Danish Environmental Protection Agency with the purpose of elaborating a detailed review and update the knowledge on the exposure and vulnerability of humans to chemical substances during the embryonic, foetal, and postnatal periods. The report is intended to form the scientific basis for future regulatory work of the Danish EPA in the protection of children and the unborn child to environmental chemical substances. Consequently, the report primarily focuses on chemical substances and regulatory aspects for which the Danish EPA has the responsibility. However, other chemical substances, such as drugs, have been included when they provide illustrative examples within the context of this report.

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## 2 Biological susceptibility

### 2.1 Susceptible periods in human development

The nature and magnitude of chemically induced damage does not depend only on the substance and dose in question, but also on the condition of the tissue with which the substance comes into contact. A given substance administered at equal dose rates but at different time points in the gestational and postnatal periods may produce essentially different types of abnormalities or functional deficits while different substances administered at the same point in time can produce identical damages. Disturbances in the normal biological cycle can lead to the early loss of the conceptus and embryo-foetal damage (i.e. malformation and functional defects).

*In utero* and postnatal developmental susceptibility to xenobiotics may depend on a number of determinants, including

- the genotype of the individual.
- the developmental stage at exposure.
- the mechanism of action of the substance.
- the kinetics of the xenobiotics in the mother, the conceptus and the child.
- the dose-effect and dose-response relationships.

(Dencker & Eriksson 1998, Harris 1997, National Research Council 1993).

#### 2.1.1 Human developmental stages

Knowledge on the normal reproductive cycle and developmental stages is important for understanding developmental toxic effects of chemical substances and their underlying modes of action.

The terminology used for the various human developmental stages is given in Table 2.1. Milestones in early organ development are given in Table 2.2 and the periods of susceptibility for persistent malformations are given in Figure 2.1 (see Annex 1).

#### *Germ cell formation and maturation*

Germ cell formation and maturation in men and women occur at quite different stages in the life cycle. In both sexes, the primordial germ cells appear at an early stage in the embryo-foetal development. However, in the female foetus, the germ cells divide during foetal growth and thus, females are born with their total number of oocytes. No new ova are formed after birth. But the oocytes are not fully developed at term. In the fourth month of gestation, the primary oocyte is arrested in the first meiotic division and the second meiotic division is not taken place until the time of ovulation during the adult females reproductive life. In contrast, the male germ cell formation and subsequent maturation does not start until around puberty and continuous throughout life.

As cells are particularly sensitive to exogenous agents when dividing, this difference between men and women may indicate that the highest risk of causing mutations in germ cells occur in the fertile period for both sexes, but in addition, the female foetus may be at increased risk around the fourth month of gestation. (Barbera 1997, LST 1989, Johnson et al. 1997).

Table 2.1 Stages in human development (from Larsen & Pascal 1998)

Developmental stage	Time period
Germ cell formation and maturation	-
Preimplantation stage	First week of pregnancy
Embryonic stage	2 - 8 weeks of pregnancy
Foetal stage	9 weeks of pregnancy - birth
Pre-term birth	24 - 37 weeks of pregnancy
Full-term birth	38 - 42 weeks of pregnancy
Perinatal stage	39 weeks of pregnancy - 1 week after birth
Neonatal stage	birth - 4 weeks
Postnatal stage	after birth
Infancy	birth - 12 months (young: 0 - 4; older: 4 - 12)
Childhood	1 year - 12 years (young: 0 - 4; older: 4 - 12)
Adolescence	>12 years - 18 years
Adulthood	>18 years

*Preimplantation stage  
conception*

At conception, the ovum is fertilised and during the next four days, the conceptus is conveyed through the oviducts to the uterine cavity. The movement of the conceptus may be affected by anatomical defects in the oviduct (malformation, formation of cicatricial tissue) and by disturbances in the woman's autonomic nervous system or hormone metabolism, particularly of sex hormones.

*blastogenesis*

During the transportation, the conceptus develops by cleavage to a blastocyst, a fluid filled vesicle. Around day 6 implantation of the blastocyst takes place. At first the blastocyst is fully dependent on the supply of nutrients via passive diffusion from surrounding secretions. It has been demonstrated that foreign substances can accumulate in the secretions within the blastocyst.

During the preimplantation period, the conceptus is in general considered refractory to exogenous insults in terms of induction of classical malformations, but exposure to certain agents may have the potential for inducing embryonic death and/or latent developmental defects (e.g. DDT, nicotine). A reasonable explanation for this is that a massive insult during this developmental stage will kill the conceptus, while, on the other hand, if only a few cells are damaged they will be replaced by other cells as the cells of the embryoblast still maintain a high degree of pluripotency (the potential to differentiate into various kinds of cells).

Although seldom in this developmental period, it has been possible to induce malformations by exposure of experimental animals to chemical substances (e.g. ethylene oxide, methylnitrosourea). (Dencker & Eriksson 1998, LST 1989, Rogers & Kavlock 1996).



*Embryonic stage  
gastrulation*

During the second and third week, gastrulation (formation of the three germ layers) and beginning of placental formation takes place. In mice, exposure to ethanol during gastrulation may lead to ocular, facial and neural malformations, which in many ways are similar to the foetal alcohol syndrome seen in human infants.

*organogenesis*

In humans, the organogenesis (used here with the same meaning as embryogenesis) occurs between approximately day 18 and 58 of gestation, ranging from the appearance of the neural plate to the closure of the palate shelves (Table 2.2). The period of organogenesis is considered to be the developmental phase most sensitive to exogenous induced classical malformations in single organs and/or syndromes of malformations. Within organogenesis, there are periods of maximal susceptibility for each forming structure (see Figure 2.1 in Annex 1). During this short period, the embryo undergoes rapid and dramatic changes and therefore, the nature of the embryo/ foetus as a target for toxicity is also changing. At week 3 of gestation, the human conceptus is in most ways indistinguishable from other vertebrate embryos. By weeks 8, the embryo is unmistakable human although the organs are not yet completely functional. The rapid changes of organogenesis require extensive cell proliferation, cell migration, cell to cell and cell to matrix interactions, and morphogenetic tissue remodelling. Differentiating cells in various organ precursors now start to develop susceptibility, for example, to xenobiotics affecting specific receptors. Loss of cells, whose function by now is often completely determined, can no longer be replaced by the surrounding cells and disturbances will result in permanent damage. Known human chemical teratogens, which are active mostly in this period, are e.g. cytostatics, thalidomide, and diethylstilbestrol. (Dencker & Eriksson 1998, LST 1989, Harris 1997, Rogers & Kavlock 1996).

*Foetal stage*

Foetogenesis, the period from 9<sup>th</sup> week of pregnancy and onwards, is characterised by tissue differentiation, growth and physiological maturation (histogenesis). Exposure to xenobiotics in this period (e.g. tetracycline, aspirin, warfarine, ethanol, lead, and methylmercury) is most likely to result in effects on growth and functional maturation and not considered resulting in major morphological malformations. The formation of the organs is not complete, but almost all the organs are present and grossly recognisable.

The further development proceeds during the foetal period to attain the requisite functionality before birth, including fine structural morphogenesis (e.g. outgrowth of renal tubules) as well as biochemical maturation (e.g. induction of tissue specific enzymes). Receptors and other molecular targets for substances affecting future functions are continuously developing, so that the foetus may be even more sensitive than the embryo to some pharmacological effects. Functional abnormalities of the central nervous system (CNS) and reproductive organs, including behavioural, mental and motor deficits and decreases in fertility are among the possible adverse outcomes. These manifestations are not necessarily apparent at birth. Until the 24<sup>th</sup>-26<sup>th</sup> week of pregnancy, the organ functions are not sufficiently developed to enable the foetus to survive outside the uterus and even at this time, the histogenesis is not yet complete in all organs. In the 36<sup>th</sup> week of pregnancy, organ differentiation is more or

less complete and the foetus is described as being fully developed. Birth normally occurs around the 40<sup>th</sup> week of pregnancy. (Dencker & Eriksson 1998, LST 1989, Harris 1997, Rogers & Kavlock 1996).

### *The Programming Hypothesis*

A number of findings reported over the last decade suggest that exposure to environmental factors in early life can increase the risk of disease later in life. For example, low birth weight, thinness and short body length at birth has been associated with increased rates of cardiovascular disease and non-insulin dependent diabetes in adult life. To explain such findings, the concept of physiologic programming or imprinting in early life has emerged. Interference with such genetic programming has been demonstrated in a variety of test systems and reflects the action of a factor during a sensitive period or window of development to exert organisational effects that persist throughout life. Programming agents may include growth factors, hormones, and nutrients. These factors may produce adaptations that permanently alter adult metabolism and responses in a direction optimising survival under continued conditions of malnutrition, stress, or other deprivation, but such responses might be detrimental during later life. (Barker 2000, Seckl 1998, Johansson 1996).

### *Placental development and function*

The placenta is the interface between the mother and conceptus. The unborn child receives all its nutrition via the placenta and also xenobiotics may enter the circulation of the conceptus through placental transfer after maternal exposure. Placental development begins immediately after implantation of the blastocyst and by the 4<sup>th</sup> week a circulation is established on both the maternal and the embryonic side of the placenta. The maternal physiology as well as the properties of the xenobiotics influence the placental transfer. The rate of transfer is affected by e.g. placental structure, placental blood flow, pH differences between the maternal and foetal blood circulation as well as the molecular size, the lipid solubility, the ionising state, and the protein binding property of the compound per se. Rodent studies have shown that during pregnancy, the pH of maternal plasma remains fairly constant, but the pH of the embryo/foetal compartment changes from slightly alkaline (relative to the maternal plasma) during early organogenesis to a more acidic environment during late organogenesis and foetal development. Therefore, placental transfer and potential accumulation of weakly acidic substances is favoured during the embryogenesis, while weakly alkaline substances are more likely to be transferred during late gestation.

The placenta is extremely permeable to chemical substances and it is now believed that almost all xenobiotics enter the foetal circulation. Chemical substances, which pass maternal membranes, are likely also to pass the placental barrier. Therefore, in general the foetus is not protected against xenobiotics that circulate in the maternal blood. (Clarke 1997, LST 1989, Hakkola et al. 1998).

### *Postnatal period*

This period appears to be vulnerable with respect to the physiological development of the nervous, immune, and endocrine/reproductive system as these systems continue to develop until adolescence. This heightens concern that toxicity during the postnatal developmental periods may have consequences throughout adult life. Particular attention has been paid to delayed functional postnatal toxicity. This effect has been ob-

served for several neurotoxic pesticides in adult animals as a result of exposure to subtoxic doses during a developmental period of high susceptibility, but there is also concern for delayed effects on the reproductive and immune systems.

#### *nervous system*

Especially the CNS should be considered a major target organ as its development is very complex and the developmental period is longer than for other organs (see Figure 2.1 - in Annex 1). Exposure to toxic agents (e.g. lead, DDT, organophosphate pesticides, PCBs) during the peri/post-natal developmental period may induce persistent functional/ behavioural effects that become manifest shortly after birth or later in life. However, experimental and clinical data have revealed that the consequences of age-related exposure to xenobiotics are difficult to predict. Thus, for DDT, it has been reported that new-born and pre-weaning rats are less susceptible than adults with respect to acute toxic effect. Concerning chronic effects (neurotoxicity), damage can be induced in the foetus at levels not causing effects in the mother. Exposure to toxic agents during the perinatal/neonatal period can also potentiate and/or modulate the reaction to adult exposure to xenobiotics. Studies in mice indicate that differences in adult susceptibility to neurotoxic substances are not necessarily an inherited condition, but may be acquired by exposure to environmental chemical substances during perinatal life when the maturational processes of the developing brain and central nervous system are at a stage of critical vulnerability.

Stages in the development of the nervous system include proliferation, differentiation, migration, synaptogenesis and axonal growth, and myelination. The major organ structures are formed as a result of cell proliferation during early organogenesis followed by cell migration in which the neurones travel to their final destination. However, cell migration is not completed until several months after birth and the cellular differentiation, which is characterised by formation of connections and myelination, is partly postnatal and lasts to the third or fourth year of life. The locations and numbers of cell receptors are very different in the immature and the adult individual.

Brain growth in children occurs rapidly during the first two years of life and at the age of two, about three-fourth of the total number of cells in the brain is present. However, in humans the full number of neurones is already obtained at the perinatal stage and the brain growth after birth is due to myelination of white matter, maturation of axonal and dendritic outgrowth, and multiplication of glial cells. The subsequent growth after the age of two years continues more slowly.

The brain size in infants and children is proportionally larger compared with adults. In a new-born child, the brain weighs about one-third of the adult brain. Compared to this, the new-born body weight only constitutes 4% of the adult weight. The cerebral blood flow per mass unit of brain weight is about 25% higher in a 10-year old child compared to a 65-year old adult. Thus, children have a higher relatively brain mass as well as a higher cerebral blood flow than adults.

The blood-brain barrier is gradually developed during gestation and is not complete until around 6 months after birth, and therefore the developing CNS is much more sensitive to toxic injury than the adult CNS.

The higher relative cerebral blood flow and immaturity of the blood-brain barrier, combined with increased unbound free fraction of xenobiotics in the infant blood (see toxicokinetics), may result in an increased exposure of the infant CNS to potential toxic agents. Further, the high lipid content in the CNS and its relatively greater mass in children may influence the distribution and storage of xenobiotics in infants. In contrast to humans, where the brain growth spurt begins during the third trimester of pregnancy and continues throughout the first two years of life, the period in rats and mice is neonatal, spanning the first three to four weeks of life. This knowledge is relevant in relation to testing of chemical substances.

#### *immune system*

There are structural and functional differences between the immune system of children and adults. The rapid maturation of the immune system that occurs during the peri- and postnatal periods is, in part, driven by unintended (food, infections, and other environmental agents) and intended (vaccination) exposure to antigens during development. Only little is known about how the developing system might be affected by xenobiotics. However, the rapid changes that occur during perinatal development indicates that exposure to chemical substances potentially can perturb the immune system in a variety of ways and at various points in the maturation process. It has been shown that impairment of the differentiation of thymocytes into immunocompetent T-cells can be induced by chemical damage to the thymic epithelial cells (e.g. 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD)). The effect seems most profound and persistent when the exposure takes place in the pre- and/or neonatal period.

The absolute size of the lymphatic organs decreases during the adolescent period. In spite of this decrease, the immune functions increase in capacity to respond as body growth progresses.

Data have shown that development of allergy from oral sensitisation apparently occurs mainly during the first years of life. The special sensitivity of infants may be related to increased intestinal uptake of allergens, and the immaturity of local and systemic immunological response. Sensitisation to food proteins may occur prenatally or postnatally. However, intrauterine sensitisation is believed to be a rare event.

Contact allergy to chemical substances may occur both in children and adults. There is probably no difference in the ability of children being sensitised compared to adults. It is generally acknowledged that sensitivity to contact allergens increases with age and environmental exposure.

#### *reproductive system*

As sex hormones cause numerous effects on a variety of tissues of males and females, including the gonads, bones, skin, hair follicles, and skeletal muscles, substances that interfere with hormonal systems may influence the development of an organism.

In the age of 10-11 years, the human female enter the pubertal period. In the puberty, the breasts enlarge and develop, primarily in response to the increase in oestradiol and progesterone levels, but the process are also influenced by glucocorticosteroids and thyroid and growth hormones. The menarche usually occurs about age 13. During puberty, a large increase in total body fat is seen.

In the male, the onset of puberty is about age of 12-13 years and the first outward physical signs are penile growth and an increase in testicular size due to increase in the diameter of the seminiferous tubules as spermatogenesis is initiated. These changes are primarily caused by an increased testosterone level. Other events related to the increased hormone production are e.g. stimulation of cell division in the epiphyseal cartilage of the long bones (leading to growth spurt), facial hair growth, deepening of the voice and increase in muscle mass.

It has been shown that a number of the chemical substances present in the environment may exert oestrogenic, anti-androgenic, or related activities on reproduction in laboratory animals (e.g. alkylphenols, DDEs, PCBs, dioxins). Similar effects on humans cannot be excluded, although there is as yet no evidence to prove a causal link between environmental endocrine modulators and humans reproductive health.

Normal masculine differentiation occurs under the influence of the SRY-gene and several other autosomal genes and androgens are required for this process. Disorders of gonadal development are frequently associated with testicular germ cell neoplasia. Oestrogens act through a specific nuclear receptor. Normal masculine differentiation occurs even in the absence of a functioning oestrogen receptor, but the individual with this receptor defect will develop poor semen quality. Oestrogen receptor deficient male mice were subfertile and few were able to sire a litter. Oestrogens are involved in the feedback regulation of gonadotropin secretion and suppression of FSH secretion during the proliferation period may result in small testes and low sperm production capacity in adult life.

During the perinatal period, the rodent Sertoli cells of the testis have been shown sensitive to thyroid hormones. Low plasma levels of thyroid hormones extend Sertoli cell proliferation and delay the differentiation, which results in an increased number of these cells in the adult testis.

Several xenobiotics (e.g. PCBs) have the ability to cause disturbances in the thyroid hormone homeostasis.

#### *references, postnatal period*

(Barbera 1997, Barker 2000, Bruckner & Weil 1999, Dencker & Eriksson 1998, LST 1989, Graeter & Mortensen 1996, ILSI 1996, WHO 1989, Johansson et al. 1996, Johnson et al. 1997, MST 1995, National Research Council 1993, Olin 1998, Renwick 1998, Roul et al. 1999, Schilter & Huggett 1998, Seckl 1998, Snodgrass 1992, Østergaard & Knudsen 1998).

#### *Lactation period*

In the sucking period, the human xenobiotic metabolising systems are still immature, but the new-born is no longer protected by the maternal metabolic system. Thus, the infant may be highly susceptible to xenobiotics in this period. However, to some extent the new-born is still protected as the breast milk has been subjected to the maternal metabolic system.

Most water-soluble substances are excreted into the milk by simple diffusion. Lipid soluble compounds are transported along with lipid molecules from plasma into the mammary gland. Several factors play a role in determining the quantity of a xenobiotic that may be transferred to breast milk. Compounds such as dioxins, DDT, PCBs, lead, mercury, and organochlorine pesticides are known to occur in breast milk. The amount of a certain substances transferred to the milk is dependent on:

- Physico-chemical factors of the substance: Degree of ionisation, molecular weight, lipophilicity, and protein binding capacity.
- Maternal factors: Dosage, frequency, and route of exposure to the compound.
- Infant conditions: Amount and frequency of feeding.

The blood plasma has a pH value about 7.5, whereas the value in milk is about 6.5. As the cellular membranes are primarily permeable for non-ionised molecules, the concentration in the milk will be higher than in the blood if the compound is slightly alkaline and lower if the compound is slightly acidic. A high protein binding capacity will lower the secretion into the milk as this results in smaller amount of free substance available for diffusion through mammary alveolar membranes.

Of concern for neonatal exposure are lipid soluble toxicants that had previously accumulated in maternal fat stores before and during pregnancy. As the body fat content returns to non-pregnant levels, the concentration of lipophilic toxicants in the rest of the body increases and this may cause enhanced potential for lactational transfer of harmful substances. (Bennett 1997, Clarke 1997, Danmarks Apotekerforening 1976, Kacew 1992).

### 2.1.2 The reproductive cycle: Comparisons between human and common experimental animals

Most of the substances to which humans are being exposed are assessed on the basis of data from animal experiments. Therefore, it is important to focus on the differences which exist between humans and the experimental animals used. Factors, which affect the extrapolation from animals to humans include:

- differences in the placental barrier
- differences in the rate of growth of the embryo/foetus
- physiological and biochemical differences affecting the intake, metabolism, and elimination of the substance administered
- differences in sensitivity to chemical disturbances in cells, tissues, organs and organ systems
- differences in the factors underlying reproductive damage.

At birth, there are large similarities between the placenta of certain experimental animals and the human placenta. However, during pregnancy the placenta changes greatly. Therefore, in order to assess the degree of embryo-foetal damage, it is necessary to know the state of the placenta at that point in organogenesis when the damage occurs.

The transport of nutrients and other compounds to the embryo or foetus will depend on the state of the placenta at any given time. During the early stages, the histotrophic supply via trophoblasts (embryonic cells which later develop into placenta) is the main form and supply consists of maternal macromolecules, which are biodegraded in the trophoblasts, secretions from the uterus, and exudate from the maternal blood. The subsequent haemotrophic supply consists mainly of the transfer across the placenta from the maternal blood to that of the embryo or foetus. This type of supply cannot take place until after the stage of organogenesis where vessels are formed and the heart begins to circulate blood. The

haemotrophic nutrition becomes the principal way around day 30 in humans and around day 9-10 in mice and rats.

Experimental animals (mice and rats) have a yolk sac placenta, which plays a very significant role during organogenesis. This is not the case for humans and monkeys in whose the yolk sac placenta is of insignificant importance. Foreign compounds may lead to malformations in rats and mice due to accumulation in the yolk sac (e.g. Trypan blue). This is not possible in humans and monkeys.

Knowledge of the different stages in the organogenesis in the various experimental animals and humans is important for the assessment of animal experiments in relation to consequences for humans. Differences in early organ development in three species are outlined in Table 2.2.

Table 2.2 Differences in development in four species (in days).

	Human	Rat	Rabbit	Guinea pig
Length of gestation	267	22	32	64-68
Blastocyte formation	4-6	3-5	2.6-6	5
Implantation	6-7	5-6	6	6
Organogenesis	18-58	6-17	6-20	-
Primitive streak	16-18	9	6.5	-
Neural plate	18-20	9.5	-	13.5
Heart begins to pump	22	10.2	-	16.5
Neuropore (cr.) closes	24-25	10.5	-	15.5
Upper/fore limb buds	29-30	10.5	10.5	16.5
Lower/hind limb buds	31-32	11.2	11	18.5
Hand plates form	35	13.4	14.5	23.7
Testes differentiation	43-44	14.5	20	26
Heart septation	46-47	15.5	-	-
Palatal closure	56-58	16-17	19-20	-
Brain growth spurt <sup>#</sup>	3. trim.⌘ - 2-3 years	0-30 a.b.*		
Testicular descent	Perinatal	> 15 a.b.*		
Sperm production and maturation	12-14 years	45-60		
Ovulation	10-14 years	35-45		

# Functional/behavioural development continues for several years

⌘ trim.: trimester

\* a.b.: after birth

The large variations among the various animal species with respect to duration of histogenesis (the maturation phase, including cell proliferation, where the organ is finally structured) have to be taken into account when extrapolating from animals to humans. With respect to certain fields, the human child is more developed at birth than e.g. the rat. The CNS is the organ, which shows the greatest difference between animals and man. A

major part of the development is prenatal in humans, but postnatal in the rat. Thus, with respect to CNS development, the new-born rat is comparable to the human foetus at the start of the third semester and the 6-10 days old rat is comparable to the neonatal infant. Consequently, the rat being experimentally exposed *in utero* and during the nursing period in toxicity studies is developmentally similar to the human development *in utero*.

(Dencker & Eriksson 1998, LST 1989, Ojeda & Urbanski 1994, Olin 1998, Rogers & Kavlock 1996).

### 2.1.3 Overall, susceptible periods

#### *Embryo-foetal period*

Chemical substances that pass maternal membranes are likely also to pass the placental barrier. As the placenta is extremely permeable to chemical substances, almost all xenobiotics enter the foetal circulation. Therefore, the foetus is generally not protected against xenobiotics that circulate in the maternal blood.

During the preimplantation period the conceptus is in general considered refractory to exogenous insults in terms of induction of classical malformations, but induction of embryonic death and/or latent developmental defects may occur. The chemically induced classical malformations and/or syndromes are mainly induced in early pregnancy during the period of organogenesis.

Exposure through the following foetal period (after 8 weeks of pregnancy) is not considered to result in major malformations, but susceptibility to substances affecting specific receptors and molecular targets still under development may lead to pronounced effects on a number of developmental processes. Informations concerning induction of such changes in humans in the foetal period are relatively scarce, but based on studies in experimental animals, it appears that this period may be even more sensitive than the embryonic stage to some growth and functional disturbances. However, in humans it may be difficult to interpret whether such damages are pre- or postnatal events.

A number of findings reported over the last decade suggest that exposure to environmental factors in early life can increase the risk of disease later in life. To explain such findings, the concept of physiologic programming or imprinting in early life has emerged. Interference with such genetic programming has been demonstrated in a variety of test systems and reflects the action of a factor during a sensitive period or window of development to exert organisational effects that persist throughout life.

#### *Peri- and postnatal periods*

The peri- and postnatal periods appear to be vulnerable periods, especially with respect to the physiological development of the central nervous system. There is human evidence that exposure to toxic agents (e.g. methyl mercury, TCDD) during the perinatal period of development may induce persistent functional/behavioural effects that become manifest shortly after birth or later in life although not morphologically apparent. After birth, the physiological development of the nervous, immune, and endocrine/reproductive systems continue to develop until adolescence. Therefore, the postnatal period should also be considered vulnerable. During the breastfeeding, the infant is exposed to chemical substances



through human milk. As the functionalities of many organs are still immature, the new-born may be particularly susceptible to xenobiotics.

## 2.2 Toxicokinetics and toxicodynamics in human development

Except for a few specific substances (primarily drugs), not very much is known about whether and why the response to a compound may differ between children and adults. Especially with respect to older children and adolescents there is a paucity of information. Differences in organ susceptibility are a function of toxicokinetic and toxicodynamic parameters, including genetic, physiological and metabolic factors, mechanism of action of the chemical, and dose-effect and dose-response relationships.

The **toxicokinetic** aspect covers absorption, distribution, metabolic biotransformation, and excretion of the substance in question.

The **toxicodynamic** aspect deals with parameters such as organ sensitivity and cytoprotective mechanisms, which determines the extent of any effect or response due to the presence of the substance at the site of toxicity.

The body's handling of substances may vary with age, but in general it appears that effects of xenobiotics on organs or end-points may be similar in children and adults. Data from drug studies in humans have shown that toxic effects in children and adults are similar qualitatively, but may differ quantitatively.

Children do not generally seem to represent a special group from a toxicokinetic viewpoint, i.e. the variability between children has a similar magnitude as the variability between adults (Renwick 1998).

In the context of this document, it is most convenient to present the two items together. The chapter is meant as a general introduction to the subject toxicokinetics/-dynamics. Therefore, case stories concerning chemical substances causing toxic effect are not given in details here, but where relevant, names of substances are briefly mentioned (in brackets). Further details can be found in section 5.

With respect to prenatal toxicology, the following toxicokinetic factors have to be taken into consideration when attempting to determine whether a chemical may reach and harm the conceptus:

- The kinetics in the mother (absorption, distribution, metabolism/elimination, and excretion).
- Transfer from the mother to the embryo/foetus via the placenta.
- The kinetics in the embryo/foetus.

Toxicokinetic conditions in the mother may have a decisive effect on the concentration of a chemical substance that reaches the embryo or foetus. During pregnancy, many physiological changes occur in the maternal organ system as a consequence of, and in order to support, the rapid growth of the foetus and reproductive tissues. These changes may in different

ways influence the intake, absorption, distribution, metabolism, and elimination of xenobiotics, and may involve the gastrointestinal tract, cardiovascular system, excretory system, and respiratory system. The gastric emptying time increases and the intestinal motility decreases, which may result in longer retention of ingested xenobiotics in the upper intestinal tract and increased possibility for enhanced absorption of xenobiotics.

The albumin concentration in plasma decreases to about two thirds of the normal level. This may lead to a higher amount of free unbound xenobiotics in the blood, hence increase the possibility for transfer via the placenta. Besides, changes in the blood concentrations of e.g. lipids, free fatty acids, and hormones during pregnancy may influence the distribution of xenobiotics in the body. By the end of human pregnancy, the total body water content has increased by up to 30% and often fat deposits have been build up. These conditions may influence the distribution of xenobiotics between the mother and the conceptus.

The cardiac output and the peripheral blood flow increases by approximately 30% during the first trimester of gestation.

The net gas respiratory minute volume is raised by about 50% due to increased tidal volume, while the pulmonary ventilation rate is not changed. Thus, volatile and airborne chemicals tend to be absorbed more readily. But this may, on the other hand, also result in faster elimination of volatile compounds from the body.

Also the renal blood flow and glomerular filtration rate are increased, which may result in enhanced renal clearance of certain xenobiotics. In experimental animals, the liver's ability to metabolise foreign substances is altered during pregnancy. In the rat, a decrease in hepatic monooxygenase and glucuronidation activities have been observed and there seems to be an overall decrease in hepatic xenobiotic biotransformation during pregnancy. This decrease in activity is almost balanced by a 40% increase in liver weight during pregnancy. A similar change in liver size is not seen in humans.

Finally, there are signs of metabolic changes and increasing activity in certain endocrine organs.

After birth, age-dependent developmental changes in physiological parameters takes place, particularly with respect to hepatic and renal function, which are described in details below. The main differences between neonates and adults are summarised in Table 2.3.

In most tissues, the cell proliferation rate is higher during development and growth compared to the fully developed organism. During infancy and adolescence, children are growing and adding new tissue rapidly, but the various organs and tissues are maturing at different rates. For example, the CNS cell population (but not the myelination) is relatively complete at two years of age and the brain achieves 50% of its adult weight by 6 months of age. In contrast, with respect to the liver, kidneys and heart, 50% of the adult weight is not achieved until the age of about 9 years. For the skeletal system, this value is reached at about 11 years of age.

The different growth rates and biochemical pathways in the various tissues causes changes in the physiological conditions which will alter the disposition of xenobiotics over time. This may imply increased organ sensitivity in foetal, neonatal, and infant organs. On the other hand, it has been speculated that the higher proliferation rate during growth may en-

hance the rate of tissue repair and thus leading to a decrease in sensitivity. Anyhow, the quantitative and qualitative implications of the difference in cell proliferation rate on organ sensitivity are unclear.

Experiences gained from toxicological studies in laboratory animals support the suggestion that it is not possible to make any general statements about age-related differences in organ sensitivity. For some chemical substances, immature animals are more sensitive than adults while in other cases, they are less sensitive, depending on the compound and its effects (see perinatal, neonatal, and postnatal period).

Differences in cell proliferation rate between children and adults are not the only parameter that may lead to differences in susceptibility. Receptors and other molecular targets for various xenobiotics are continuously developing during the embryonic, foetal, and infant periods. This may cause age-dependent differences in the outcome of receptor-xenobiotic interactions and even result in opposite effects of chemical substances in infants and adults.

In relation to organ sensitivity, the literature is primarily directed towards cancer and effects on the immune, reproductive, and nervous systems (see perinatal, neonatal, and postnatal period).

From animal experiments it has been shown that transplacental exposure to chemical substances can cause tumour development in the offspring. With respect to humans, it has been shown that girls born from mothers exposed to diethylstilboestrol during pregnancy were at increased risk for developing vaginal cancer. Classical animal experiments have indicated an enhanced perinatal sensitivity for genotoxic, but not non-genotoxic carcinogens. For some genotoxic chemicals, dramatically increased sensitivity can be observed if the compound is administered to animals during a critical period within few days after birth (e.g. dimethylbenz[a]-anthracene (DMBA)). It has been suggested that this critical period may be when the liver is actively expressing H-ras and there is rapid growth and cell proliferation.

(Clarke 1997, LST 1989, Graeter & Mortensen 1996, Larsen & Pascal 1998, National Research Council 1993, Renwick 1998, Østergaard & Knudsen 1998).

### 2.2.1 Absorption

#### *Gastro-intestinal system*

The gastro-intestinal tract is a major entry of xenobiotics to the body. A number of physiological changes occur in the gastrointestinal tract after birth, which could influence the absorption of xenobiotics (see Table 2.3). Thus, the relatively high gastric pH in new-born infants is one factor that may influence the absorption of xenobiotics. It has also been shown that intestinal absorption of various compounds is higher in neonate and young mammals compared with adults (e.g. lead). Decrease to the low-level uptake seen in adults occur about the time of weaning and has been associated with structural and functional maturation (reduction in pinocytotic activity) of the intestinal epithelial cells. Further, it has been suggested that development of a more selective intestinal absorption takes place with age and alterations of the diet.

Table 2.3 Examples of changes in physiological parameters.

<b>Absorption</b>	
Gastric pH	Raised at birth (pH 4-5) but falls to adult values within months after birth
Gastric emptying	Variable and irregular until 6-8 months of age
Digestive enzyme activity	Lower at birth, increases during the first year
Intestinal absorption	Higher at birth, decreases during the first months
Bacterial flora	Established soon after birth but change gradually over time
Respiratory rate	Decreases during adulthood
Surface area to body weight ratio	Decreases 2.5-fold from birth to adult stage
Pulmonal surface area	Increases > 20-fold from birth to 8 years of age
<b>Distribution</b>	
Body water: Total body water Extra cellular fluid volume Intra cellular fluid volume	At birth: 75% of body weight cf. 40-60% in adults Make up 40% cf. 20% in adults Make up 33% cf. 40% in a 6 month infant
Muscle	Lower relative mass in infants
Body fat	Increases 17-36-fold from birth to adult stage
Brain	Higher relative mass, lower myelin content, higher blood flow and reduced blood-brain barrier in infants
Plasma proteins	Lower protein binding capacity at birth, increase in capacity during the first year
<b>Elimination</b>	
Liver mass	Higher relative mass in infants
Oxidation	Lower levels of cytochrome P450 isoenzymes in early infancy
Ester hydrolysis	Lower levels in early infancy
Glucuronidation	Lower levels at birth
Renal function	Lower glomerular filtration and tubular secretion in early infancy

The complexity of factors makes it difficult to predict the net effect of maturation on absorption of chemical substances. The maturation of the gastrointestinal tract occurs within about 6 months and by late infancy,

most of the processes are comparable to that of the adult. Another important parameter for intestinal absorption is the biliary excretion, which depend on e.g. the enterohepatic circulation. Bile flow depends on the adequate synthesis, conjugation, secretion, and recirculation of bile acids. The two major bile acids have been found elevated in blood from one to four days old neonates. The levels then gradually declined over the next four to six months to adult values. The possible consequence of deficient bile excretion is inefficient intestinal fat digestion and inhibition of biliary excretion. Impaired fat digestion could be of toxicological importance when lipophilic chemicals are ingested.

(LST 1989, National Research Council 1993, Plunkett et al. 1992, Renwick 1998, Roberts 1992, Østergaard & Knudsen 1998).

### *Skin*

The larger surface area to body weight ratio compared with adults may have considerable implications for the exposure to xenobiotics by skin absorption in children. The new-born has approximately a 2.5-fold greater ratio than adults and still in 13-year old children, the ratio is greater. This may have considerable implications for children whose skin is exposed to environmental chemical substances as xenobiotics often are lipid soluble and therefore easily absorbed via the skin.

The most important barrier to dermal absorption is the stratum corneum (the horny outer layer of the skin). Studies have shown that the structure of the stratum corneum is not different in infants and adults and the overall thickness remains relatively constant throughout postnatal development. However, until 3-5 days following birth, the epidermis is unkeratinised. This means a reduced barrier function in this period, which may be an important factor in relation to new-born's dermal contact with xenobiotics (e.g. iodine, hexachlorophene, and aniline dye). After keratinisation has taken place, the percutaneous absorption appears to be comparable between infants and adults. Premature infants have been shown to exhibit an increased skin permeability for a period of more than 3-5 days. (National Research Council 1993, Plunkett et al. 1992, Renwick 1998, Snodgrass 1992, Østergaard & Knudsen 1998).

### *Respiratory tract*

The respiratory tract is the entrance for many chemicals (e.g. pesticides) and most xenobiotics are absorbed through the alveolar surface by simple passive diffusion.

The lung is in a period of growth and maturation during infancy and the various changes with age may be of consequence in relation to pulmonary uptake of xenobiotics. During the first years of life, there is a marked increase in the alveolar surface area. The new-born infant has approximately 10 million alveoli. By the age of 8 years, the number is 300 million, which is the adult number. The gas exchange area (alveolar surface) increases from approximately 3 m<sup>2</sup> at birth to 75 m<sup>2</sup> in adulthood and thus, the gas exchange area increases more than twenty-fold from infancy to adulthood. The full complement of mature cell is not realised in the lung until adolescence, as there is a progressive increase in the amount of elastic collagenous fibres in the alveolar wall until 18 years of age. The respiration rate decreases during adolescence from 40 breaths/minute in infants to 15 in adults whereas the tidal volume, on a body weight basis, is about the same for children and adults (10 ml/kg b.w./breath). Thus, the amount of inhaled air per unit of time, on a weight basis, in resting children is nearly three times that of the resting adult and thereby, children have a higher exposure to xenobiotics than

adults. The approximately 20-fold smaller lung surface area and 3-fold higher respiratory minute volume/kg b.w. imply that children have a more than 60-fold higher respiratory minute ventilation rate (expressed as ml inhaled air/kg b.w./m<sup>2</sup> of lung surface area/minute) and thereby, a greater possibility for inhalation of xenobiotics than adults. These conditions could mean, at least with respect to local effect, that the vulnerability to xenobiotics by inhalation (e.g. oxygen, ozone) may be greater in children, particularly prematures, whose lungs are significantly less mature than the infant born at term. The lungs of a child might be able to tolerate the acute effects resulting from a toxic exposure, but subsequently develop chronic changes as a result of influence of the toxic event on lung maturation. However, it should be mentioned, that animal experiments have shown that for some xenobiotics (e.g. chlorphenteride), new-borns may be less sensitive.

A few studies concerning systemic absorption in experimental animals have indicated that lipid soluble compounds (procainamide, sulfosoxazole) were absorbed at similar rates by neonates and adults indicating that the properties of the alveolar epithelium do not change with age. However, lipid insoluble compounds (p-aminohippuric acid, tetraethylammonium bromide) were absorbed about twice as readily by 3-12 days old rats compared to adults. It is not known whether this difference can be extended to man.

(National Research Council 1993, Plunkett et al. 1992, Snodgrass 1992.)

### 2.2.2 Distribution

Plasma protein binding capacity is of importance for the distribution of foreign substances in the body. Xenobiotics often become bound to the albumin fraction and this may reduce the extent of tissue distribution in the body. The body composition of infants differs from that of adults (see Table 2.3) and this could influence the distribution of foreign substances.

The higher water content in infants means that the volume of distribution for hydrophilic xenobiotics may be twice as large in neonates compared with adults. In a 4-month old infant, the water content is at a level comparable with an adult. The amount of body fat is often lower in children compared with adults and this may in the same manner lead to differences in the distribution volume for hydrophobic compounds. Concerning prematures, their fat content is 1% of the body mass compared with 15% in a full term child. The relative body fat content begins to increase quite rapidly about 5-7 years of age. In females, this increase continues throughout adolescence and into adulthood. In males, there is usually a decrease in fat content during the mid to late adolescent years. Postpubertal females have approximately twice as much body fat as their male counterparts. There is a corresponding decrease in the female body water, expressed as percentage of body mass. The relatively high body fat in postpubertal girls may result in increased retention of lipid-soluble substances (e.g. DDT, PCBs and dioxins).

As mentioned above, the plasma protein binding capacity (primarily albumin) is of importance for distribution of xenobiotics. This is well known from the clinical pharmacology. Infants seem to have lower concentrations of mature albumin in the blood and therefore a decreased

protein binding capacity. Adult values are seen about the age of 10-12 months. In addition, the content of free fatty acids in the blood is high in neonates and this may lead to a replacement of xenobiotics from albumin. The lowered capacity will increase the free fraction of xenobiotics in the blood and in this way affect the extent of distribution. A higher circular blood flow rate in children compared to adults may also influence the tissue distribution of xenobiotics. For some drugs, it has been shown that adult values are reached about one year of age.

(National Research Council 1993, Plunkett et al. 1992, Renwick 1998, Snodgrass 1992, Østergaard & Knudsen 1998).

### 2.2.3 Metabolism

The level of expression of xenobiotic metabolising enzymes shows a wide inter-individual variability depending on the age and tissue investigated. The most important parameter for the elimination of a wide range of xenobiotics is the activity and concentration of the key xenobiotic metabolising enzymes in the liver. 90-98 % of the xenobiotics are metabolised in this organ.

The liver undergoes various morphological and functional changes perinatally, including differentiation of hepatocytes and emergence of constitutive enzymes. The enzyme systems responsible for xenobiotic metabolism are usually grouped in phase I oxidative enzymes, consisting mainly of oxidative cytochrome P450 (CYP) enzymes, and phase II conjugating enzymes, such as uridine diphospho- (UDP)-glucuronosyltransferases, sulfotransferases, and glutathione S-transferases.

The phase I enzymes produce more polar products and may promote formation of reactive metabolites. The phase II enzymes catalyse reactions where the more polar metabolites are conjugated with water-soluble endogenous molecules which results in conjugates being more easily excreted. The toxicological consequences of exposure to chemical substances will depend on the balance between the phase I and II reactions. In humans, 14 CYP families have been described and the main site of expression of xenobiotic metabolising CYPs is the liver. The members of the CYP families 1 and 3 are considered to a great extent to metabolise xenobiotics, while the other families participate in the metabolism of endogenous substrates.

It should be mentioned that infants as well as adults show individual variability in xenobiotic metabolic capacity. This variability is to a large extent genetically determined, but environmental factors (e.g. exposure to chemical substances) can also play an important role in modifying the metabolic capacity of individuals

(Cresteil 1998, Hakkola et al. 1998, Renwick 1998, Østergaard & Knudsen 1998).

#### *Embryo-foetal period*

Animal models show that the metabolism of some, but not all, xenobiotics is mediated mainly by extraembryonic tissues and reflects the metabolic phenotype of the mother rather than that of the conceptus. The embryo/foetus of common experimental animals is relatively deficient in its

capability to metabolise drugs and other foreign substances until near the time of birth or even in postnatal life.

In contrast, the existence of a xenobiotic metabolising system in the human foeto-placental unit has now been established. The individual enzymes responsible for the metabolism of foreign compounds in the human conceptus are, however, still poorly characterised. The liver of the human foetus possesses a relatively well developed metabolic capacity towards xenobiotics. The xenobiotic metabolising enzymes start their development in mid gestation and maybe as early as the first trimester of gestation. The isoforms CYP3A7, 3A4 and 4A1 are expressed in significant amounts in the foetal liver. Other isoforms have been detected (CYP 1A1 and 3A5), but only at very low levels. They are mostly active on endogenous substances, but especially CYP 3A4 and 3A7 metabolise many foreign compounds and may play a major role in the foetal metabolism of xenobiotics. The CYP 2E1 seems not to be active in the foetal liver.

The prenatal expression pattern is clearly different from that in the adult liver. The reactions are fewer in number and their levels are generally lower. The total amount of P450 per mg microsomal protein in the foetal liver constitute 20 to 70% of the level in the adult liver. The P450 isoforms develop independently and show different onset. Some CYP forms are expressed in higher amounts in the foetal than in the adult liver whereas other P450 isoenzymes are low at birth and increase with age. Concerning esterase activity in the foetal human liver, it has been shown that it constitutes one third of that in adults.

Among phase II enzymes, epoxide hydrolase (which converts epoxides into dihydrodiols) is active in the foetal liver and account for 50% of the adult activity. The glutathione S-transferase  $\pi$  is abundant in the foetal liver, but regresses after birth. The  $\alpha$  and, especially, the  $\mu$  forms of S-transferases are present at rather low levels in the foetal liver. UDP-glucuronyl-transferase activities towards exogenous molecules and bilirubin were found to be extremely low in the foetal liver.

In the human foetus, CYP protein has been found in the adrenals. The content may be higher than that of the foetal liver and thus the adrenals may be able to metabolise xenobiotics.

Further, several other embryonic and foetal tissues including lungs, kidneys and cardiac tissue exhibit low metabolic activity for xenobiotics, among which are many carcinogens and mutagens.

It has been shown that a xenobiotic metabolising system exists in the human placenta and substances that pass through the placenta may therefore be metabolised during transfer. However, the metabolic profile of the placenta is clearly more restricted compared to the maternal liver and the individual enzymes responsible for the metabolism of foreign compounds are still poorly characterised.

The total microsomal P450 content/mg protein in the human full-term placenta constitutes 10-30% of the level in the adult liver. Multiple enzyme systems have been suggested to participate in the placental bio-transformation of xenobiotics and the CYPs constitute such a system. Most of the placental P450 consist of steroid metabolising CYP forms, especially CYP19 (aromatase). Xenobiotic metabolising CYPs represent only a minority of the total P450 and, apart from cigarette smoke-induced CYP1A1 activities, placental activity toward foreign compounds is low.



It has been shown that CYP1A1 metabolises and activates several compounds that are considered to be carcinogenic in humans. Besides the CYP activities, phase II conjugation capacity has been demonstrated in placenta. However, several of the enzymatic activities present in the maternal liver are absent or exist at very low levels in the placenta. It is suggested that individual genetic factors influence the enzymatic activity in placenta.

Although metabolic capacity has been discovered both in the foetus and placenta, the contribution of their metabolising enzymes to the total kinetics of drugs or xenobiotics administered to the mother is probably minimal. This is due to the relatively small size and low metabolic activity of the embryo/foetus and placenta compared with the mother. The maternal liver remains the major capacity of metabolism throughout pregnancy.

However, although the total capacity of the foetal liver to convert xenobiotics to both toxic and non-toxic metabolites is low, local toxic consequences, resulting from metabolism in the foetal compartment, may occur.

(Cresteil 1998, Hakkola et al. 1998, Renwick 1998).

#### *Infant and child*

The hepatic xenobiotic metabolism is still immature in humans at birth, especially in the pre-term neonate, but matures rapidly over the first months of life. Adult levels of most enzyme systems are achieved at 3-6 months of age. However, some investigations have shown that the total P450 content was about one-third of the adult value until one year of age. The isoforms CYP2D6 and 2E1 surge within hours after birth, but are probably not seen in the human foetal liver. CYP3A4 increases and 2C develops during the first weeks after birth and CYP1A2 is expressed after one month. Differences are observed in *in vitro* rate of postnatal maturation of different demethylation pathways indicating involvement of different isoenzymes. It has been shown that esterase activity was at the same level in 1-24 months old infants and adults.

Only few data are available in the literature regarding the ontogeny of Phase II enzymes in human tissues. The glutathione S-transferases  $\alpha$  and  $\mu$  are, as mentioned, present at rather low levels in the foetal liver, but the amount increases within three months after birth. UDP-glucuronyl-transferase activities towards exogenous molecules and bilirubin were extremely low in the foetal liver as well as in neonates aged less than 10 days. However, the majority of UDP-glucuronyl-transferase isoforms present in adult liver microsomes had developed within three months after birth.

Based upon rat studies, it has been suggested that conjugation of simple substrates is high at birth and then declines to adult levels, whereas the activity towards the more bulky substrates is low at birth and then increases. In agreement with this is the fact that neonates are more susceptible than older individuals to the bulky substance chloramphenicol due to diminished glucuronide conjugation. Glucuronic acid conjugation of many drugs reaches the adult values around 6 months of age. The evidence of impaired sulphate conjugation is less clear.

With respect to acetylation, studies have shown that infants have a limited capacity for acetylation of certain compounds and in some cases the adult phase II pattern is not reached until the age of two years.

Similar enzymatic changes take place in neonatal laboratory animals over the first 2-3 weeks of life prior to weaning. There seems to be a general pattern in phase I enzyme activity, in which there is an increase in activity postnatally that reaches a maximum level within the first weeks of life. In rats, the *in vitro* hepatic phase II activities increase 16-fold between birth and day 20. There seems to be differences in the expression of the various enzymes. Conjugation of simple substrates is high at birth and decreases to adult levels after 7 days. In contrast, the activity towards bulkier substrates is low at birth and increases after 20 days.

Metallothioneins are important in the binding and detoxification of toxic metals. There are low concentrations in most tissues in adult animals but levels can be increased by exposure to e.g. metals. The concentrations of metallothioneins in the liver increase dramatically in rats in the three days prior to birth and high levels are maintained for 7 days after birth following which there is a rapid decrease towards adult concentrations. A similar profile was found in neonatal mice and human liver samples.

Children have a higher basal level of metabolism than adults as the metabolic rate is inversely related to body size. The smaller body size, the greater surface to body mass ratio and the higher metabolic rate. A decrease of 66% in the surface area to body mass ratio is seen when an individual develops from infant to adult. The discrepancy in the ratio of surface area to body weight between children and adults is 2.3 at birth, decreasing to 1.8, 1.6, 1.5, and 1.3 at 0.5, 1, 5, and 10 years, respectively. The generally higher rates of metabolism will result in more rapid elimination of the parent compound and in greater or more rapid formation of metabolites. Further, the mass of the liver in relation to the whole body is larger in infants and children compared with adults. This will also contribute to the relatively higher metabolic activity observed in infants and children.

Expressing exposure as mg/kg b.w., which is traditionally used, may represent an extra safety margin because in scaling down from an adult to a child, the equivalent dose for an infant or child, based on surface area, will be higher than that based on body weight. A dose equivalent to 10 mg per adult would be 1.37 mg per one year-old infant based on body weight, but 2.32 based on surface area. Thus, the use of body surface area gives a better adjustment for parameters such as intermediary metabolism and basal metabolic rate.

The above indicates that in the neonate and young infant, the metabolic activity is low and therefore, the individual may be very sensitive to xenobiotics in this period. On the other hand, toxicity is quite often caused by the production of toxic intermediates. Since enzymes involved in their production (most notably the different P450 forms, but also conjugation enzymes) are not fully developed during the early infant period, compounds requiring biotransformation to become toxic may sometimes be less toxic to these individuals.

About 6 months of age, the adult levels of enzyme activity are almost obtained. Due to the higher basal metabolic rate in children, the metabolic activity may be even higher in children than in adults. However, whether this results in more, equal or even less sensitivity to xenobiotics in the older infant and child depends on the nature of the compound. It is difficult to generalise about age-dependent deficiencies in the metabolism of xenobiotics because the various enzyme systems mature at different time points. The age at which metabolism is similar to the adult value, may be different for each compound.

(National Research Council 1993, Plunkett et al. 1992, Renwick 1998, Snodgrass 1992, Wells & Winn 1997, Østergaard & Knudsen 1998).

#### 2.2.4 Excretion

Xenobiotics are predominantly eliminated from the body by renal and biliary excretion. Volatile compounds may be exhaled via the respiratory system.

##### *Renal excretion*

Renal excretion is the principal pathway for elimination of substances and is dependent on glomerular filtration, tubular reabsorption, and tubular secretion. The renal functions are immature in humans at birth and for a variety of xenobiotics, the renal clearance is low in new-borns (e.g. certain antibiotics), especially in the pre-term neonate. However, rapid maturation takes place over the first three to six months of life, followed by a gradual increase in function up to 6 years of age. The ability to concentrate urine is low at birth, but reaches the adult level at about 16 months of age.

All glomeruli are developed at birth, but immature and not fully functional. Histological examination of the kidneys has shown that in the neonatal period, the glomerular basal membrane is rather thick and becomes thinner in the weeks after birth. The glomerular filtration in new-borns is approximately one-third of the adult value, but seems to mature within the first months after birth. The glomerular filtration rate increases about 4-fold during the first 72 hours after birth in full term infants and adult values are in general reached by 3-6 months of age.

The tubular reabsorption is probably developed already at birth, whereas the maturation of tubular secretion seems to take five to eight months. Creatinine clearance is reduced particularly in pre-term infants. The renal clearance of a radiolabelled marker revealed a rapid increase in renal function during the first 12 weeks after birth followed by a gradual increase up to 6 years of age. Renal blood flow (expressed per gram of kidney) increases within the first 5 months. The value is found to be constant between one and 16 years of age.

Besides the influence of the maturation processes, the amount of compound cleared by the kidney is influenced by the degree of protein binding and the renal blood flow.

Postnatal changes similar to humans take place in the neonatal rat during the first weeks of life, prior to weaning. Glomerular filtration rate in 10 days old rats is about one-half that of adults. Effective renal blood flow

(per gram of kidney) and glomerular filtration rates are low in 4-week old rats, but approach adult values by 7 weeks of age.

The immature renal function seen in neonates leads to decreased elimination and prolonged serum half-lives for any compound that relies on renal excretion. About the age of 6 months, most of the renal functions have reached adult levels. At this time, the elimination of xenobiotics (at least drugs) may show a very varying pattern. The renal clearance may be higher as well as lower in infants compared with adults, depending on the compound.

#### *Biliary excretion*

During the first months of life, the new-born infant has a decreased ability to conjugate and eliminate substances in the bile. This may lead to accumulation of xenobiotics in the body and consequently toxic reaction (e.g. hexachlorophene).

Animal studies have in a similar way shown that new-born and young rats have a lower capacity to excrete certain compounds into the bile. Further, studies with non-metabolised as well as metabolised agents (e.g. via glucuronidation) have shown that both the parent compound and the metabolite is excreted more slowly in neonatal rats compared to adults.

#### *Respiratory excretion*

The respiratory minute ventilation, on a body weight basis, is about three times higher in children than adults. This may cause differences in the excretion rate of volatile substances between infants and adults.

(National Research Council 1993, Plunkett et al. 1992, Renwick 1998, Snodgrass 1992, Østergaard & Knudsen 1998).

### **2.2.5 Overall, toxicokinetics and toxicodynamics**

Age-related differences in toxicokinetics/-dynamics occur in both experimental animals and humans, particularly in relation to hepatic xenobiotics metabolism and renal function. Except for a few specific substances, not very much is known about whether and why the response to a compound may differ between age-groups. In general, it appears that effects of xenobiotics on organs or end-points may be similar in children and adults e.g., liver necrosis observed in adults will also be observed in children.

As regards toxicodynamics, age-dependent differences are primarily related to the specific and unique effects that chemical substances may have on the development of the embryo, foetus and child in that the physiological development of the nervous, immune, and endocrine/ reproductive systems continue to develop until adolescence. Furthermore, receptors and other molecular targets for various xenobiotics are continuously developing during the embryonic, foetal and infant periods. This may cause age-dependent differences in the outcome of receptor-xenobiotic interactions and even result in opposite effects of xenobiotics in infants and adults.

During pregnancy, many physiological changes occur in the maternal organism as a consequence of, and in order to support, the rapid growth of the foetus and reproductive tissues. These changes may in different ways

influence the intake, absorption, distribution, metabolism, and elimination of xenobiotics.

Metabolic capacity has been discovered both in the human foetus and placenta, but the contribution of their metabolising enzymes to the total pharmacokinetics of drugs or xenobiotics administered to the mother is probably minimal. The human foetus and the placenta possess metabolic capacity, but the contribution of these metabolising entities to the total kinetics is probably minimal.

In the early infancy, the organs are still rather immature and various maturation processes are taking place. The complexity of all these factors makes it difficult to predict the net effect on absorption, distribution, and elimination of chemical substances. However, the maturation of the gastrointestinal system, liver, and kidneys has generally taken place within 6-12 months after birth. By late infancy, most processes related to metabolic activity and excretion are probably comparable to that of the adults for most compounds. Because of the immature function of the organs, the neonates and young infants may have lower biotransformation and elimination capacities. This may render these individuals less able to detoxify and excrete xenobiotics and thereby more vulnerable to toxicants. On the other hand, if toxicity is caused by toxic intermediates produced via biotransformation, young infants may be less sensitive.

In the subsequent late infancy and childhood, metabolism and excretion of xenobiotics may be equal to or even higher than in adults due to the higher basal metabolic rate and relative liver size. It is difficult to generalise about age-dependent deficiencies in the metabolism of xenobiotics because the various enzyme systems mature at different time points. The age at which metabolism is similar to the adult value may be different for each compound.

Concerning the lungs, prematures may be more vulnerable to xenobiotics (at least with respect to local toxic effect) as their lungs are significantly less mature compared with the infant born at term.

With respect to dermal absorption, it should be stressed that although children do not show increased percutaneous absorption, the surface to body weight ratio is higher compared with adults until the age of > 10 years. This may lead to enhanced susceptibility.

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# 3 Exposure

In order to estimate the risks which chemical substances may pose to the unborn child, infants and children, it is necessary to know the possible exposure routes and exposure patterns for different types of substances in the various age groups. The exposure route and pattern varies between the different age groups. Thus, exposure of the unborn child and the young infant is dependent on the exposure of the pregnant/lactating woman, whereas exposure of infants and children is also influenced by differences in nutritional/energy requirements, activity level, and the location where activities are taking place. The natural curiosity of infants and children may significantly increase their exposure to a wide variety of chemical substances.

An additional aspect in relation to the time at which exposure occurs is that exposure to environmental xenobiotics prenatally or during childhood may result in functional defects or predisposition to development of certain diseases which may affect the individual during the whole life span.

In the following, several types of exposure will be addressed:

- Exposure of the unborn child
- Postnatal exposure
  - oral exposure:
    - breastfeeding
    - infant formula
    - drinking water
    - diet and beverages
    - non-dietary ingestion
  - exposure via inhalation
  - exposure via dermal contact

There is nearly always possibility for simultaneous exposure via several routes. For example, substances such as lead, mercury, and persistent organic pollutants may be present in foodstuffs, drinking water, and ambient air and thus result in simultaneous exposure via oral ingestion, inhalation, and dermal contact.

Exposure may occur to different types of chemical substances for instance:

- persistent organic pollutants
- pesticides
- metals
- food additives
- pharmaceuticals
- vapours/fumes
- combustion products/particulate matter
- plasticizers

- alcohol
- environmental tobacco smoke
- cosmetics
- household chemicals

### 3.1 Exposure of the unborn child

Most substances entering the bloodstream of the pregnant mother will be distributed to the embryo/foetus (see section 2). The exposure of the unborn child will thus be a reflection of the exposure pattern of the mother: her dietary habits and lifestyle, whether she drinks alcohol, smokes, is under medical treatment, or is subjected to occupational exposure. Persistent substances accumulated in the body of the mother (from previous exposures) may be redistributed, thus leading to exposure of the unborn child. An example is lead deposited in the bones of the mother, which is distributed to the foetus during gestation.

The embryo-foetal exposure to chemical substances depends on the amount the mother is, and has been (if the substance is accumulated in the body), exposed to and on the extent to which the substance passes the placenta. Organic mercury and lead compounds pass the placenta and reach the unborn child, while cadmium to some extent will be withheld in the placenta. During embryogenesis, the placental transfer of weakly acidic substances is favoured, while during late gestation, pH of the foetal compartment changes and favours the transfer of weakly alkaline substances (see section 2).

Regarding medical treatment of pregnant women, benefits and risks of the intended treatment should be carefully evaluated for the mother and the unborn child before the treatment is actually given.

### 3.2 Oral exposure

#### 3.2.1 Breastfeeding

The infant may be exposed to chemical substances through breastfeeding, because many substances, especially lipophilic ones, which the lactating mother is exposed to or has been exposed to at an earlier point in her life, are secreted into the breast milk. Some substances may even concentrate in breast milk.

Breast milk has a lipid content of 3 to 4%. The intake of breast milk of infants (4-6 weeks of age) has been quantified to an average intake of about 750 g/day (EPA 1997; Butte et al. 1984)

PCB and dioxins are examples of substances, which are liberated from the fat tissue of lactating mothers and secreted into the breast milk. In Denmark, an investigation has been performed to determine the content of dioxins and PCB in the breast milk of Danish mothers. The average concentration of dioxins + PCB was 30 pg TEQ/g fat (TEQ = 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity equivalents) corresponding to an average daily intake of the sucking child of 150 pg TEQ/kg b.w./day. A

TDI of 1-4 pg TEQ/kg b.w./day (dioxins and dioxin-like PCBs) has been set (WHO 1999) as an average daily intake for the whole lifetime. (SST/FDIR 1999).

Data from recent Danish and Swedish studies show reductions in the concentrations of organochlorine compounds such as PCBs and DDT in human milk throughout the last decade. In contrast hereto, the concentration of organobromine compounds such as polybrominated diphenyl ethers (PBDEs) has increased continuously with levels showing an exponential increase from 1972 and a doubling time of 5 years. (SST/FDIR 1999, Norén & Meironyté 1998).

### 3.2.2 Infant formulas

The mean intake of infant formula is slightly higher than for breast milk. A Swedish study showed that the mean breast milk intake at the age of 6 weeks is 746 g/day compared to an intake of cow's milk formula of 823 g/day and an intake of soy formula of 792 g/day (Köhler et al. 1984 - quoted from EPA 1997).

Infant formulas are based on cow's milk or on soy products and may be contaminated as described in section 3.2.4. Infant formulas can be divided into those "ready to feed" and those consisting of dry powder to be mixed with water immediately before use. Water is the major ingredient in infant formula. The water used for industrial manufacturing of "ready to feed" formulas is purified e.g. by active carbon filtration (NRC 1993) and is not anticipated to contain any chemical substances of concern. The water used for infant formula in households is most likely tap water and may contain chemical substances which may result in an unacceptable exposure of the infant (see section 3.2.3).

### 3.2.3 Drinking water

Drinking water may contain high concentrations of naturally occurring substances such as fluoride, copper, and nitrate as well as contaminants such as persistent organic pollutants and pesticides. Therefore, drinking water may constitute a health risk for all population groups; however, infants and young children are at the highest risk, since they, on a body-weight basis, may consume up to 5 times more water than adults (Lawrie 1998). Intake values for regulatory purposes are given in Table 3.3.

Generally, it is very difficult to estimate the intake of chemical substances from drinking water, because the concentrations and types of substances vary from one geographical site to another and also depend on whether ground water or surface water is the source of drinking water.

Fluoride is one example of a natural constituent in drinking water. A concentration of about 1 mg/l of fluoride in drinking water may give rise to discoloration of the tooth enamel and adverse skeletal changes may be observed at concentrations around 3 to 6 mg/l. Fluoride also provides a protective effect against dental caries, especially in children; this pro-

tective effect increases with concentrations of fluoride in drinking water up to about 2 mg/l.

Nitrate is another example of a natural constituent in drinking water and drinking water obtained from certain wells may contain high levels of nitrate either due to direct contamination or as a result of bacterial contamination. When the concentration of nitrate exceeds 50 mg/l (the limit value in Denmark), drinking water will be the major source of total nitrate intake, especially for bottle-fed infants. High nitrate levels pose a particular health risk to infants. Infants have a higher pH in the stomach which favours the reduction of nitrate to nitrite. Nitrite is involved in the oxidation of normal haemoglobin to methaemoglobin, which is unable to transport oxygen to the tissues. Haemoglobin is more easily oxidised in infants compared to older children and adults and the enzymatic system for reduction of methaemoglobin is not fully developed in infants.

Other contaminants in the drinking water may present due to the release from the water pipes and installations. Copper is one example where infants may have an increased risk of experiencing toxic effects. The concentration of copper in the body is held relatively constant by homeostatic mechanisms; however, children under one year of age are probably more susceptible than adults to copper toxicity because the homeostatic mechanisms may not have fully developed.

#### 3.2.4 Diet and beverages

Infants and young children have a dietary pattern different from that of adults. They have a higher food intake per kg bodyweight and they also have other food preferences and needs. In the Nordic dietary recommendations (Nordic Council of Ministers 1996) it is stated that the percentage of energy from fat in the diet of infants younger than 6 months should be minimum 40%, for children from 1 to 3 years 30 to 35%, and for adults less than 30% of the total energy intake.

Comprehensive British surveys (between 1986 and 1993) have shown that, on a body weight basis, the nutritional requirements (energy, protein, and water) of infants and young children are 2-5 times higher than for adults; the largest difference is seen for water intake.

The average consumption by young children (aged 1½ to 4½) of the main food categories (fruit, vegetables, bread, cereals, meat, fish, eggs) was 1.7 to 2.7 times higher, on a body weight basis, than that by adults.

Dairy products (including cheese and yoghurt but excluding milk), puddings, sugar and confectionery were found to be of particular significant importance in the diet of the young child, with an intake up to 5 times the equivalent adult figure (after correction for body weight). For beverages, the difference between young children and adults were even more marked; excluding milk, young children obtained over 80% of their fluid intake from soft drinks (75% was squashes and 25% was carbonated drinks) giving an average level of consumption which was 16 times the equivalent adult figure. (Lawrie 1998)

A Danish survey (National Food Agency 1996) showed that, relative to their bodyweight, children drink up to nine times as much milk as adults,

and that they eat more cereals (including bread and porridge), fruit, vegetables, products of animal origin and sweets than adults, see Table 3.1.

Table 3.1 Mean intake of food commodities in Denmark 95<sup>1</sup>

Commodity (g/kg b.w. per day)	1 - 3 years (14.1 kg b.w.)	7 - 10 years (31.7 kg b.w.)	15 - 18 years (63.0 kg b.w.)	35 -44 years (72.4 kg b.w.)
Milk (not yoghurt a.o.)	27.7	14.8	7.3	3.1
Cheese	0.8	0.7	0.5	0.5
Bread	6.2	4.1	2.4	2.1
Cereals <sup>1</sup>	1.1	0.6	0.2	0.2
Porridge	3.4	0.7	0.1	0.1
Fruit incl. fruit juice	12.4	5.7	2.9	2.0
Vegetable incl. Potato	8.0	5.9	3.7	3.3
Rice <sup>2</sup>	0.7	0.4	0.2	0.2
Meat and meat products	2.9	2.9	1.8	1.7
Poultry	0.9	0.4	0.2	0.2
Fish	0.8	0.6	0.3	0.3
Sweets, ice cream, ice lolly	1.8	1.1	0.5	0.2
Soft drink and fruit juice (with natural sugar)	13.8	7.2	2.9	1.2
Soft drink and fruit juice (sugar free, light)	7.8	4.8	1.6	0.7
Tap water <sup>3</sup>	7.8	5.7	3.8	5.2
Bottled water	0.7	0.8	0.8	1.1
Tap water and bottled water	8.5	6.5	4.6	6.3

<sup>1</sup>Estimated from "Danskernes kostvaner 95" (National Food Agency 1996) including unpublished data.

<sup>2</sup>Dry weight

<sup>3</sup>Tap water consumed directly from the tap. Tap water used for cooking and dilution of e.g. soft drink concentrates is not included.

#### *Cow's milk*

Cow's milk is a traditional constituent of the childhood diet and young children (1 to 3 years) on the average consume approximately nine times as much as adults (35-44 years). The predominant exposure to chemical substances through cow's milk and milk products are in form of residues from veterinary medicinal products and from substances present in the feed of the dairy cow.

#### *Products of animal origin*

Younger children eat relatively more meat, fish and poultry than adults. Meat and poultry may be contaminated with chemical substances, which pass from the soil, possibly through the feed, to the animals. In fish, par-

ticularly in fat fish, lipid-soluble persistent substances (e.g., methylmercury, dioxins, PCBs, and chlorinated pesticides) typically accumulate through the food chain.

### *Cereals*

Cereals may contain pesticide residues and chemical substances from soil. The pesticide residues from herbicides, fungicides, insecticides, and growth regulators used on cereals are normally concentrated in the bran, which is often removed during processing. Organo-phosphates and pyrethroids form the major part of residues after post-harvest treatments. Certain metals tend to concentrate in special crops, e.g., cadmium is found in high concentrations in sunflower seeds, which are used in large amounts in some types of bread. Cadmium is also found in other cereals. In a Swedish study (Eklund & Oskarsson 1999), the highest intake, 0.44  $\mu\text{g Cd/kg b.w. per day}$  on a body weight basis, was found in 6-month-old children, consuming the recommended amount of wheat-, oat- and milk-based formulas; this intake is below the PTWI (Provisionally Tolerable Weekly Intake) of 7  $\mu\text{g/kg b.w.}$  (1  $\mu\text{g/kg b.w. per day}$ , for comparison only) (WHO 1989).

### *Fruit*

Fruit is a food commodity, which may contain pesticide residues because a large part of the production is treated with pesticides during growth. The pesticide residues will often be reduced through washing, cooking, or by removal of the peel. Thus the highest exposure occurs when children eat unwashed whole fresh fruit.

### *Vegetables*

In general, young children eat more vegetables relative to their body-weight than adults and data from the Danish survey (National Food Agency 1996) indicate that children eat a higher quantity of vegetables harvested from the soil (potatoes and carrots) than harvested above the soil (leafy vegetables). Higher levels of pesticide residues are found on vegetables harvested above the soil than from the soil because they are treated directly.

All vegetables may contain chemical substances present in the soil and some substances may even accumulate in the vegetables. Examples of chemical substances, which have been detected in vegetables, are metals, chlorinated solvents, pesticides and polyaromatic hydrocarbons.

### *Soft drinks and fruit juice*

According to both a Danish (National Food Agency 1996) and a British (Lawrie 1998) survey, children between 1 and 3 years in average consume, relative to their body weight, more than ten times the amount of soft drinks and fruit juice compared to adults. The relatively higher consumption of soft drinks and fruit juices means that children are exposed to relatively higher amounts of the food additives used in these drinks such as artificial sweeteners and colouring agents. Some soft drinks are diluted in the home and this also means a relatively higher intake of tap water. Tap water used for this purpose is not included in the figure for tap water in Table 3.1.

### *Sugar*

Children have a higher intake of sugar compared to adults. This is not considered to lead to higher exposure to chemical substances because sugar is refined to a degree where almost no contaminants are found.

### *Essential elements*

In cases of essential elements such as copper and fluoride, the relationship between intake and risk has a U-shaped curve, with risks from defi-

ciency associated with low intakes and risk of toxicity associated with high intakes. The range of optimal intakes to meet the biological requirement as well as to prevent risk of toxicity are for some substances rather narrow.

### 3.2.5 Non-dietary ingestion

Children and infants are by nature curious and continuously examine their environment. One way in which they investigate their surroundings is by putting objects into their mouth. During outdoor playing activities, children get into contact with soil both by dermal contact and by ingestion.

A consequence of children's oral examination of their environment is the risk of intoxication which in some cases is fatal. In Denmark, around 800 to 1300 children are every year (the number has decreased during the last years) referred to hospitals due to accidental intoxications; however, only very few cases are fatal (3-4 per year). Acute intoxications are primarily due to unintentional exposure to household chemicals, organic solvents, drugs, irritating gases, asphyxiants in fumes, and poisonous animals, plants and fungi. The most frequently reported intoxication among young children during the nineties is ingestion of liquid paraffins (lamp oil); however, the number of these cases has been reduced during the last few years probably because of general information to consumers and because of exclusion of attractive colours and fragrances in lamp oils. (Ishøy & Jensen 1999).

General prophylactic measures include the introduction of child resistant closures to several products, including household chemicals and organic solvents.

Exposure to chemical substances may also occur through oral contact with toys and other products used by children such as soothers, teats, glue, finger paint, or cosmetics made for children. Soothers may lead to exposure to chemical substances due to migration of substances from the different materials used (plastic, silicone, or natural rubber); one example is migration of mercapto benzothiazole (MBT) from natural rubber.

Toys may contain a number of chemical substances such as plasticisers (e.g. phthalates), metals, and organic solvents, which may migrate from the toy to the saliva when the child is sucking or chewing on the material.

Soil ingestion is related to the hand-to-mouth activity and is most profound in the age range from 1 to 3 years. Several factors may influence the intake of soil. The major factor is the access to free soil surfaces, others include the climate, which affects the time being spent outdoors, and whether the ground is frozen or covered with snow. There is an uneven distribution of soil ingestion as many children eat small amounts while a few eat large amounts (Larsen 1998). Average figures for soil and dust ingestion are reported to be in the range of 39 to 271 mg/day (Osimitz 1999). A study with Dutch children aged 2 to 4 years showed a mean value of 105 mg/day (range 23 to 362 mg/day) compared to a value for hospitalised children of 49 mg/day (Clausing et al. 1987). For occasional single exposure, a figure of 10 g/day is given for children deliberately

eating large amounts of soil (EPA 1997). Intake values for soil ingestion for regulatory purposes are given in Table 3.4.

### 3.3 Exposure via inhalation

Children at rest inhale a relatively larger volume of air compared to adults. A further increased inhalation volume is due to a higher activity level during play. In Table 3.2, estimates of daily inhalation volumes (based on oxygen consumption associated with energy expenditures) are shown for different age groups. Inhalation volumes used for regulatory purposes are given in Table 3.6.

*Table 3.2 Daily inhalation rates calculated from food-energy intakes (Layton 1993 - quoted from EPA 1997).*

Age	Body weight (kg)	Daily inhalation rate (m <sup>3</sup> /day)	Inactive (m <sup>3</sup> /day)	Active (m <sup>3</sup> /day)
Children				
< 1 year	7.6	4.5	2.35	6.35
1 - 2 years	13	6.8	4.16	9.15
3 - 5 years	18	8.3	4.98	10.96
6 - 8 years	26	10	5.95	13.09
Male 35-50 years	82	15	10.25	18.45
Female 35-50 years	66	10	7.80	11.7

Children are exposed to indoor air pollutants e.g., from exposure to tobacco smoke, wood burning, cooking, vapours from household and hobby products, vapours emitted from furniture and building materials, and housedust to which many chemical substances are adsorbed.

Due to a greater amount of time spent outdoor and relatively higher inhalation volume compared with adults, children are exposed to a higher degree to ambient air pollutants such as particulate matter, PAHs, nitrogen dioxide, and ozone (Larsen et al. 1997). In busy roads, children inhale air from a level near the ground which make them more exposed to vehicle exhaust from the traffic.

### 3.4 Exposure via dermal contact

For whole body exposures, children and infants have higher exposures than adults because of their greater surface to bodyweight ratio. Many of the substances in the environment of infants and children, which pose a problem in relation to non-dietary ingestion, may also pose a problem when getting in contact with the skin e.g., household chemicals, childcare products, and products for personal hygiene.



Clothes to which there is an extended and close contact, may contain chemical substances. For nappies, in addition to a long contact time, there is also an occlusive effect.

Cosmetics are widely used, may be applied several times a day, and may be used on large skin areas. For infants and young children, the exposure to cosmetics is related to their parent behaviour. As the child grows older, it will gradually take over the use of cosmetics for personal hygiene and other products intended for application to the skin such as face paint. If the product is applied on damaged skin, there will be a greater absorption of the constituents. Likewise, when a product is applied on the skin and covered with a nappy, a higher absorption will occur because of the occlusive effect.

More and more products are marketed directly for children, some of which may contain chemicals getting in contact with the skin e.g., face paint, child cosmetics, wet tissues, and plasticine.

### 3.5 Overall

Children are subjected to a relatively higher exposure to chemical substances compared to adults because of physiological differences, differences in dietary needs or food preferences, or different activity patterns. The following aspect should be emphasized:

- The unborn child shares the exposure pattern of the mother. Most substances to which the mother is exposed will be passed on to the unborn child. Substances accumulated in the mother's tissues may be re-distributed and passed on to the unborn child.
- The breastfed child shares the exposure pattern of the mother. Substances accumulated in the fatty tissue of the mother will be passed on to the child through the breast milk.
- Drinking water may be contaminated with chemical substances or contains naturally occurring substances at levels which may pose a risk to infants and young children since they, on a bodyweight basis, may consume up to 5 times more water than adults.
- Nutritional requirements (energy, protein, and water) of infants and young children are 2-5 times higher than for adults.
- Relative to their bodyweight, children drink up to nine times as much milk as adults. They eat more of the main food categories (fruit, vegetables, bread, cereals, meat, fish, eggs) and sweets than adults.
- Children consume large amounts of soft drinks containing artificial sweeteners and colouring agents.
- Soil ingestion is most profound in the age range from 1 to 3 years. Small children may in single occasions deliberately eat as much as 10 gram of soil.

- Children inhale a relatively larger volume of air compared to adults and may therefore to a higher degree be exposed to indoor and ambient air pollutants.
- More and more products aimed directly at children are put on the market, some of which may contain chemical substances getting in contact with the skin e.g., face paint, child cosmetics, wet tissues, and plasticine.
- Because of their curiosity and behavioural pattern, children may more easily be involved in emergencies due to oral, inhalational, or dermal exposure to household chemicals or other chemical products containing dangerous substances.

When estimations of exposure of infants and children are made, special attention should be directed towards the fact that infants and children have a different activity pattern from adults. Child specific activities include hand to mouth activity, different food preferences, and their curiosity-driven examinations of their surroundings.

Tables 3.3 to 3.6 give an overview of volumes of intake used by different authorities and organisations in derivations of guidance values for drinking water (Table 3.3), soil (Table 3.4 for oral ingestion; Table 3.5 for dermal contact), and ambient air (Table 3.6):

*Table 3.3 Values used for drinking water intake*

	<b>Children</b>	<b>Adults</b>
MST (1990)	$\frac{3}{4}$ - 4 years old:	
average	1 litre/day	2 litres/day
maximum	2 litres/day	4 litres/day
WHO (1994)	-	2 litres/day
EPA (1997)	10 kg or less: 1 litre/day	2 litres/day

*Table 3.4 Values used for soil ingestion*

	<b>Children</b>	<b>Adults</b>
MST (1990)	$\frac{3}{4}$ - 4 years old:	
average	0.2 g/day	0.025g/day
maximum (excl. pica)	3 g/day	0.1 g/day
pica child	10 g/day <sup>a</sup>	
WHO (1994)	-	0.020 g/day
EPA (1997)		
mean	0.1g/day <sup>b</sup>	0.05 g/day
upper percentile	0.4 g/day <sup>c</sup>	

	<b>Children</b>	<b>Adults</b>
pica child	10 g/day <sup>d</sup>	

- <sup>a</sup> To be used in acute exposure assessments.
- <sup>b</sup> 0.2 g/day may be used as a conservative estimate of the mean
- <sup>c</sup> Study period was short; therefore, these values are not estimates of usual intake.
- <sup>d</sup> To be used in acute exposure assessments. Based on only one pica child.

*Table 3.5 Values used for dermal soil contact*

	<b>Children</b>	<b>Adults</b>
MST (1990)	<sup>3</sup> / <sub>4</sub> - 4 years old:	
average	1 g/day	0.1 g/day
maximum	10 g/day	1 g/day
WHO (1994)	-	-
EPA (1997)	-	-

*Table 3.6 Values used for respiratory volume*

	<b>Children</b>	<b>Adults</b>
MST (1990)	<sup>3</sup> / <sub>4</sub> - 4 years old:	
average	10 m <sup>3</sup> /day	20 m <sup>3</sup> /day
maximum	12 m <sup>3</sup> /day	30 m <sup>3</sup> /day
WHO (1994)	10 years old: 15 m <sup>3</sup> /day	22 m <sup>3</sup> /day <sup>a</sup>
EPA (1997)	-	20 m <sup>3</sup> /day

- <sup>a</sup> figure given is for an average adult (male: 23 m<sup>3</sup>/day; female 21 m<sup>3</sup>/day) with 8 hours of resting and 16 hours of light/non-occupational activity.

### 3.6 References

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## 4 Testing methodology

Well-planned and documented epidemiological studies have a clear advantage over studies in experimental animals in providing the most relevant information on health effects in humans, thus avoiding extrapolation from experimental animals. However, owing to the lack of adequate epidemiological data for most chemical substances, toxicological studies in animal species play an important role in hazard identification and risk assessment.

### 4.1 The use of epidemiology

#### 4.1.1 Types of epidemiological studies

In general there are two groups of basically different types of studies in epidemiology. One group includes different types of epidemiological experiments e.g. clinical trials (with patients as subjects), field trials (with healthy subjects) and community intervention trials (with the intervention assigned to groups of healthy subjects). For various reasons these types of experiments are hardly ever used in human toxicology (except for the registration of adverse effects in phase 2 and 3 of clinical trials). In the other group are the non-experimental trials of which there are three primary types: the cross-sectional study, the case-control study and the cohort study.

#### *Cross-sectional study*

In the cross-sectional study both the exposure and the effect are measured at the same time in a defined population (all persons in a limited area or a random sample). Because of the lack of longitudinal data this type of studies is often insufficient to elucidate a cause-effect relationship especially if there is a time delay before the effects occur. The advantage is that the data are collected within a limited time resulting in a relatively short time interval between generation of the hypothesis and the answer.

#### *Case-control study*

In the case-control study different exposures in two selected groups of people, the cases (the ill) and the controls (the reference group of well persons) are compared. This type of study is relatively simple and economical to carry out. The cases are defined by the investigator (case definition). A classic example of a case-control study was the discovery of the relation between thalidomide and unusual limb defects in babies born in the Federal Republic of Germany in 1959 and 1960; the study, undertaken in 1961, compared affected children with normal children. Of 46 mothers whose babies had typical malformations, 41 had taken thalidomide between the fourth and ninth weeks of pregnancy, whereas none of the 300 control mothers, whose children were normal, had taken the drug at these stages. In a case-control study the association of an exposure and an effect is measured by calculation of the odds ratio, which is the ratio of the odds of exposure among the cases to the odds of exposure among the controls.

#### *Cohort studies*

In cohort studies (follow-up studies) the risk factor (exposure) in a defined group is defined and measured. Then the group is followed over time for development of the disease (or another end-point parameter). The ratio between the risk in the exposed group and the risk in the unexposed group is the Relative Risk (RR). In this kind of studies the entire population is known, which is not always the case in the case-control study. The disadvantage of this type of study is that they are usually very big and expensive. On the other hand they provide the best information about the causation of disease and the most direct measurement of the risk of development of disease.

#### 4.1.2 **Bias**

The quality of the result of epidemiological investigations depends on the validity and accuracy of the data on the exposure as well as the effect. The validity depends on the number of systematic errors when data are collected (bias). The accuracy depends on the number of random errors. The two have a tendency to counterbalance each other. Information- and selection bias give rise to misclassification. Whether the study population is selected in a truly random manner or not is crucial for the results of epidemiological trials. Selection bias (e.g. healthy worker effect) might mask an association between an exposure and a health effect. A small vulnerable group might appear insignificant in a big less vulnerable group (maybe they are already dead due to the exposure) and a small heavily exposed group might appear insignificant in a big less exposed group. A low rate of participation increases the risk for selection bias.

#### 4.1.3 **Confounding**

In a study of the association between exposure to a cause (or risk factor) and the occurrence of disease, confounding can occur when another exposure exists in the study population and is associated both with the disease and the exposure being studied. A problem arises if this extraneous factor – itself a determinant or risk factor for the health outcome – is unequally distributed between the exposure subgroups. It could be age, gender, smoking habits, socio-economic status etc. One way of reducing the effect of confounding is by matching cases and controls with regard to the potential confounders.

#### 4.1.4 **Controls**

In case-control studies the controls act as representatives for the background exposure. In order to fulfil this they have to be chosen independently of their exposure and they should have been submitted to the same selection mechanisms as the cases (i.e. as potential cases). When selecting the control group there is a potential for selection bias. Random controls selected from the community often introduce recall bias. They are less prone to recall a certain exposure in the past than cases, resulting in a drift towards a false positive association. Problems of similar kinds arise when using other not randomly selected controls as family, friends or cases with another end-point.

#### 4.1.5 Strength

In general, case descriptions are suitable to show a possible effect of an exposure but they are insufficient to evaluate a risk. Case-control studies may be limited by the small study groups with few cases and rare specific exposures. Cohort studies may be limited by the rare occurrence of effects associated with the exposure in question. Hence, the strength of the statistical calculations are often limited in case-control and cohort studies.

Traditional epidemiology is often challenged by incorrect information about exposure and effect, resulting in a low sensitivity. This, and a small gap between the exposure in the exposed and the unexposed group, makes it difficult to study the effects of low dose exposures. To get statistically significant results either large study populations, a strong cause-effect relationship or a very rare effect parameter are required. A small study population may in itself be the reason for overlooking a relevant association. This makes it risky to conclude that there is no cause-effect relationship when the study indicates that the exposure-effect relationship is not existing. In reviewing the literature there is an additional risk of publication bias, as both the scientists and the editors are more prone to publish positive correlations than negative results.

In general epidemiological studies has a number of advantages as compared to animal experiments: they study effects in humans, they are often cheaper, they can be based on already existing data, and they can use intellectual and psychological end-points.

## 4.2 Test methods in experimental animals

### 4.2.1 Reproductive toxicity tests

Many different experimental methods for investigating toxic effects of chemicals on reproduction and development are in use. Several tests are standardised and guidelines have been issued by various governmental agencies and international organisations, others are still undergoing scientific evaluation. In the following sections, standardised and regulatory accepted methods are discussed concerning procedures and endpoints with emphasis on effects induced during the prenatal and postnatal period. Table 4.2 summarises the tests discussed. In the last section the effects assessed during the prenatal and postnatal period is compared to the possibility for detection of effects in toxicity testing in adults, e.g. in repeated dose toxicity studies.

Other test than those included can reveal effects which indicate a potential of a chemical to interfere with normal reproduction, e.g. the dominant lethal test, fertility assessment by continuous breeding, and repeated dose toxicity testing where the gonads are subjected to pathological examination. These tests, however, provide only information on effects of dosing adult animals.

During recent years many in vitro test systems have been proposed as alternatives to animal testing for developmental toxicity. These tests may

be useful for screening of closely related chemicals and for pinpointing mechanisms underlying developmental effects, but they cannot replace animal testing. Consequently, they are not considered in the following sections.

*Table 4.2 Overview of in vivo tests for reproductive toxicity testing.*

Test	Exposure period	Endpoints in offspring	Guideline(s)
Generation studies	Continuously over one, two or several generations	Growth, development and viability Histopathology of sex organs, brain and target organs Fertility Proposal: oestrous cyclicity and sperm quality	OECD TG 415 One-generation Study OECD TG 416 Two-generation Study
Prenatal Developmental Toxicity Study (Teratology study)	Usually during organogenesis Proposal: from implantation to the day before birth	Resorptions Foetal growth Morphological variations and malformations.	OECD TG 414
Developmental Neurotoxicity Study (Behavioural teratology studies)	During pregnancy and lactation	Birth and pregnancy length Physical and functional maturation Behavioural changes due to CNS and PNS effects Brain weights and neuropathology	OECD TG 426 Developmental Neurotoxicity Study (draft 1999)
Reproduction/ Developmental toxicity screening test	At least three dose levels from 2 weeks prior to mating until day 4 postnatally	Fertility Pregnancy length and birth Foetal and pup growth and survival until day 3	OECD TG 421 and 422

Modified from Hass et al. (1994).

### *Design considerations in reproductive toxicology studies*

When designing experimental *in vivo* studies in reproductive toxicology some important factors must be considered such as selection of animal species, dose, time of dosing, route of exposure, and housing and handling of the animals.

The animal species usually used for screening studies are rats, mice and rabbits. There are important species differences between humans and rodents especially concerning the timing of brain development in relation to the birth of the offspring. These differences must be taken into account when extrapolating from animal data to the human situation.

The dose levels used in animal studies are often higher than the expected exposure levels in humans. There are several reasons for this. Firstly, the limited number of animals per group limits the sensitivity of the experi-



mental studies; secondly, the human exposure may continue for a long time, even throughout life, while the exposure period in experimental studies is shorter; and thirdly, comparisons of human and animal data seem to indicate that humans are more sensitive than the commonly used laboratory animals when the dose is expressed as mg/kg (Jakobsen & Meyer 1989). The highest dose level should normally be chosen with the aim to induce (slight) general toxicity in adult animals but not death (OECD 1983, OECD 1994).

The time and duration of treatment in reproductive toxicity tests influences the types of effects found. Tests for developmental toxicity are, for example, often restricted to the period of organogenesis, i.e. days 6-15 for mouse and rats, and days 6-18 for rabbits. This period is sensitive to induction of structural malformations while functional effects may be induced at all stages of development. Ideally, treatment in the mouse, rat and rabbit should continue from day 6 of pregnancy through to weaning, though there may be advantages in separating prenatal and postnatal phases in different groups of animals (Barlow & Sullivan 1975).

The route of exposure in studies designed for hazard assessment should ideally be the same as the expected exposure of humans. In the workplaces the main routes of exposure are via inhalation or uptake through the skin. However, oral exposure is often used and recommended in guidelines (OECD 1983, OECD 1994). An important difference between inhalation exposure and e.g. oral exposure is that there is no first-pass effect in the liver for inhaled chemicals, i.e. the chemical in the bloodstream reach the placenta before the liver. Therefore, extrapolation from experimental inhalation studies to inhalation exposure of humans is easier than extrapolation from oral studies. Inhalation studies are, however, not easily performed during the birth period and the early neonatal period. Therefore, inhalation exposure is often postponed a few days before expected birth and only in some cases continued after birth at day 2 or 3.

Standardisation of litter size to 8 pups (culling) was previously commonly used in generation studies, and the procedure is described in the guidelines for generation studies (OECD 1983). An argument in favour of culling is that since pup weight is related to litter size, culling might lead to a more uniform pup weight at weaning. However, in a study of data from approximately 500 litters culling seemed only to increase the mean pup weight in the litters, while the variation remained unaffected (Palmer 1986). Actually culling results in elimination of 25-40% of the offspring and may introduce bias for example by random elimination of runts (Palmer 1986).

Postnatal effects, especially on body weight and behaviour of the pups, may be induced via effect on the mother. For example, lactation or maternal care may be affected and potentially any alteration in maternal physiology and behaviour may in turn influence the offspring. To control for this, cross-fostering techniques may be used where prenatally exposed litters are reared by non-exposed mothers and vice versa. This obviously requires more animals and demands more resources, and is therefore not normally used in screening studies.

Indications of gender differences in response level were among other examples seen in the Collaborative Behavioral Teratology Study (CBTS)

in the USA (Kimmel & Buelke-Sam 1985). There were no indications that female behaviour were more variable than male behaviour in the CBTS study. Therefore, there is no reason to exclude females because of possible response variability due to oestrus cycling. Especially in a screening situation, behaviour of both sexes should be monitored (Kimmel & Buelke-Sam 1985).

Housing and handling of animals may influence the results. For example, frequent handling of rats during infancy may alter their physical response to stress and their behaviour in tests for emotionality and learning. To control for environmental influences, the conditions under which the animals are kept must be standardised within experiments with respect to variables such as temperature, humidity, noise level, lighting, cages, handling, and cage cleaning (Barlow & Sullivan 1975).

#### *One-, two-, and multi-generation studies*

Guidelines for carrying out one-, two-, and multigeneration studies have been published by the US-FDA, OECD (1983), and the EEC, amongst others. In the EEC, the test is requested for new industrial chemicals (i.e. introduced after 1980) with a production volume reaching 100 tons per year.

The purpose of generation studies is to examine successive generations to identify possible increased sensitivity to a chemical, effects on the fertility of male and female animals, pre-, peri-, and postnatal effects on the ovum, foetus and progeny, including teratogenic and mutagenic effects, as well as peri- and postnatal effects on the mother.

In a one-generation study, the test substance is administered in graduated doses to groups of males and females. Males should be dosed during growth and for at least one complete spermatogenic cycle (approx. 56 days in the mouse and 70 days in the rat) in order to elicit any adverse effect on spermatogenesis. Females should be dosed for at least two complete oestrus cycles in order to elicit any adverse effect on oestrus. The test substance should be given continuously during mating and for the females also during pregnancy and the nursing period.

The main idea in two- and multigeneration studies is to incorporate dosing during the time of the organogenesis of the ovaries and testis and to investigate whether a chemical causes increasing toxicity and reproductive problems during subsequent generations. The dose levels and dosing period are similar to the one-generation study but continues until the third generation is weaned. The endpoints assessed in the offspring are viability, growth and histopathology of sex organs, brain and identified target organs. In a revised proposal for the OECD TG 416 Two-generation reproductive toxicity study, assessment of effects on sperm quality and oestrous cyclicity in offspring have been added (OECD 1999).

There are some limitations of generation studies concerning endpoints such as neonatal death and malformations, since the commonly used laboratory animals may eat dead or seriously malformed pups immediately after birth. An effect may, therefore, only be indicated indirectly by a smaller litter size. If only a few pups were malformed or dead, the reduction in litter size will be small compared to the normal variation in litter size and may therefore go undetected or not reach statistical significance. Preimplantation

losses and resorptions are indicated in an indirect way as a decreased litter size and the sensitivity for these effects may be rather low.

Birth weight and postnatal growth can be assessed, but it is important to include variations due to different litter sizes or different sex distribution in the litters in the analysis. A change in offspring body weight is a sensitive indicator of developmental toxicity, in part because it is a continuous variable. In some cases, weight reduction in offspring may be the only indicator of developmental toxicity in a generation study. While there is always a question remaining as to whether weight reduction is a permanent or transitory effect, little is known about the long-term consequences of short-term foetal or neonatal weight changes. Therefore, weight reduction should be used to establish the NOAEL (OECD 1989).

*Teratology study, prenatal developmental toxicity*

This is the most used *in vivo* method for studying developmental toxicity. Current guidelines include those issued by USFDA, OECD and EEC (Meyer et al. 1989). In the EEC, the test is requested for new industrial chemicals (i.e. introduced after 1980) with a production volume reaching 100 tons per year.

In this protocol, pregnant animals are dosed during the period of organogenesis, since this period is most sensitive to the induction of structural, anatomical malformations. The day before expected birth the foetuses are removed and examined. The main endpoints recorded include resorptions, retarded growth, and visceral and skeletal anomalies.

The teratology test was designed to detect malformations. In the past, there was a tendency to consider only malformations or malformations and death as relevant endpoints in teratology studies. Today it is assumed that all of the four manifestations of developmental toxicity (death, structural abnormalities, growth alterations, and functional deficits) are of concern (OECD 1989). As a consequence, the name of the test is changed to "prenatal developmental toxicity test" and the exposure period is extended to the day before birth in a revised proposal for the OECD guideline 414 (OECD 1999).

The sensitivity of the test for detection of rare events such as malformations is limited, due to the use of a relatively small number of animals. With the normal group sizes of 20 pregnant rats, it is not possible to identify any increase in major malformations unless high dose levels are administered or the substance studied is highly embryo/foetotoxic (Palmer 1981). To assess the developmental toxicity of a chemical, it is therefore important to include information on other developmental effects such as minor anomalies, variations, foetal death and growth. In addition, malformations of organs developing after the period of major organogenesis, e.g. the sex organs and the brain, may at present not be detected in the teratology study. An example is the suspected endocrine disrupter dibutyl phthalate where exposure during the period of male sexual differentiation resulted in major disturbances in the morphological and functional development of the male reproductive system (Mylchreest et al. 1999).

Teratology studies are very suitable for the demonstration of intra-uterine death after implantation (resorptions). In studies where dosing is started before implantation, preimplantation loss may also be assessed.

Foetal weight can be assessed rather exact in a teratology study, but it is important to include variations due to different litter sizes or sex distribution in control vs. exposed groups in the analysis.

*Developmental neurotoxicity studies, postnatal studies*

A number of chemicals are known to produce developmental neurotoxic effects in humans and other species. A guideline for developmental neurotoxicity study was issued by USEPA in 1991 and a revised US guideline was proposed in 1995 (USEPA 1991). During recent years a proposal for OECD TG 426 Developmental Neurotoxicity Study has been developed based on the US guideline (OECD 1999).

Developmental neurotoxicity studies are designed to develop data on the potential functional and morphological hazards to the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation.

The protocol is designed to be performed as a separate study, however, the observations and measurements can also be incorporated into e.g. a two-generation study.

The evaluation of the offspring consists of observations to detect gross neurological and behavioural abnormalities, assessment of physical development, reflex ontogeny, motor activity, motor and sensory function, and learning and memory; and evaluation of brain weights and neuropathology during postnatal development and adulthood.

The behavioural functions assessed cover many important aspects of the nervous system, however, some functions of relevance for e.g. endocrine disruption such as social interaction and mating behaviour are not included in guidelines at present.

The limitations mentioned in the section on generation studies concerning endpoints such as neonatal death, malformations, preimplantation loss and resorptions apply also to developmental neurotoxicity studies.

Other potentially relevant postnatal endpoints as e.g. kidney function, liver function and immunotoxicity, are not included in guidelines.

*Reproduction / developmental toxicity screening tests*

Recently, the OECD introduced guidelines for screening tests for reproductive toxic effects, i.e. the Reproduction/Developmental Toxicity screening tests, as part of the Screening Information Data Sets (SIDS) for high production volume (HPV) chemicals (OECD 1994). The "Combined repeat dose and reproduction/developmental toxicity screening test" is a combination of a 28 days toxicity study and a reduced generation study whereas the "Preliminary reproduction toxicity screening test" is a reduced generation study.

The purpose of the test is to generate limited information concerning the effects of a test substance on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of conceptus and parturition. It is not suggested as an alternative to or as a replacement for the existing test guidelines for generation and teratology studies.

The dosing of the animals is initiated 2 weeks prior to mating and continued until the end of the study on postnatal day 4. The number of animals per group is at least 10 animals of each sex and is expected to provide at least 8 pregnant females per group. Effects on fertility and birth are registered. Live pups are counted and sexed and litters weighed on days 1 and 4 postpartum.

The test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting postnatal manifestations of prenatal exposure or effect induced during postnatal exposure.

The value of a negative study is more limited than data from generation and teratology studies due to the lower number of animals per group, the shorter period of exposure as well as the limited number of endpoints measured.

#### 4.2.2 Comparison to assessment of effects in adult animals

Repeated dose toxicity testing in adult animals provide information on the potential for systemic toxicity by investigations of growth, clinical symptoms, haematology, biochemistry, organ weights, pathology and histopathology of organs.

Reproductive toxicity testing can provide information on a number of developmental effects, such as malformations, growth retardation, foetal and postnatal death, fertility, and functional effects on the CNS. However, the investigations of systemic effects are not similar to the repeated dose toxicity studies in adults, since haematology and biochemistry is not investigated. In addition, the investigations of organ weight, pathology and histopathology are limited to the brain, sexual organs and identified target organs. Consequently, systemic effects induced during pre- or postnatal development on e.g., liver and kidneys may not be investigated.

In most cases, the effects of chemicals have not been assessed in the two-generation study, the prenatal developmental toxicity study as well as the developmental neurotoxicity study. In these cases, some of the developmental effects mentioned above will not be covered. For example, information on developmental effects on fertility and sex organs are only provided in the two-generation study, while effects on brain development is investigated only in the developmental neurotoxicity study.

In order to have a sufficient background to determine the sensitivity of the developmental period compared to adulthood there is a need for studies where end-points are investigated similarly for both age groups. Ideally, this would require a two-generation study incorporating developmental neurotoxicity end points and supplemented with similar investigations of systemic effects in offspring as in repeated dose toxicity studies.

### 4.3 Test procedure for medicinal products

Guidelines on Clinical Investigation of Medicinal Products in Children have been issued by the European Committee for Proprietary Medicinal Products (CPMP 1997a). According to the guidelines, children should not be given medicines which have not been adequately evaluated for use in that age group. The rationale for this is that adequate evaluation of medicinal products for use in children cannot be achieved in adult studies because there are physiological differences between children and adults, and because children suffer from different diseases from adults, or show a different natural history for the same disease.

Furthermore, the guidelines state the scientific data required before medicinal product testing in children:

When paediatric patients are included in clinical trials, safety data from previous adult human exposure should generally be available before paediatric clinical trials are started.

In all cases, in addition to appropriate repeated dose toxicity studies, all reproductive toxicity studies and the standard battery of genotoxicity tests should be completed prior to the initiation of trials in paediatric populations. Juvenile animal safety studies should be considered on an individual basis.

The need for carcinogenicity testing should be addressed prior to long term exposure in paediatric clinical trials, taking into account the duration of treatment and/or cause for concern.

The ICH Guideline on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (CPMP 1997b) also points at juvenile animal safety studies when previous animal data and human safety data are insufficient.

The ICH draft Guideline on Clinical Investigation of Medicinal Products in the Paediatric Population (CPMP 1999) provides an outline of critical issues in paediatric drug development and approaches to the safe, efficient, and ethical study of medicinal products in the paediatric population. The specific clinical study issues addressed include considerations when initiating a paediatric program for a medicinal product, timing of initiation of paediatric studies during medicinal product development, types of studies (pharmacokinetic/pharmacodynamic, efficacy, safety), age categories for studies, and ethics of paediatric clinical investigation.

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## 5 Specific substances, examples of exposure and effects

This section will illustrate some differences in biological susceptibility and/or exposure between children and adults by giving a brief overview of selected chemical substances for which such differences have been reported in the literature. It should be stressed that the section is only intended for presenting some illustrative cases and therefore by no means should be considered as an exhaustive overview covering all cases and aspects.

### 5.1 Alcohol

Consumption of alcohol during pregnancy is associated with a great potential for developmental defects in the unborn child.

Alcohol is distributed with body water, and adverse effects in most organs have been reported. The critical target organs are the central nervous system and the liver. (Andrews & Snyder 1991, ILSI 1999).

A distinct dysmorphic condition, the foetal alcohol syndrome (FAS), has been associated with alcoholism in the pregnant mother. The abnormalities most typically associated with FAS include central nervous system dysfunction characterised by mental deficiency and microcephaly, growth deficiencies (both length and weight), a characteristic cluster of facial abnormalities (short palpebral fissures, hypoplastic upper lip with thinned vermilion, diminished or absent philtrum, deficient eye growth, short nose), and variable major and minor malformations (cardio-vascular and skeletal defects).

The critical gestational stage at which the foetus is most vulnerable to the effects of alcohol is not yet known; however, it may be about the time of conception or early pregnancy, when heavy maternal drinking is particularly risky.

The severity appears to be related to the extent of alcohol consumption by the mother during pregnancy. Data are insufficient to state a threshold and no level of maternal drinking can be established as absolutely safe for the foetus. The National Board of Health in Denmark (personal communication), recommends that pregnant women generally should avoid drinking alcohol and maximum 1 glass a day corresponding to around 12 g of alcohol per day.

The pathogenesis of FAS still remains undefined. However, three main, nonexclusive mechanisms that may explain the genesis of FAS include impaired placental/foetal blood flow, deranged prostaglandin balance, and direct effects of alcohol (or acetaldehyde, the primary metabolite) on cellular processes.

(Schardein 2000a, Andrews & Snyder 1991, ILSI 1999).

Many studies in laboratory animals clearly demonstrate that alcohol is a potent developmental toxicant inducing growth deficiency, mortality, and malformations similar to those seen in humans. Acetaldehyde, the primary metabolite of ethanol, is teratogenic in mice and induces growth

retardation and malformations in rat foetuses similar to those of ethanol when injected into the maternal animal during organogenesis. (Schardein 2000a).

Animal models have provided evidence that foetal alcohol effects are dose-dependent and have clearly demonstrated that the peak maternal blood alcohol concentration and the pattern of drinking are the most important determinants of the magnitude of alcohol-related birth defects. Different aspects of development may be sensitive to different levels of alcohol. (ILSI 1999).

## 5.2 Tobacco smoke

Suspicious that exposure to tobacco smoke could be hazardous to reproductive function and foetal development date back to early in the present century. It is now generally considered that smoking during pregnancy increases the risk of perinatal mortality, lowers mean birth weight, increases the risk of spontaneous abortion, and has a significant influence of risks of premature delivery, placenta previa, and abruptio placentae. However, it appears that congenital malformations are not associated with smoking. (Schardein 2000b).

Exposure to environmental tobacco smoke (passive smoking) has been reported to be associated with a variety of respiratory disorders in children, including asthma, wheezing, dyspnoea, cough, bronchitis, pneumonia, otitis media, and laryngospasm with anaesthesia induction. Children exposed to passive smoke in the home have more days of school absence, bed confinement, and restricted activity than children in a smoke-free environment. (McCance & Huether 1998).

Tobacco smoke contains more than 3800 different compounds. About 10% of these constitute the particulate phase, which contains nicotine and tar. The remaining 90% contains volatile substances such as carbon monoxide, carbon dioxide, cyanides, various hydrocarbons, aldehydes, and organic acids. Although all of these substances affect the smoker to some degree, nicotine is generally considered to be the primary substance responsible for the pharmacological responses to smoking.

The nicotine content is approximately 3 mg/cigarette smoked in 5 to 10 minutes. Pharmacologically, it is a classic cholinergic agonist initiating nervous stimulation and then blocking cholinergic receptors. By releasing catecholamines, nicotine increases heart rate, oxygen consumption, utilisation of free fatty acids, hypoglycaemia, all of which can be expected to affect foetal development. Nicotine freely crosses the placenta but levels in foetal tissues remain relatively low compared with those of maternal tissues, except in the early stages of development. (Schardein 1993b, IARC 1986).

Smoking reduces birth weight of offspring and the consensus of over 200 published studies is that smokers' babies weigh, on the average, 170 to 200 g less at birth than non-smokers' babies, with about twice as many babies weighing less than 2500 g at birth. Reduced birth weight is primarily due to intrauterine growth retardation, rather than prematurity. The cause of the growth retardation in utero remains highly controversial, but the available published information supports the direct effects of

nicotine and carbon monoxide as factors causing intrauterine hypoxia as the most likely mechanism for the effect of smoking on birth weight. There seems to be a dose-response relation as it has been calculated that the decrease in birth weight is about 8 to 9 g for each cigarette smoked daily. Smoking fewer than seven to ten cigarettes per day or early cessation of smoking in pregnancy results in foetal body weights that do not differ significantly from those of nonsmokers.

Increased perinatal or neonatal mortality is also associated with smoking during pregnancy. Perinatal mortality increases 20% for less than one-pack-per-day and 35% for more than one-pack-per-day and in some studies, the risk is more than doubled. The incidence is proportional to the birth weight. Prematurity, anoxia, and placental complications (abruptio placentae and placenta previa) are generally regarded as being responsible for most of this increase.

Spontaneous abortion rates may also be higher in mothers who smoke. The frequency of abortion appears to be directly related to the number of cigarettes smoked with the risk perhaps twofold greater among heavy (more than one pack per day) smokers than in nonsmokers. Heavy smokers also appear to abort earlier in pregnancy.

Data are conflicting concerning a possible causal association between congenital malformations and cigarette smoking. Of 21 studies published since 1970, 11 studies reported positive associations for congenital malformations; cardiac defects, cleft lip and palate, and anencephaly were most often identified as specific malformations associated with smoking. The remaining 10 studies failed to associate smoking in pregnancy and malformations.

The effects of maternal smoking on functional development have not been defined precisely but several reports suggest some causal association between smoking and impaired function. New-borns of smoking mothers have been reported to perform poorer in two operant tasks (head turning and sucking), to be less visually alert, and to have atypical sleep patterns. Childhood hyperkinesis is reportedly more common among children of smoking mothers. Maternal smoking may also have an adverse effect on learning. Recent studies have shown that these types of effects are not limited to offspring of active smokers alone. (Schardein 2000b).

Some of the developmentally toxic effects observed in humans have been observed in laboratory animals as well. Foetal growth retardation is also a characteristic in rats and rabbits, in the absence of malformations, at human exposure levels. Increased stillbirths have also been observed in rabbits exposed to the equivalent of 20 cigarettes per day during pregnancy. (Schardein 2000b).

### **5.3 Ambient air pollution**

In the past decades the incidence of respiratory allergic diseases in children has increased significantly in the Western world. Current data do not indicate that ambient air pollution attribute significantly to this rise, however, air pollution may cause significant increases in respiratory symptoms and their intensity in persons suffering from asthma and other respiratory diseases.

Children in this respect may be considered as a special risk group as they breathe more air relative to their body weight and lung surface, and thus receive proportionally higher doses of air pollutants systemically and locally. Furthermore children spend more time outdoor, and are more active, resulting in mouth-breathing and increased respiratory rate (see section 2).

Recently Jedrychowski et al. (1999) in a Polish study followed groups of children in two different areas, and over time compared their growth in lung function. They found a significantly greater growth in lung function among the boys from the area with low level air pollution compared to boys from the area with the higher air pollution level. For girls a similar difference did not reach the level of significance. The authors suggested that air pollution may lead to retardation in pulmonary function growth during the pre-adolescent years.

Several epidemiological studies indicate that children are susceptible to ambient air pollution. Increasing levels of ambient ozone levels have been shown to be associated with a decrease in lung function in children (Krzyzanowski et al. 1992, Hoek et al. 1993a, Hoek et al. 1993b, Braun-Fahrlander et al. 1994, Stern et al. 1994, Kinney et al. 1996).

In the study by Krzyzanowski et al. (1992), children together with asthmatics and adults spending long time outdoor were identified as having the most significant decline in lung function in relation to ozone exposure.

Burnett et al. (1994) studied the association between ambient air pollution levels and the number of daily hospital admissions at 168 hospitals in the Ontario area, representing 8.1 million people. Summer respiratory admissions were found to be closely related to ozone levels, and children was found to be especially affected as 15% of the admissions for children were associated with ambient air pollution compared with 4% for the elderly population.

Recently Loomis et al. (1999) and Woodruff et al. (1997) found increased infant mortality associated with ambient levels of fine particles. Loomis et al. (1999) found that a  $10 \mu\text{g}/\text{m}^3$  increase in mean level of fine particles during a period of three days was associated with an 6.9% increase in infant mortality. The authors noted that this particle-related excess mortality observed among children was greater in relative risk terms than excess mortality observed among elderly people in several other studies.

In relation to average particle level (long term exposure) Woodruff et al. (1999) compared post-neonatal mortality in areas with different levels of air pollution. They found a significantly increased odds ratio of 1.10 for postneonatal mortality (adjusted for other covariates) in highly polluted areas compared to low pollution areas (mean particle levels of  $44.5 \mu\text{g}/\text{m}^3$  and  $23.6 \mu\text{g}/\text{m}^3$ , respectively). The increased mortality was primarily due to respiratory-related mortality and sudden infant death syndrome. The authors suggested that children may be considered as a susceptible group in relation to particulate air pollution.

## 5.4 Pesticides

### 5.4.1 Organophosphates

Organophosphorus insecticides are normally esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids. More than 100 different organophosphorus insecticides are known. Organophosphorus insecticides exert their acute effects in both insects and mammals by inhibiting acetylcholinesterase (AChE) in the nervous system with subsequent accumulation of toxic levels of acetylcholine (ACh), which is a neurotransmitter. Delayed neuropathy is initiated by attack on a nervous tissue esterase distinct from AChE. (WHO 1986a).

Many thousands of cases of acute poisoning by organophosphorus insecticides have been recorded, the majority being due to parathion and methyl parathion (WHO 1986a).

Great differences in response to parathion exposure have been observed among individuals but children are generally more sensitive than adults. In a number of cases, the oral dose of parathion was known to be exactly 900 mg (around 15 mg/kg b.w.), and it was uniformly fatal. However, in one case the ingestion of 120 mg parathion (around 2 mg/kg b.w.) led rapidly to the death of a man. On the other hand, children (5-6 years old) died after eating 2 mg of parathion (around 0.1 mg/kg b.w.) In instances in which parathion-contaminated food was eaten by people of different ages, death occurred mainly or exclusively among children. Also the epidemiological evidence indicates that parathion is generally more toxic to children than to adults. (Gallo & Lawryk 1991).

The acute toxicity of different organophosphates ranges from highly toxic to only slightly toxic (oral LD<sub>50</sub>-values for the rat range from less than 1 mg/kg b.w. to over 3000 mg/kg b.w.) (WHO 1986a). Several studies have reported higher sensitivity based on lethality in young animals compared to adults following acute exposure to organophosphorus insecticides. The age-related differences in sensitivity may differ qualitatively and quantitatively with different organophosphates and varying exposure conditions (e.g., high vs. low dose, acute vs. repeated). A study by Liu et al. (1999) showed that repeated exposures to chlorpyrifos were associated with relatively similar degrees of cholinesterase inhibition among the age groups (neonatal, 7 days of age vs. adult, 90 days of age). In contrast, cholinesterase activity and muscarinic receptor binding were generally more reduced in neonatal relative to adult brain regions following repeated exposures to methyl parathion.

Many organophosphorus insecticides are embryotoxic at doses that are toxic for the mother, but only few teratogenic effects have been reported (WHO 1986a).

Parathion is toxic to the foetus causing prenatal and postnatal death of the young and reduced weight gain of the surviving young; no developmental abnormalities have been observed. The greater susceptibility of the foetus is in spite of the fact that inhibition of cholinesterase activity in foetal blood is less than that in maternal blood following dosing of the mother. Weanlings are also more susceptible than adults to parathion due

to their poorly developed microsomal enzymes and also greater inherent susceptibility of the young brain. (Gallo & Lawryk 1991).

In reproduction studies of methyl parathion at maternally toxic dose levels (ChE inhibition), no consistent effects on litter size, number of litters, pup survival rates, and lactation performance were observed; no primary teratogenic or embryotoxic effects were noted. (WHO 1993b).

In a toxicokinetic study in rats orally exposed to chlorpyrifos during late gestation (gestational days 14 to 18), the concentration of 3,5,6-trichloro-2-pyridinol (TCP - the only metabolite detected) in the foetal brain was two- to fourfold higher than the concentration in the maternal brain. The concentration of TCP in the maternal liver was approximately fivefold higher than in the foetal liver. Thus, the foetal nervous system may be exposed to a higher concentration of chlorpyrifos than the maternal nervous system when the dam is exposed during late gestation. Cholinesterase activity levels were determined in liver and brain from maternal and foetal rats. Maternal liver cholinesterase was two-fold more inhibited than foetal liver activity. Similarly, the maternal brain exhibited more cholinesterase inhibition than foetal brain at 5 hours after the last dose of chlorpyrifos. Foetal brain cholinesterase activity recovered to control levels by 24 hours after the last dose, whereas the maternal brain activity was still inhibited (44%) 5 days after the last dose. (Hunter et al. 1999).

In USA, chlorpyrifos is one of the most commonly used pesticides in the indoor environment today. A recent study (Gurunathan et al. 1998) showed that after a single broadcast spraying of chlorpyrifos in the indoor environment, chlorpyrifos continued to accumulate on children's toys and hard surfaces 2 weeks after spraying. Based on this study and other research studies, the estimated chlorpyrifos exposure levels for children from indoor spraying are approximately 20 to 120 times above the current recommended reference dose (US-EPA) of 3 g/kg b.w./day for chlorpyrifos exposure to children from all sources. (Davis & Ahmed 1998). In Denmark, pesticides are not used very much in the indoor environment (MST 2000 - personal communication).

#### 5.4.2 Carbamates

A considerable number of reproduction and teratogenicity studies have been carried out with different carbamates and various animal species. Generally, the foetal effects included an increase in mortality, decreased weight gain in the first weeks after birth, and induction of early embryonic death. Certain carbamates also induce teratogenic effects, mainly at high dose levels applied by stomach tube. When the same dose levels was administered with the diet, no effects were seen. (WHO 1986b).

#### 5.4.3 Lindane

Lindane is the  $\gamma$ -isomer of 1,2,3,4,5,6-hexachlorocyclohexane (HCH). Lindane interacts with cellular membranes and may produce several generalised cytotoxic effects associated with impaired membrane function. (ATSDR 1997).

Several cases of fatal poisoning and of non-fatal illness caused by lindane have been reported. The toxic or lethal dose appears to vary considerably; under certain conditions, 10 to 20 mg/kg b.w. can represent a lethal hazard to humans, but higher doses can be tolerated when followed by appropriate medication. Some information indicates that children are more sensitive to lindane than adults. (WHO 1991b).

The acute toxicity in experimental animals is moderate (oral LD<sub>50</sub>-values for rats and mice range from 60 to 250 mg/kg b.w. depending on the vehicle used). Young animals are generally more sensitive than adults. (WHO 1991b).

Lindane had no teratogenic effect in experimental animals after oral or parenteral administration. Foetotoxic and/or maternal toxic effects were observed with doses of 10 mg/kg b.w. and above when given by oral gavage. (WHO 1991b).

#### 5.4.4 Paraquat

Paraquat is the dichloride salt of 1,1'-dimethyl-4,4'-bipyridinium ion. Following exposure to paraquat, a characteristic lung injury is observed. A large number of cases of suicidal or accidental poisoning from paraquat has been reported. Among individuals who experience lung damage the mortality rate is high. The minimum lethal dose is stated to be about 35 mg/kg b.w.; however, some individuals have survived after ingesting 10-20 g of paraquat (about 140-280 mg/kg b.w.).

The acute toxicity in experimental animals is moderate (oral LD<sub>50</sub>-values for rats range from 100 to 200 mg/kg b.w.). Young rats were more resistant to the toxicity of paraquat than older rats; and some authors have paralleled this resistance with that of young rats to oxygen toxicity. One study (Smith & Rose 1977b) found a more than 40% increase in cumulative mortality in 180 g rats compared with 50 g rats, after oral dosing with paraquat at 175 mg/kg b.w.; according to the authors, the difference in renal function between young and mature rats accounted for the difference in paraquat toxicity.

Oral administration of high doses of paraquat to pregnant rats, mice, or rabbits on various days of gestation produced no evidence of teratogenicity, but did produce slight embryotoxicity; however, the doses used induced significant maternal toxicity. (WHO 1984).

### 5.5 Drugs

Several drugs are known to induce various adverse effects in children including the unborn child. The classical example is the known human teratogen thalidomide which caused unusual limb defects in babies born of mothers who had taken thalidomide between the fourth and ninth weeks of pregnancy. A few other examples are given here.

#### 5.5.1 Chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic having an antibacterial spectrum and potency very similar to those of the tetracyclines. It is well

absorbed from the gastrointestinal tract, metabolised in the liver, and excreted rapidly in the urine predominantly as metabolites. Chloramphenicol crosses the placenta and the concentration in the foetus varies from 30 to 80% of the concentration in maternal blood. Chloramphenicol is also secreted into the milk.

Premature babies and infants (up to one month of age) do not have the detoxifying liver enzyme (glucuronyltransferase) and are therefore extremely sensitive to the serious toxic effects associated with exposure to chloramphenicol. The symptom complex observed in this age group, often referred to as the “grey baby syndrome”, consist of gastrointestinal effects (vomiting and abdominal distension), dyspnoea, cyanosis, and vascular collapse.

Because of the serious toxicity such as aplastic anaemia (complete suppression of bone-marrow activity) observed in adults and in foetuses and the “grey baby syndrome” in infants following systemic administration, chloramphenicol is only indicated for the treatment of very serious infections; it is contraindicated for pregnant and lactating women. No data on teratogenic effects in humans are available.

(Lægemiddelkataloget 1996a, Goth 1978).

Chloramphenicol caused closure defects, among other abnormalities, in rats and non-specific malformations in rabbits. The teratogenic activity in the rat was attributed to interference with activity of the electron transport systems and oxidative energy formation in the embryo during embryogenesis. In mice and rhesus monkeys, no teratogenic effects have been noted under the regimens employed. Some postnatal behavioural effects, including reduction in learning ability, have been described in mice following prenatal treatment. (Schardein 2000c).

### 5.5.2 Sulfonamides

Sulfonamides are derivatives of sulfanilic acid and used for treatment of infections predominantly of the urinary tract. Only one sulfonamide, sulfamethizole, is registered in Denmark.

Sulfonamides are rapidly and extensively absorbed following oral administration, metabolised (acetylation or glucuronidation) in the liver and excreted in the urine predominantly as metabolites. Sulfonamides cross the placenta and are secreted into milk.

The most common adverse effects (dermatitis and drug fever) of sulfonamides are related to acquired hypersensitivity. Serious toxic effects (icterus because of damaged liver cells, aplastic anaemia, neutrocytopenia, acute haemolytic anaemia) are infrequently reported.

Because of the risk of development of icterus in new-borns and young infants (age up to one month), sulfonamides are contraindicated to pregnant women during the last 4 weeks before expected delivery, to lactating women, and to infants (age up to one month).

(Lægemiddelkataloget 1996b).

One study of 458 women taking sulfonamides over the entire pregnancy reported that there were more congenital malformations among their offspring than in the young of untreated controls. Four other studies found no relation to sulfonamide therapy in early pregnancy and malformations.



A large collaborative study found no significant malformations associated with the use of specific sulfonamides, including sulfamethizole. Sulfonamides have shown a mixed teratogenic potential in experimental animals with about one-third indicating activity. (Schardein 2000d).

### 5.5.3 Diethylstilboestrol (DES)

DES is an artificial non-steroid oestrogen used as an antineoplastic agent for treatment of prostate cancer. DES is absorbed rapidly following oral administration, metabolised in the liver and excreted in the urine and faeces. (Lægemiddelkataloget 1996c, Schardein 2000e).

DES is a transplacental carcinogen in humans and development of adenocarcinomas in the cervix and vagina have been observed in young females of mothers treated with DES during pregnancy.

DES was apparently thought to be efficacious in the definitive and preventive treatment of abortion and premature delivery. For this reason, it was given to a large number of pregnant women, being approved for use in pregnancy by the US-FDA in 1947. Early in 1970, seven cases of vaginal adenocarcinoma were reported in young women aged 15 to 22 and it was found that the patient's mothers had ingested oestrogen, DES specifically, in the first trimester of their respective pregnancies many years earlier. In 1971, a registry of clear cell adenocarcinoma of the genital tract in young females was established. By June 1997, 695 cases of clear cell adenocarcinoma were listed in the registry, two-thirds of which were associated with prenatal DES exposure. In all of the patients who have vaginal and cervical carcinoma, maternal ingestion of DES occurred before the 18<sup>th</sup> week of pregnancy and thus, early first-trimester exposure appears to be mandatory in its subsequent toxicity. A peak in the age incidence curve of DES-related cases has been observed at about 19 years, with the age range (latency) being 7 to 30 years.

By far, most of the cases reported have been in the USA, but case stories have also been reported from other countries; however, countries where DES was never used (e.g., Denmark and West Germany) did not have cancer cases.

A mechanism for the vaginal lesions has been theorised. DES may act to sensitise the proliferating stroma of the lower müllerian duct so that it is incapable of fostering upgrowth of urogenital sinus epithelium to spread over and replace the epithelium covering the vagina and cervical protoco by 18 weeks when this event should occur. DES may also preferentially affect the stroma of the developing cervix.

(Schardein 2000e).

## 5.6 Polychlorinated biphenyls (PCBs)

Because of their high persistence, and their other physical and chemical properties, PCBs are present in the environment all over the world. The general population is exposed to PCBs mainly through contaminated food (aquatic organisms, meat, and dairy products). Infants are exposed through the mother's milk and it has been estimated that the nursing period contributes about 1.3% of the life-time intake. (WHO 1993a).

In Denmark, an investigation has been performed to determine the content of dioxins and PCBs in the breast milk of Danish mothers. The average concentration of total PCB was 469 ng/g fat corresponding to an average daily intake in the sucking child of 2.4 µg/kg b.w./day. In the report, a NOAEL of 0.33 µg/kg b.w./day was considered for behavioural effects. However, these two figures should not be compared as the TDI relates to a life long exposure. (SST/FDIR 1999).

In general, PCBs appear to be rapidly absorbed, particularly via the gastrointestinal tract after oral exposure; information on the rates of human absorption is limited. PCBs are rapidly cleared from the blood and accumulate in the liver and adipose tissue. There is evidence of placental transport, foetal accumulation, and distribution to milk. (WHO 1993a).

There are great difficulties in assessing human health effects separately for PCBs and polychlorinated dibenzofurans (PCDFs) since, quite frequently, PCB mixtures contain PCDFs. Therefore, in many cases, it is not clear which effects are attributable to the PCBs themselves and which to the much more toxic PCDFs. Much of the data of the human toxicity come from large episodes of intoxication, e.g., the Yusho and Yu-Cheng episodes. The most striking signs of intoxication in Yusho and Yu-Cheng patients include hypersecretion in the eyes, pigmentation of the nails and mucous membranes, and acneiform eruptions of the skin.

Furthermore, oedema of the extremities, liver enlargement and disorders, central nervous disturbances, respiratory problems, and changes in the immune status were also observed. In children of Yusho and Yu-Cheng patients, diminished growth, dark pigmentation of the skin and mucous membranes, gingival hyperplasia, xenophthalmic oedematous eyes, dentition at birth, abnormal calcification of the skull, rocker bottom heel, and a high incidence of low birth weight were observed. (WHO 1993a).

The Michigan Maternal and Infant Study has reported adverse developmental health outcomes in new-born infants of mothers who consumed more than 12 kg of contaminated Great Lakes fish. Statistically significant decreases in infant's birth weight, gestational age, and head circumference compared to controls were observed. The infants also exhibited neurodevelopmental and behavioural deficits based on tests of visual recognition and memory at 7 months and 4 years of age. At 11 years of age, many of the neurobehavioural deficits had persisted, e.g., poorer short- and long-term memory and lower IQ scores. (Johnson et al. 1999).

Lesions induced in experimental animals exposed to PCB mixtures or individual congeners concern the liver, skin, immune system, reproductive system, oedema, and disturbances of the gastrointestinal tract and thyroid gland.

The acute toxicity of PCBs is generally low in rats; young animals appear to be more sensitive than adults.

The Rhesus monkey is the most sensitive species with regard to general toxicity and particularly with regard to reproductive toxicity. PCBs adversely affected the reproductive performance of female Rhesus monkeys, mated with control males after 6 months of dietary exposure to a toxic dose (0.09 mg/kg b.w./day) and continuation of the exposure for an average of up to 10 months. Neonates of nursing mothers exposed to PCBs (during gestation and lactation of the first generation) showed ad-

verse effects similar to those seen in their mothers and, in addition, persistent behavioural disturbances as well as several other adverse effects. PCBs may also bind to the cytoplasmic oestrogen receptor. Effects have also been observed on the oestrus cycle of female rats and monkeys, on the sex organs of male rats, and on the implantation rate of fertilised ova following exposure of female mice or male rats.

Available studies in rats and monkeys did not indicate any teratogenic effects (malformations), when animals were dosed orally during organogenesis at doses that produced foetotoxicity and/or maternal toxicity. (WHO 1993a).

## 5.7 Polychlorinated dibenzo-p-dioxins (PCDDs)

Consumption of food (including human milk) is the most important pathway for exposure to PCDDs for the general population (adults and children) representing over 90% of the total daily intake. Other pathways include inhalation and direct contact with PCDDs. Exposure of infants and young children may be very high because of their relatively high consumption of milk, including breast milk. (ATSDR 1998).

In Denmark, an investigation has been performed to determine the content of dioxins and PCB in the breast milk of Danish mothers. The average concentration of dioxins + PCB was 30 pg TEQ/g fat (TEQ = 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity equivalents) corresponding to an average daily intake of the sucking child of 150 pg TEQ/kg b.w./day. A TDI of 1-4 pg TEQ/kg b.w./day (dioxins and dioxin-like PCBs) has been set (WHO 1999) as an average daily intake for the whole lifetime (SST/FDIR 1999).

Humans can absorb PCDDs by the inhalation, oral, and dermal routes of exposure; absorption is vehicle-dependent and congener-specific. For most mammalian species, the liver and adipose tissue are the major storage sites of PCDDs. Tissue deposition is congener-specific and depends on the dose, the route of administration, and age; 2,3,7,8-substituted PCDDs are the predominant congeners retained in tissues. PCDDs are very slowly metabolised. The major routes of excretion are the bile and the faeces; smaller amounts are excreted via the urine. In mammalian species, lactation is an effective way of elimination PCDDs from the liver and other tissues. Human studies show that infants may absorb up to 95% of the amount ingested via breast milk. (ATSDR 1998).

A wide variety of effects have been observed in adults exposed to 2,3,7,8-TCDD. The primary targets appear to be the skin, liver, thyroid, and cardiovascular, endocrine, and immune systems; an increased cancer risk has also been observed. It is likely that these organs/systems will also be sensitive targets in children. Children exposed to 2,3,7,8-TCDD appear to be more sensitive than adults to the dermal effects (chloracne). A number of human studies have investigated the potential of 2,3,7,8-TCDD to induce developmental effects. In one study, no significant increases in the incidence of birth defects have been observed in the children of parents living in Seveso at the time of the accident or during the next 6-year period. In contrast, other studies have found increases in specific types of defects, although the total number of defects was not significantly altered. (ATSDR 1998).

In animal oral toxicity studies, toxic effects of PCDDs have been observed in most organs/systems. The studies clearly demonstrate that the developing organism is very susceptible to the toxicity of PCDDs, in particular 2,3,7,8-TCDD. Prenatal or perinatal exposure has resulted in structural malformations (e.g., cleft palate, hydronephrosis), functional alterations (e.g., damage to the immune system, impaired development of the reproductive system), decreased growth, and foetal/new-born mortality in several animal species. The organ system most sensitive during development is the reproductive system (alterations in androgen levels, secondary sex organs, spermatogenesis, fertility, and sexual behaviours). Also neurobehavioral effects are observed. Additionally, several animal studies provide evidence that exposure to 2,3,7,8-TCDD via mother's milk alone can adversely affect the developing animal. (ATSDR 1998).

### 5.8 Polybrominated diphenyl ethers (PBDEs)

PBDEs are widely used in a variety of materials and products including textiles, many types of electronic devices, cabins for and circuit boards in personal computers and TV sets, electrical cables, switches and capacitors, and building materials. Furthermore, PBDEs are found in several foods of animal origin (fish, meat, and cow's milk) (Darnerud et al. 1998, WHO 1994, WHO 1997b).

In Swedish human milk samples, the concentration of PBDEs has increased continuously with levels showing an exponential increase from 1972 and a doubling time of 5 years (Norén & Meironyté 1998).

Studies on the reproductive toxicity of PBDEs are limited. Only one study on fertility (decaBDE in rats) is available; no treatment-related effects in reproductive performance or maturation of pups were reported. The developmental toxicity studies available on decaBDE, octaBDE, and pentaBDE are equivocal. Foetotoxicity (resorptions, delayed ossification) but no malformations have been observed in rats exposed to decaBDE (one study) and octaBDE (two studies) and in rabbits exposed to octaBDE (one study) at dose levels which did not induce maternal toxicity. In contrast to these findings, foetotoxicity was observed in rats exposed to pentaBDE (one study) only in the presence of maternal toxicity. (WHO 1994, Darnerud et al. 1998).

A very recent study investigating possible neurobehavioural effects in neonatal mice (10 days old) following a single exposure to pentaBDE suggests differences in behavioural patterns between treated and control mice (Eriksson et al. 1998).

### 5.9 Phthalates

Phthalates are high production volume chemicals widely used as additives in PVC plastics. Due to the ubiquitous use, phthalates are found everywhere in the environment.

In Denmark, the intake of the phthalates DEHP, DBP, and BBP has been analysed in a number of samples (29) from a double portion study where

adults participated; estimates of the mean and maximum intake were 0.13-0.29, 0.02-0.03, and 0.19-0.3 mg/person/day for DBP, BBP, and DEHP, respectively. For children, the mean and maximum intake of phthalates from infant formulae has been estimated to <0.042, 0.0006-0.0009, and 0.009-0.021 mg/child/day (assumed child weight of 3 kg) for DBP, BBP, and DEHP, respectively. (Petersen 1999).

Some phthalates affect fertility and reproduction in rodents of both sexes and also produce developmental effects in the offspring.

Generally, phthalates with side chains of 4 to 6 carbon atoms in length, e.g., di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), and butyl benzyl phthalate (BBP), affect the reproductive system of male rodents whilst phthalates with side chains shorter than 4 carbon atoms and longer than 6 carbon atoms appear to be without effect. Observed effects include marked reductions in the weights of the testes and accessory sex glands, decreased numbers of spermatocytes, degeneration of the seminiferous tubules, a reduction in testicular zinc and iron levels and serum testosterone levels, an increase in testosterone levels in the testes, sloughing of germ cells, and vacuolisation of Sertoli cells (DEHP). Species differences have been observed as the reproductive system of the male rat appears to be more sensitive than that of the mouse, which appears to be more sensitive than that of the hamster, guinea pig, and non-human primates.

The effects on the male reproductive system are influenced by the age at which the animal is exposed. Studies of DEHP have shown that developing and sexually immature male rats are more sensitive to DEHP-induced testicular toxicity than sexually mature rats and that the onset of the effects in young animals is more rapid. Furthermore, exposure (DEHP, DBP) of rats prenatally and during suckling has produced irreversible testicular damage at dose levels inducing only minimal effects in adult animals. (CSTEE 1998, WHO 1997, Nielsen & Larsen 1996, Mylchreest & Foster 1998).

Numerous studies have shown that some phthalates induce embryotoxic and teratogenic effects in the offspring. DEHP is embryotoxic and teratogenic in mice and embryotoxic in rats at maternally non-toxic dose levels (CSTEE 1998, Nielsen & Larsen 1996). DBP generally induce foetotoxic effects in rats and mice in the absence of maternal toxicity, and teratogenic effects only at high maternally toxic doses (WHO 1997a).

In very recent studies in rats exposed to DBP during the prenatal and early neonatal periods, a number of effects were seen in the male offspring, including decreased anogenital distance, absent or underdeveloped epididymis and seminal vesicles, cleft phallus (hypospadias), decreased reproductive organ weights, and widespread germ cell loss in the testis. In contrast, vaginal opening, age at first oestrus, and oestrous cyclicity were not affected in the female offspring indicating that DBP is not oestrogenic but rather antiandrogenic. (Mylchreest & Foster 1998).

## 5.10 Lead

In the non-smoking adult general population, the major exposure pathway for lead is from food and water. The level of dietary exposure to lead de-

depends upon many factors, including foodstuffs consumed, processing technology, use of lead solder, lead levels in water, and use of lead-glazed ceramics.

For infants and young children, food, air, water and dust/soil are the major potential exposure pathways. For infants up to 4 or 5 months of age, air, breast milk, formulae and water are the significant sources of lead exposure. For infants and young children, lead in dust and soil often constitutes a major exposure pathway. Lead levels in dust depend upon such factors as the age and condition of housing, the use of lead-based paints, lead in petrol and urban density. The intake of lead will be influenced by the age and behavioural characteristics of the child and the bioavailability of lead in the source material. (WHO 1995).

A recent Swedish study (Berglund et al. in press - quoted from CSTE 2000) has shown that food is now the main source of lead exposure even in young children living in areas with high soil lead concentrations, i.e. downtown Stockholm (<10-330 mg/kg in soil) and mining areas (20-5000 mg/kg). It was concluded that lead in soil and dust contributed little to the total intake of lead.

In humans, lead adversely affects several organ systems and organs, including the nervous, haematopoietic, reproductive, and cardiovascular systems, the liver, the kidney, and the gastrointestinal tract. Neurodevelopmental effects and subcellular changes, particularly the effects on haem synthesis, appear to be the most sensitive endpoints. (WHO 1995).

Cognitive and sensory motor deficits have been shown in children to be associated with blood lead levels as low as 100 to 150 µg/l. An average IQ decrement between 1 to 3 points with increasing blood lead levels from 100 to 200 µg/l corresponds to 20% or less of the standard deviation of a typical IQ distribution. These and more recent data indicate, that even below 100 µg/l effects might occur and that no clear threshold for effects has been identified. (CSTE 2000).

Children, in comparison with adults, are more susceptible to lead in several respects. Children have a greater absorption of ingested lead than adults, resulting in a higher body burden from a given external exposure. About 40 to 50% of dietary lead is absorbed from the gastro-intestinal tract in infants and young children compared to around 5 to 10% in adults. Absorption of lead from ingested dust and soil is somewhat lower than from food, approximately 30% in infants and young children. It also appears that children are generally more sensitive to the toxicological effects of lead at a given internal exposure level (measured as the blood lead level) as the lowest observed effect levels (LOAELs) for various end-points (e.g. slowed nerve conduction velocity, impaired neurobehavioural function, encephalopathy, anaemia, reduced haemoglobin) are lower in children than in adults. (WHO 1995, WHO 1996).

## 5.11 Mercury

The general population is primarily exposed to inorganic mercury and methyl mercury through the diet. In most foodstuffs, mercury is largely in the inorganic form. Fish and fish products are the dominant source of methyl mercury in the diet. Air and water can also contribute significantly to the total daily intake of total mercury. Furthermore, dental

amalgam may also be a source of exposure to inorganic mercury due to release from amalgam restorations. (WHO 1990, WHO 1991a). No specific information was found concerning exposure of infants and children.

Methyl mercury is a well-established neurotoxicant that can cause serious adverse effects on the development and function of the human central nervous system, especially when exposure occurs prenatally (Harada 1995). The neurotoxic potential was first described from industrial exposures as the Hunter-Russell syndrome, and then reappeared in the fishing town of Minamata, Japan, in the early 1950s (Igata 1993). Most surprisingly, while unaffected themselves by mercury toxicity, many pregnant women exposed to mercury-contaminated fish bore infants that suffered from severe congenital poisoning (Harada 1995, Igata 1993). The characteristics of this form of developmental neurotoxicity are now relatively well known at high exposure levels, where a cerebral palsy syndrome occurs. In less severe poisoning, blindness, deafness, and mental retardation may be apparent. In a poisoning incident in Iraq, a dose-response relationship was established between maternal hair-mercury concentrations during pregnancy and the prevalence of severe psychomotor retardation in the children (Marsh et al. 1990). This evidence from poisoning outbreaks clearly documents the hypersusceptibility of the developing nervous system with regard to this neurotoxicant.

Current concerns relate to the neurotoxic risks at lower exposure levels prevalent in fishing communities (Grandjean et al. 1997). A birth cohort of 1000 Faroese children was examined at age 7 years, where clinical examination did not reveal any clear-cut abnormalities associated with the cord-blood mercury concentrations. However, mercury-related neuropsychological deficits at this age occurred in the domains of language, attention, and memory, and to a lesser extent in visuospatial and motor functions. The associations could not be explained by various possible confounders such as polychlorinated biphenyls (PCBs) from seafood, and they remained after exclusion of highly-exposed children with a maternal hair-mercury concentrations above 10 µg/g. This limit was thought to represent an upper safe level as based on the data from Iraq (WHO 1990).

Supporting evidence has now emerged from a fishing community in Madeira, where 149 children from the first grade in school showed mercury-related delays in the electrical signals of the brain, as recorded by the evoked potentials technique (Murata et al. 1999). A similar pattern was seen in the Faeroes (Grandjean et al. 1997). Other cross-sectional studies in Brazil (Grandjean et al. 1999) and French Guyana (Cordier et al. 1999), have shown mercury-associated developmental effects in agreement with the Faroese findings. However, a prospective study in the Seychelles has not revealed any clear adverse effects related to maternal hair-mercury concentrations, but results beyond 5 years of age are not yet available (Davidson et al. 1998).

Although the question as to the safety of fish consumption during pregnancy has not been settled, the preponderance of evidence indicates that the unborn child is much more susceptible to methyl mercury neurotoxicity than adults are. This substantial age-dependency may be a more

general phenomenon for neurotoxicants, as similar evidence is available for other substances, especially lead and PCBs (Steuerwald et al. 2000).

### 5.12 Copper

In the general population, the major route of exposure to copper is oral. Variations in dietary copper intake reflects different dietary habits as well as different agricultural and food processing practices used world-wide. In some cases, drinking water may make a substantial additional contribution to the total daily intake of copper, particularly in households where corrosive waters have stood in copper pipes. All other intake for copper (inhalation and dermal) are insignificant in comparison to the oral route. Women using copper-containing intra uterine devices (IUDs) are exposed to only minor amounts from this source. (WHO 1998a).

Copper is an essential element and adverse health effects are related to deficiency as well as to excess intake. The relationship between intake and risk has a U-shaped curve, with risk for deficiency associated with low intakes and risk for toxicity associated with high intakes. The range of acceptable intakes which meet the biological requirement without causing toxicity may be rather narrow.

New-borns appear to absorb copper more readily than adults whereas the aged appear to absorb copper less efficiently. Copper can cross the placental barrier and is taken up by the foetus. The copper concentration in the liver of new-borns is 6-10 times higher than in adults but decreases during the first 3 months of life.

The concentration of copper in the body is kept relatively constant by homeostatic mechanisms, and toxic effects of long-term ingestion of excess copper are not frequently observed in the general population. However, children below one year of age are probably more susceptible than adults to copper toxicity because the homeostatic mechanisms may not have fully developed. A number of cases (some of them fatal) in which development of liver damage (early childhood cirrhosis) has been related to excess intake of copper from drinking water has been described. (Nielsen 1997).

### 5.13 Boric acid

The most frequent and appreciable general population exposures to boron are likely to be from ingestion of food and, to a lesser extent, from ingestion of drinking water. Other potential sources include absorption of boron from cosmetic and medical preparations through mucous membranes or damaged skin; and the inhalation, dermal absorption, or accidental ingestion of boron-containing household cleaning products, pesticides, or fertilisers. (WHO 1998b).

Boric acid is readily absorbed from the gastrointestinal and respiratory tracts. The absorption is essentially complete (approximately 95% in humans) following ingestion. Dermal absorption across intact skin is negligible in all species evaluated, including humans (infants and adults). Boric acid is rapidly excreted mainly (95%) by the kidneys. (WHO 1998b).



Only a few human studies have been conducted to assess health effects associated with exposure to boron compounds, including boric acid. Fatalities among young children have resulted from skin absorption of boric acid used as dusting powder on diapers; in 120 reported cases of poisoning, the mortality was 52.5% (Deichmann & Gerade 1969). Accidental use of boric acid solution in the preparation of baby formula has resulted in poisoning in infants and a lethal dose of 2 to 3 g has been reported. Based on the reported lethal doses, which are not well documented in the literature, infants appear to be more sensitive than adults to boron compounds. (WHO 1998b).

After repeated oral administration to experimental animals, growth inhibition, organ weight changes, and testicular damage are the most striking effects observed.

The testis is the critical target organ with adverse effects being observed ranging from inhibited spermiation to degeneration of the seminiferous tubules with variable loss of germ cells, to complete absence of germ cells resulting in atrophy and transient or irreversible loss of fertility but not of mating behaviour.

Developmental toxicity has also been demonstrated in experimental animals with lower foetal bodyweight being the critical effect (WHO 1998b).

#### 5.14 Nitrate and nitrite

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion is the stable form for oxygenated systems although it can be reduced to nitrite by microbial action.

In general, vegetables will be the main source of nitrate intake in the general population. When the nitrate concentration in drinking water exceeds 50 mg/l (the limit value in Denmark), drinking water will be the major source of total nitrate intake, especially for bottle-fed infants. For the bottle-fed infant, daily intake from formula made with water containing 50 mg/l of nitrate would average about 8.5 mg/kg b.w. per day. (WHO 1996).

Ingested nitrate is readily and completely absorbed from the gastrointestinal tract and rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva, where it is reduced to nitrite by the oral microflora. Bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract. (WHO 1996).

The toxicity of nitrate to humans is thought to be solely the consequence of its reduction to nitrite. The major biological effect of nitrite in humans is its involvement in the oxidation of normal haemoglobin to methaemoglobin, which is unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifest when methaemoglobin concentrations reach 10% of that of haemoglobin and above; the condition, called methaemoglobinaemia and also known as the “blue baby syndrome”, causes cyanosis and, at higher concentrations, asphyxia. The haemoglobin of new-borns and young infants is more susceptible to

methaemoglobin formation than that of older children and adults. (WHO 1996).

## 5.15 References

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## 6 Regulations

Chemical substances are present around us and consequently we are all exposed to a number of such substances e.g., via the environment, food and beverages, cosmetics, and through a number of different products which many of us use daily. Additionally, adults and young people may be exposed to chemical substances via the working environment and children by contact with toys and childcare products (see section 3).

In order to assure the best possible protection against adverse effects resulting from exposure to chemical substances, several regulations within the different areas have been issued. This part of the report briefly summarises the most relevant Danish regulations on chemical substances with focus on the protection of children and pregnant women, including their unborn child, exposed to chemical substances.

It should be noticed that the regulations of chemical substances are based on the existing knowledge with regard to adverse health effects and risks related to the use of the substances. It must be emphasised, that for the great majority of substances used, very little - if any - knowledge exists regarding the relevant exposure scenarios and/or vulnerability of children, including the unborn child.

### 6.1 Environmental contamination

In Denmark, health based limit values (quality criteria) are set for chemical substances in soil, drinking water and ambient air according to the principles laid down in the Guideline for health based evaluation of chemical substances in drinking water (MST 1992a) and in Appendix A of the Industrial Air Pollution Control Guidelines (MST 1992b). The principles as they are used today will briefly be outlined here:

When the available toxicological data have been evaluated, the hazard considered most important, “the critical effect”, for setting the quality criteria for a given substance is identified. Generally, the effects are considered to be of more concern the lower the concentration or dose at which they occur, and the effect observed at the lowest concentration or dose level often forms the basis for the calculation of a tolerable concentration or tolerable daily intake (TDI).

In doing this, a “no observed adverse effect level” (NOAEL) or when this is not possible a “lowest observed adverse effect level” (LOAEL) is identified. Having identified a NOAEL (or a LOAEL), three safety factors (uncertainty factors)  $SF_I$ ,  $SF_{II}$ , and  $SF_{III}$  are used for the calculation of the tolerable concentration or TDI.  $SF_I$  accounts for interspecies variation in susceptibility and  $SF_{III}$  accounts for the quality and relevance of the data.  $SF_{II}$  accounts for differences in intraspecies susceptibility, i.e. this safety factor is used for the protection of groups in the population that may be more susceptible than the general population such as children, pregnant women and their unborn child, elderly or sick people; in general a default value of 10 is used for  $SF_{II}$ .



Following calculation of the tolerable concentration or TDI, the health based limit values are derived for the relevant media by division of the value for the tolerable concentration or TDI by a given standard exposure level of the relevant media. For derivation of the limit value for drinking water and ambient air, a daily standard exposure for an adult person is used. Thus, no specific attention is normally given to exposure of children. For soil exposure, however, the standard exposure used for calculation of the limit value is a standard exposure for soil exposure of children (values for daily soil ingestion or soil in contact with skin), as exposure to soil is considered especially important (and critical) for children. To ensure that the total daily intake for various media does not exceed the tolerable concentration or TDI, a certain percentage (often 10%) of the tolerable daily exposure level can be assigned to the relevant media and in this way account for other important exposure routes such as exposure through the food.

## 6.2 Chemical substances and products

### *Classification and labelling*

Within the EU, regulations on classification and labelling of chemical substances are based on total harmonised directives (EEC 1967, with amendments). In Denmark, the Statutory Order on classification, packaging, labelling, sale and storage of chemical substances and preparations (MEM 1997) implements the relevant EU Council Directives into national legislation. The Statutory Order provides the regulations for evaluating the inherent properties of a chemical substance or preparation in relation to hazard identification and assessment.

The hazard identification and assessment cover a number of important toxicological endpoints such as acute toxicity, irritative and corrosive properties, sensibilisation, repeated dose toxicity, reproductive and developmental effects, and genotoxic and carcinogenic properties. Chemical substances considered dangerous according to the regulations are classified and labelled with symbols and indications of danger, nature of special risks (R-phrases) and safety advises (S-phrases).

Classification is normally based on results from specific animal experiments according to EU guidelines (Annex V to EEC 1967, with amendments). In most of these studies, no specific attention is paid to whether specific age groups could be identified as sensitive subgroups.

A number of R- and S-phrases are, however, specifically related to protection of children and/or pregnant women:

Chemical substances which are classified as reproductive toxicants in category 1 (substances known to impair fertility or to cause developmental toxicity in humans) and category 2 (substances which should be regarded as if they impair fertility or cause developmental toxicity in humans) are labelled with R60 “may impair fertility” or R61 “may cause harm to the unborn child”, respectively.

Chemical substances which are classified as reproductive toxicants in category 3 (substances which cause concern for human fertility or for humans owing to possible developmental toxic effects) are labelled with

R62 “possible risk of impaired fertility” or R63 “possible risk of harm to the unborn child”, respectively.

Chemical substances which are known or suspected to be secreted into the breast milk at toxic levels are labelled R64 “may cause harm to breastfed babies”.

The S-phrase S2 “keep out of the reach of children” is assigned to every classified chemical substance or preparation.

*Packaging, sale and storage*

The Statutory Order on classification, packaging, labelling, sale and storage of chemical substances and preparations (MEM 1997) also include regulations which ensure that children will not come into contact with most of the dangerous chemicals.

Chemical substances and preparations which are classified as carcinogenic, mutagenic, or reproductive or developmental toxicants in category 1 and 2 must not be sold to the general public.

Chemical substances and preparations which are classified as “very toxic” (Tx) and “toxic” (T) can only be sold to individuals of the general public who have a special permission from the police.

Chemical substances and preparations must not be marketed in containers which are considered to attract children’s attention. For certain classified chemical substances and preparations, special closures (child-resistant fastenings) should be used for the containers and such products should be stored out of the reach of children.

*Risk assessment in EU and OECD*

Within the EU and OECD, specific programs on risk assessment for chemical substances are on-going. In the EU, a directive (EEC 1993a) provides the regulation on risk assessment of new notified chemical substances and two Council regulations (EEC 1993b, EEC 1994a) on risk assessment of existing substances. Denmark takes part in the risk assessment programs according to the EU regulation, but no specific regulation have been issued in Denmark to implement this regulation.

The risk assessment process, in relation to both human health and the environment, entails a sequence of actions: effect assessment (hazard identification and dose (concentration) - response (effects) assessment), exposure assessment (estimation of the concentrations/doses to which human populations or environmental compartments are or may be exposed) and risk characterisation (estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance). The risk characterisation is carried out separately for three subgroups of the population: workers, consumers, and man exposed indirectly via the environment; only the last two subgroups include children. In the risk characterisation for consumers and man exposed indirectly via the environment, the interspecies variation (see 6.1) is taken into account when considering the margin of safety (the ratio between the NOAEL or LOAEL and the estimated exposure).

For some specific substances (e.g., phthalates), children have been considered as a specific subgroup of consumers as children may be exposed to these substances in a way which is not considered relevant for adults.

No specific EU or OECD guidelines for risk assessment for children and pregnant women and their unborn child have been issued.

*Assessment based on available data*

Classification and risk assessment are based on available data. In general, the available data on a specific chemical substance seldom allow for an evaluation with respect to whether children or the unborn child should be considered more susceptible than adults. Concerning fertility and teratogenicity, data exist only for 20-30 % of the 2500 high production volume chemicals, and for thousands of chemicals produced in smaller quantities this percentage is expected to be considerably lower (Danish Board of Technology 1996).

*Testing of chemical substances*

*new chemical substances*

For new notified chemical substances (substances which were not on the market within the EU at any time in the 10 years prior to 18 September 1981), step-wise, tonnage related data requirements have been issued. The number of tests required is dependant on the supply level. The results of one study should generally be evaluated before another study is initiated.

*base-set testing*

Base-set testing is demanded for a new substance produced or imported in amounts of 1-10 tonnes/year per producer or importer in an EU member state. No specific tests to detect adverse effects on reproduction are required. However, it is indicated that there is a requirement at this level to "screen" new substances for their potential to cause reproductive toxicity.

*level 1 testing*

Level 1 testing is additional to the base-set testing and includes a fertility study (one species, one generation, males and females, most appropriate route of administration) and a teratogenicity study (one species, most appropriate route of administration). Level 1 testing may be required when supply levels reach 10 tonnes/year per producer or importer (or 50 tonnes/year cumulative per producer or importer in the EU) and shall be required at supply levels of 100 tonnes/year (500 tonnes cumulative) unless the notifier can give good reason why a particular test is not appropriate or an alternative scientific study would be preferable.

*level 2 testing*

Level 2 testing is required for high production volume chemicals, i.e. when supply levels reach 1000 tonnes/year (5000 tonnes cumulative) and includes fertility study (three-generation study; only if an effect on fertility has been established at Level 1), developmental toxicity study on peri- and post-natal effects, and teratogenicity study (species not employed in the respective Level 1 study).

*less than 1 tonne/year*

For new substances produced in amounts less than 1 tonne/year, no tests on reproductive toxicity are required.

*existing chemical substances*

For existing high production volume chemical substances (substances which were on the market within the EU at any time in the 10 years prior to 18 September 1981 and produced in amounts above 1000 tonnes/year), the data to be made available shall at least include the base-set required for new substances. Any gaps in the base-set data should be filled, unless the producers or importers can justify not providing it.

### 6.3 Cosmetics

The use of cosmetics by different age groups, including infants and children, has increased during the last years. Consequently, exposure to chemical substances via cosmetics is increasing and it has been suggested that this is a contributing factor for the increased incidence of al-

lergy observed among the general population. Illustrative examples of the increasing use for infants and children include bath and shower preparations, suncare products, wet tissues, hair care products (including colours and bleaches), and special cosmetics intended for children.

Within the EU, regulations on cosmetics are based on totally harmonised directives (EEC 1976a, with amendments). In Denmark, the Statutory Order on cosmetic products (MEM 1998a) implements the relevant EU Council Directives into national legislation. The regulation on exposure to chemical substances in cosmetic products is built up of different lists:

Annex 1 lists the different cosmetic products which are regulated.

Annex 2 lists the chemical substances which are not allowed intentionally as ingredients in cosmetic products.

Annex 3 lists the chemical substances to be used in cosmetic products according to specific limitations.

Annex 4-6 are positive lists for pigments, preservatives and UV-filters, respectively.

In order to protect children against exposure to specific chemical substances in cosmetics, a few specific substances have been banned from cosmetic products intended for children below 3 or 12 years of age.

The evaluations of chemical substances to be used in cosmetic products are discussed in the EU Scientific Committee on Cosmetics. In the Committee's evaluation of a specific substance, the safety margin for expected daily use is estimated; however, no specific considerations are taken regarding the use of the substance in products which will be used for children.

#### **6.4 Toys and childcare products**

Within the EU, regulations on toys are based on total harmonised directives. The Statutory Order on safety regarding toys and products which can be mistaken for foodstuffs (FST 1995) implements the EU Council Directives (EEC 1987, EEC 1988a, with amendments) into national legislation. According to these regulations, toys may generally not contain dangerous chemical substances (those substances which are classified according to the EU Council Directive (EEC 1967) on classification, packaging and labelling of chemical substances and preparations) in such amounts, which will give rise to development of adverse effects in children. Furthermore, specific migration limits have been set for 8 metals (antimony, arsenic, barium, cadmium, chromium, lead, mercury, selenium). It should be noted, however, that a safe migration limit for a substance in a specific toy only can be set on the basis of a realistic worst-case exposure scenario with respect to toy exposure. The scenarios may differ considerably for different kind of toys and the possibility for exposure during use (and misuse).

Denmark has prohibited the use of phthalates in toys and childcare products for children of the age of 0 to 3 years (MEM 1999).

EU has adopted measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of 6 specific phthalates (DINP, DEHP, DBP, DIDP, DNOP, BBP) (EU 1999).

Several other national regulations have been issued in order to protect children from exposure to chemicals through their use of toys.

## 6.5 Food additives, pesticides and contaminants in food

### 6.5.1 Food additives

In Denmark, food additives are regulated by the Statutory Order on food additives (FM 1997) which implements the relevant EU Council Directives (EEC 1978, EEC 1988b, with amendments) into national legislation. The use and use levels of permitted food additives are given in "Positivlisten" (VFD 1997). The establishment of the use levels is based on the ADI (acceptable daily intake) which is estimated from the "no observed adverse effect level" (NOAEL) considered from evaluations of studies on experimental animals or, in cases where a NOAEL cannot be identified, the "lowest observed adverse effect level" (LOAEL), by applying a safety factor of normally 100. This safety factor is considered to take into account the intraspecies variation, i.e. to take into account that the observed effects may vary in severity within the population, thereby protecting children and pregnant women against possible adverse health effects from exposure to the substance.

The ADI is not considered to cover the use of food additives in infant formula, since the usual toxicity test regimen does not cover this situation; therefore, specific evaluations are carried out for the use of food additives in infant formulas.

#### *Budget method*

Having obtained the ADI, the use levels are derived by applying the so-called 'Budget method'. The Budget method, which has been developed by the National Food Agency, now the Danish Veterinary and Food Administration, is based on considerations of the total daily intake of energy (calories) and liquid. The intake of liquid per kg body weight varies with age, being highest in small children, thereafter declining rapidly during childhood and more slowly in adult age. The Budget method uses the recommended liquid intake at the age of two, 100 ml/kg b.w., as the basis for the calculations of maximum intakes. Intake of food (energy) per kg body weight is also dependent on age. The Budget method uses the energy requirement of 100 kcal for a one-two year old child as a basis for the limit of food intake which will sufficiently protect adults also. (Larsen 1992).

### 6.5.2 Pesticides

#### *Sale and use*

Before sale and use, the active substance in the formulated pesticide product has to be authorised in the EU, whereas the formulation itself has to be authorised in the actual Member State for each crop. The active substances are evaluated according to the EU regulations (EEC 1967,

with amendments) on classification, packaging and labelling of chemical substances and preparations, see 6.2. In Denmark, the formulations are evaluated according to the Statutory Order on pesticides (MEM 1998b) which implements the relevant EU Council Directives (EEC 1991, EEC 1976, with amendments) into national legislation.

The evaluation of the formulations are based on risk assessment for specific uses and therefore generally only apply to adult workers. Although children and pregnant women may also be exposed to formulated pesticides, e.g. following their use in gardens and indoor such uses are generally not included in the evaluation of the formulations.

To protect the general population, the most toxic formulations which are labelled as “very toxic” or “toxic” according to the EU regulations on classification, packaging and labelling of chemical substances and preparations (see 6.2), are not allowed for use in gardens, public areas, kindergartens etc. This includes substances which are classified as reproductive or developmental toxicants in category 1 and 2. Formulations are banned in Denmark when the substance is carcinogenic or mutagenic in category 1 and 2. Also when exposure is greater than the AOEL (Acceptable Operator Exposure Level), use of the product cannot be approved. The AOEL is based on the no effect level (NOAEL) from animals studies with a safety factor of at least 100. Formulated products classified as “irritant” with the R-phrase R41 “risk of serious damage to eyes” are not allowed in Denmark.

A separate risk evaluation is not done for re-entry in private homes after professional application of products not allowed for private use. However, in green-houses, where relevant, this evaluation is done for re-entry exposure.

Specific considerations related to protection of children and/or pregnant women are taken into account in the evaluation of a given formulation. Data from studies on reproductive and developmental effects as well as effects in sucking pups of the active substance(s) are requested for the authorisation.

Furthermore, when estimating the ADI for the active substance one of the safety factors, often set to 10, takes into account the intraspecies variation, i.e. that the observed effects may vary in severity within the population.

### *MRLs in foodstuffs*

EU establishes harmonised maximum residue levels (MRLs) for pesticides in foodstuffs, which are in force in all Member States. In Denmark, the Statutory Order on maximum residue levels for pesticides in foodstuffs (FM 1999a) implements the relevant EU Council Directives (EEC 1976b, EEC 1986a,b, with amendments) into national legislation; the MRLs for a number of substances are given in the Annexes of the Statutory Order. The establishment of MRLs is based on the principles of Good Agricultural Practice (GAP<sup>1</sup>) and the estimated food consumption per person. From the MRL the TMDI (theoretical maximum daily intake) is calculated and compared with the ADI estimated according to the

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<sup>1</sup> GAP in the use of pesticides “includes the nationally authorised safe uses of pesticides under actual conditions necessary for effective and reliable pest control. It encompasses a range of levels of pesticide applications up to the highest authorised use, applied in a manner which leaves a residue which is the smallest amount practicable.”

same practice as already mentioned for food additives, see 6.5.1. If the TMDI exceeds the ADI, more refined methods are used to calculate a more realistic intake using e.g. the actual median residue levels determined after GAP supervised trials, and reduction factors from the processing of food. If the ADI is still exceeded, then the proposed MRLs cannot be endorsed.

For pesticides with a highly acute toxicity, acute reference doses are also estimated and compared with the MRL to protect against such effects.

The Danish Veterinary and Food Administration has analysed the residue levels of pesticides in many different foodstuffs. The estimated intake of the most frequently detected pesticides indicates that the average intake for adults is maximally a few percent of the ADI; therefore, it may be considered that most children also are protected against possible adverse health effects from exposure to the actual pesticides in foodstuffs.

In the EU, a general limit value of 0.01 mg/kg of residues of individual pesticides in processed baby foods and infant formulae has been issued (EEC 1999a,b). This limit value is considered to provide a very high degree of protection of children against adverse health effects from exposure to pesticides. Furthermore, specific pesticides (listed in Annexes to the Directives) must not be used in agricultural products intended for the production of baby foods and infant formulae. These regulations will be implemented in a revision of the Statutory Order on pesticides.

### 6.5.3 Veterinary medicinal products

EU establishes harmonised maximum residue levels (MRLs) for veterinary medicinal products in milk and foods of animal origin, which are in force in all Member States (EEC 1990, with amendments); the MRLs for a number of substances are given in the Annexes to the Council Regulation (EEC 1990, with amendments).

MRLs for veterinary medicinal products are established on the basis of the ADI assuming that a person weighing 60 kg consumes a certain amount of meat and fish and 1.5 l of milk (cheese and butter included) (“standard food package”). By using this “standard food package” in relation to children, the milk intake is underestimated as children often drink more than 1.5 l of milk (see section 3); however, the intake of meat and/or fish will often be overestimated as children generally consume less of these foods than adults. Thus, the ADI is generally not exceeded for children under normal circumstances. However, if an MRL is only set for milk (e.g. products for intramammary administration) and the ADI is solely allocated to this commodity; the underestimation of the milk intake for children may consequently result in exceeding the ADI even if the level of the given residue in milk is within the MRL.

### 6.5.4 Contaminants

Chemical substances arising from different sources may occur in foodstuffs. In Denmark, the Statutory Order on certain contaminants in foodstuffs (FM 1999b) implements the relevant EU Council Directives (EEC

1993c, EEC 1997, with amendments) into national legislation; limit values for some metals (lead, cadmium, mercury and tin), nitrate, and mycotoxins (aflatoxin and ochratoxin A) are given in the Annexes. The evaluation of contaminants in foodstuffs are carried out according to the same practice as already mentioned for food additives, see 6.5.1. However, a TDI (tolerable daily intake) instead of an ADI is estimated to signal that the contaminants are not added to foodstuffs deliberately. Furthermore, the database for evaluation of contaminants is less comprehensive than for food additives and pesticides and therefore, higher safety factors are often applied when the TDI is estimated.

Since 1983, the Danish Veterinary and Food Administration has analysed the levels of a number of contaminants in relevant foodstuffs. The levels of the analysed contaminants have significantly decreased during recent years and no specific dietary advice to pregnant women are considered necessary.

## 6.6 Working environment

In Denmark, the general regulations for employers and workers are given in the Danish Working Environment Act (1998) which include specific regulations regarding young people; these regulations are further specified in the Statutory Order on young people's employment (AM 1996). Specific regulations are also given for pregnant women in the Statutory Order on the performance of the work (AM 1994). These regulations have been issued to protect young people and pregnant women against exposure to chemical substances and preparations via their working environment.

### *Young people*

The specific regulations for young people below the age of 18 is given by the Statutory Order on young people's employment (AM 1996); the Statutory Order goes beyond the relevant EU Council Directive (EEC 1994b).

Generally young people below the age of 18 are not allowed to work with or in other ways be exposed to specific chemical substances and materials listed in Annex 2a to the Statutory Order. Most of the listed chemical substances are covered by the EU regulations (EEC 1967, with amendments) on classification, packaging and labelling of chemical substances and preparations (see 6.2) and the following are included:

- substances classified as "very toxic" (Tx), "toxic" (T), "corrosive" (C), "explosive" (E)
- substances with the R-phrases R12, R39, R45, R46, R60, R61
- substances classified as "harmful" (Xn) and at least one of the following R-phrases R40, R42, R48, R62, R63
- substances labelled as "irritant" (Xi) and R43
- substances and materials included on the Danish Working Environment Service's list of chemicals and processes considered as carcinogenic as well as materials containing more than 0.5% of the chemicals
- organic solvents included on the Danish Working Environment Service's list of organic solvents as well as materials containing more than 0.5% solvents.



The specific regulations for pregnant women are given by the Statutory Order on the performance of the work (AM 1994); the Statutory Order implements certain regulations in the relevant EU Council Directives (EEC 1989, EEC 1992, with amendments). Other regulations are implemented by collective agreements or other legislation.

Generally, a specific evaluation of the pregnant woman's working environment, including the influence of exposure to chemical substances, is requested to identify possible influence on the pregnancy. If this evaluation identifies any influences, a more detailed evaluation is requested regarding the nature, extent and duration of the influence(s) to identify any risks for the pregnant woman and the unborn child. If any risk is identified, the working environment of the pregnant woman should be improved or, in cases where such an improvement is not possible, the pregnant woman should be removed from the actual working environment. Annex 4 to the Statutory Order lists the chemical substances which must at least be considered when evaluating the risk during pregnancy and lactation. Included are:

- chemical substances and materials classified and labelled with R40, R45, R46, R60, R61, R62, R63, and R64
- chemical substances and materials included in the Danish Working Environment Service's Statutory Order no 300, 1993 regarding carcinogens
- chemical substances and materials included in the Danish Working Environment Service's Statutory Order no 52, 1988 regarding materials containing volatile chemicals, including solvents
- pesticides
- volatile anaesthetics
- mercury
- lead
- cytostatics
- carbon monoxide
- chemical substances, which pose a proven risk by dermal uptake.

## **6.7 Drugs**

### **6.7.1 Registration of drugs**

In EU, there is at present no mandatory requirement for pharmaceutical companies to investigate new medicinal products in children.

The pharmaceutical industry is however encouraged to investigate the safety and efficacy of a product in children, if it is likely to be of therapeutic benefit to this age group (CPMP 1997). In case sufficient documentation is provided, the license may cover treatment of children.

### **6.7.2 Principles for dosing children, and pregnant and lactating women**

The unborn child as well as infants are considered potentially more susceptible to adverse effects of drugs than adults. The principles for medical treatment of children and pregnant and lactating women are outlined

below. The principles and recommendations are described in the catalogue of registered pharmaceuticals in Denmark, *Lægemiddelkataloget* (1997).

### *Children*

According to *Lægemiddelkataloget* (1997), schematic dosing of infants and children is difficult and is generally practised as mg drug per kg body weight.

Because of the differences between children and adults in response to drugs, simple proportionate correction in the adult dose for body weight may not be adequate to determine a safe and effective dose for children, especially for the very young. Dosage requirements, cardiac output, hepatic and renal blood flow, and glomerular filtration rate in children and in adolescents of widely differing sizes have been found to correlate better with body surface area than body weight. According to this concept, a child's dose can be calculated from the formula:

$$\text{child's dose} = 1.4 \times \frac{\text{surface area child (m}^2\text{)}}{1.8 \text{ m}^2} \times \text{adult dose}$$

where 1.8 m<sup>2</sup> is the surface area of an average 70-kg adult. The surface area of a child can be determined from its body weight using the observation that surface area is proportional to body weight to the 0.7 power. (Rowland & Tozer 1995).

Another approach for determining the dose for children is to test the drug in this age group and the Committee for Proprietary Medicinal Products (CPMP) has issued a Note for Guidance on Clinical Investigation of Medicinal Products in Children (CPMP 1997).

Drug therapy in new-borns and particularly premature babies requires special attention because of the very rapid changes in renal and hepatic function in these early stages of life. Therefore, dosing of this age group is generally referred to paediatric specialists.

### *Pregnant women*

Most chemical substances, including drugs, taken by pregnant women can cross the placenta and enter the tissues of the developing embryo and foetus with consequent risk of teratogenic effects in the form of malformations or other developmental effects (see section 2 and 3 for further details).

Relatively few chemical substances and drugs are known human teratogens; however, a potential for inducing teratogenic effects by the remainder cannot be fully excluded as the mechanisms by which teratogenic effects can be induced are poorly understood and are probably multifactorial. Therefore, every drug should be considered as a potential human teratogen and medical treatment of pregnant women and women who plan pregnancy should be avoided if possible. However, some women with chronic diseases are dependent on medical treatment. Regarding medical treatment of pregnant women with chronic diseases, benefits and risks of the treatment should be carefully evaluated for the mother and the unborn child and in case of medical treatment, these women have to be very carefully monitored during their pregnancy. (*Lægemiddelkataloget* 1997).

As a general practise, medical treatment during pregnancy should be based on the following principles (Lægemiddelkataloget 1997):

- Avoid new drugs because preclinical studies do not fully exclude a risk to the human foetus.
- Avoid medical treatment during the first trimester where the risk for malformations are greatest.
- Manage medical treatment by monitoring the plasma concentration.
- Avoid combination of drugs.

### *Lactating women*

Most drugs administered to lactating women can be detected in the breast milk (see section 2 and 3 for further details). The concentration of drugs in the breast milk is highly variable and is dependent on many different factors; however, the knowledge within this area is still very limited. Therefore, as a general practise, medical treatment of lactating women should be avoided. If medical treatment cannot be avoided, the sucking child should be very carefully followed. (Lægemiddelkataloget 1997).

## **6.8 The Precautionary principle**

The precautionary principle is a principle that opens up for regulatory action on the basis of suspicions for harmful consequences rather than waiting for cases or a full scientific evidence. In the context of this report, the use of the precautionary principle may be relevant for the aim of protecting defined vulnerable groups in the population against potential hazardous exposures for which the harmful consequences are not fully understood.

The first actual mentioning of the precautionary principle in connection with legislation was in German legislation in 1976, where it is called the Vorsorgeprinzip: “Environmental policy is not fully accomplished by warning off imminent hazards and the elimination of damage which occurred. Precautionary environmental policy requires furthermore that natural resources are protected and demands on them are made with care.” Since then, and in various wording, the precautionary principle has been incorporated in a series of international treaties (e.g., the EU Amsterdam Treaty from 1998) and declarations. In many of these the principle has been connected mainly to hazardous chemical substances, but there are also examples of broader approaches to environmental issues as such. (MST 1998).

In Danish legislation the precautionary principle is not explicitly mentioned, but it is often reflected in the introductory comments to various environmental acts, the central ones being the Environmental Protection Act, the Chemical Substance and Product Act, the Marine Environment Act, and the Gene Technology Act. (MST 1998).

Both in Denmark and abroad, there has been increasing focus on the precautionary principle in recent years. In line with the increasing complexity of environmental issues, politicians, enterprises, and authorities are expected to allow this doubt to benefit the environment and through this public health. However, it is not clear either in Denmark nor in other

countries, what the principle specifically involves and how it should be applied in the day-to-day work. To have a debate on the issue and to give a better understanding of the principle, the Danish EPA held a conference on 29 May 1998 which was attended by industry people, researchers, green organisations, politicians, authorities, and other interested parties. The conference provided no clear answers and no easy solutions. However, in general there was broad agreement among the presenters and during the debates that the precautionary principle must be regarded as a political norm for both legislation and administration. It was expressed in the discussion that there was a great need for more guidance on how to apply the precautionary principle in practice. (Danish EPA 1998).

Recently, the Commission of the European Communities has published a communication on the precautionary principle (EU 2000).

## 6.9 Overall

As mentioned in section 1, the Danish Government initiated a cross-ministerial overview concerning the administrative measures for protecting children and pregnant women. From this overview, which also covered the chemical areas described in this section, it was concluded that in specific areas increased effort should be made to increase the protection of children and pregnant women and several future initiatives in different ministerial sectors were proposed.

With respect to environmental factors and chemical regulations, it was concluded that more specific focus on protection of children and/ or pregnant women should be put in regulatory procedures in relation to: the use of chemicals in cosmetics and toys; risk assessment procedures in the EU; risk assessment procedures in relation to the national derivation of limit values for air, soil and water; and the procedures for approval of pesticides (especially pesticides for domestic use) and for risk assessment of food additives and food contaminants.

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## 7 Summary, conclusions and recommendations

Concern has been raised that infants and children are at higher risk than adults from exposure to environmental chemicals. In particular, this important discussion has focused on the potential higher exposure of infants and children and increased vulnerability to the detrimental effects of chemicals. In 1998, the Danish government initiated a cross-ministerial overview concerning “protection of children and pregnant women against dangerous substances” and a conference was held in October 1998 in order to pay more attention to this topic and to discuss and identify possible needs for further development. The overall impression from the discussions at the conference was that children and pregnant women should generally be recognised as special risk groups with respect to effects from exposure to chemical substances. As a consequence, the present project was initiated by the Danish EPA.

### 7.1 Summary

#### 7.1.1 Aim of the project

The purpose of the project was to elaborate a detailed review and update the knowledge on the exposure and vulnerability of humans to chemical substances during the embryonic, foetal and postnatal periods (up to the age of about 18 years). The report is intended to form the scientific basis for future regulatory work of the Danish EPA in the protection of children and the unborn child to environmental chemical substances. Consequently, the report primarily focuses on chemical substances and regulatory aspects for which the Danish EPA has the responsibility. However, other chemical substances, such as drugs, have been included when they provide illustrative examples within the context of the report.

#### 7.1.2 Biological susceptibility

Age-related differences in organ susceptibility are a function of toxicokinetic and toxicodynamic parameters, including genetic, physiological, and metabolic factors, mechanism of action of the substance, and dose-effect and dose-response relationships. The toxicokinetic aspects cover absorption, distribution, metabolic conversion and excretion of a given substance. The toxicodynamic aspects cover parameters such as organ sensitivity and cytoprotective mechanisms, which determine the extent of any effect or response due to the presence of the substance at the site of toxicity.

*Susceptible periods in human development*  
*embryo-foetal period*

Chemical substances that pass maternal membranes are likely also to pass the placental barrier. As the placenta is permeable to chemical substances, almost all xenobiotics enter the foetal circulation. Therefore, the foetus is generally not protected against xenobiotics that circulate in the maternal blood.



During the preimplantation period, the conceptus is generally considered refractory to exogenous insults in terms of induction of classical malformations, but induction of embryonic death and/or latent developmental defects may occur (e.g., from exposure to DDT or nicotine). The chemically induced classical malformations and/or syndromes are mainly induced in early pregnancy during the period of organogenesis. Well-known examples of such teratogens are cytostatics, thalidomide, and diethylstilboestrol.

Exposure to chemicals (e.g., ethanol and methylmercury) during the subsequent foetal period (after 8 weeks of pregnancy) is not considered to result in major malformations, but susceptibility to substances affecting specific receptors and molecular targets still under development may lead to pronounced effects on a number of developmental processes. Informations concerning induction of such changes in humans in the foetal period are relatively scarce, but based on studies in experimental animals, it appears that this period may be even more sensitive than the embryonic stage to some growth and functional disturbances. However, in humans it may be difficult to interpret whether such damages are caused by pre- or post-natal events.

A number of findings reported over the last decade suggest that exposure to environmental factors in early life can increase the risk of disease later in life. To explain such findings, the concept of physiologic programming or imprinting in early life has emerged. Interference with such genetic programming has been demonstrated in a variety of test systems and reflects the action of a factor during a sensitive period or window of development to exert organisational effects that persist throughout life.

#### *peri- and post-natal periods*

The peri- and post-natal periods appear to be vulnerable periods, especially with respect to the physiological development of the central nervous system. There is human evidence that exposure to toxic substances (e.g., methyl mercury, TCDD) during the perinatal period of development may induce persistent functional/behavioural effects that become manifest shortly after birth or later in life although not morphologically apparent. After birth, the physiological development of the nervous, immune, and endocrine/reproductive systems continue to develop until adolescence. Therefore, the post-natal period should also be considered vulnerable. During the breastfeeding, the infant is exposed to chemical substances through human milk. As the functionalities of many organs are still immature, the new-born may be particularly susceptible to xenobiotics.

#### *Toxicokinetics and toxicodynamics*

Age-dependent differences in susceptibility to a chemical substance may be due to differences in either toxicokinetics or toxicodynamics, or both. Age-related differences in toxicokinetics/toxicodynamics occur in both experimental animals and humans. Except for a few specific substances, not very much is known about whether and why the response to a compound may differ between age-groups.

#### *toxicodynamics*

In general, it appears that effects of xenobiotics on organs or end-points may be similar in children and adults e.g., liver necrosis observed in adults will also be observed in children. As regards toxicodynamics, age-dependent differences are primarily related to the specific and unique effects that chemical substances may have on the development of the embryo, foetus and child in that the physiological development of the

nervous, immune, and endocrine/ reproductive systems continue to develop until adolescence. Furthermore, receptors and other molecular targets for various xenobiotics are continuously developing during the embryonic, foetal and infant periods. This may cause age-dependent differences in the outcome of receptor-xenobiotic interactions and even result in opposite effects of xenobiotics in infants and adults.

#### *toxicokinetics*

During pregnancy, many physiological changes occur in the maternal organism as a consequence of, and in order to support, the rapid growth of the foetus and reproductive tissues. These changes may in different ways influence the intake, absorption, distribution, metabolism and elimination of xenobiotics. The human foetus and the placenta possess metabolic capacity, but the contribution of these metabolising entities to the total kinetics is probably minimal.

In early infancy, the organs are still rather immature and various maturation processes are taking place. The complexity of all these factors makes it difficult to predict the net effect on absorption, distribution and elimination of chemical substances. However, the maturation of the gastrointestinal system, liver and kidneys has generally taken place within 6-12 months after birth. By late infancy, most processes related to metabolic activity and excretion are probably comparable to that of adults for most compounds. Because of the immature function of the organs, neonates and young infants may have lower biotransformation and elimination capacities. This may render these individuals less able to detoxify and excrete xenobiotics and thereby more vulnerable to toxicants. On the other hand, if toxicity is caused by toxic intermediates produced via biotransformation, young infants may be less sensitive.

In the subsequent late infancy and childhood, metabolism and excretion of xenobiotics may be equal to or even higher than in adults due to the higher basal metabolic rate and relative liver size. It is difficult to generalise about age-dependent deficiencies in the metabolism of xenobiotics because the various enzyme systems mature at different time points. The age at which metabolism is similar to the adult value may be different for each compound.

### 7.1.3 Exposure

Infants and children may be exposed to certain chemicals in the environment to a larger extent than adults as they, on a body weight basis, breathe more air, drink more water, and eat more food than adults. In addition, their behavioural patterns, such as being naturally curious, playing close to the ground, and putting their hands and objects into their mouth, can increase their exposure to chemicals in the environment. Children also have more years of life ahead of them than adults, so they have more time to develop those chronic diseases that take several decades to appear, and which may be triggered by early environmental exposure. Diseases with long latency periods include some forms of cancers such as mesothelioma from asbestos, skin cancer from sunlight, and leukaemia from benzene. Also, substances which accumulate in the organism may be of concern in this respect.

#### *Unborn child*

Most chemical substances entering the bloodstream of the pregnant mother will be distributed to the embryo/foetus. The exposure of the un-

born child will thus be a reflection of the exposure pattern of the mother. Persistent substances accumulated in tissues and organs of the mother (from previous exposures) may be redistributed to the unborn child (e.g., lead deposited in the bones of the mother).

*Infants and children  
human milk*

The infant is exposed to chemical substances through breast-feeding. Persistent organohalogenated substances (e.g., PCBs, dioxins and brominated flame retardants) accumulate in adipose tissue and are excreted into human milk at levels providing a comparably much higher intake in breast-fed infant than adults.

*drinking water*

Contamination of drinking water due to pollution by xenobiotics (e.g., pesticides and other industrial chemicals) as well as high levels of natural constituents (e.g., fluoride, copper and nitrate) constitute a potential health risk for all individuals. Infants and children are, however, at a higher risk since they, on a bodyweight basis, consume up to 5 times more water than adults.

*diet and beverages*

Infants and young children have a dietary pattern different from that of adults as they have a higher food intake per kg body weight and often also have special food preferences and needs. On a body weight basis, the average consumption of the main food groups (fruit, vegetables, bread, cereals, meat, fish, eggs) has been reported to be about 2-2.5 times higher in young children (aged 1½ to 4½) than in adults. For dairy products (excluding milk) and confectionery (including sugar), consumption up to 5 times the corresponding adult figures have been reported. For milk and beverages, the consumption by young children was reported to be up to 9 times and 16 times, respectively, higher than by adults.

*air*

Children inhale a relatively larger volume of air compared to adults and at the same time they often have a higher activity level (e.g., during playing) thus increasing their respiration rate. In some situations, children also have a higher exposure to e.g. vehicle exhaust as they, due to their height, inhale the air at a level closer to the vehicle emissions.

*exposure via dermal  
contact*

The use of cosmetics by different age-groups, including infants and children, has increased during recent years and consequently, exposure to chemical substances via cosmetics is increasing. Examples of products which are increasingly used by infants and children include child care products, bath and shower preparations, suncare products, wet tissues, hair care products (containing colours and bleaches), and special cosmetics meant for children (e.g., face paints). Cosmetics and child care products may be used repetitively and on a relatively large surface area of the skin. If skin irritation or skin damage is present, the absorption of chemicals from these products may be increased.

*behavioural differences*

Infants and children are by nature curious and continuously examine their environment. Especially oral exposure to chemical substances due to hand-to-mouth activities (e.g., ingestion of contaminants from soil and dust) and object-to-mouth activities (e.g., ingestion of phthalates migrating from toys, and mercapto benzothiazole migrating from soothers and teats made of natural rubber) are specific to infants and young children and thus present a risk of higher exposures to chemical substances for this age group compared to adults. Accidental exposures to a number of

different chemical substances and products, primarily household chemicals, organic solvents, drugs, irritating gases, asphyxiants in fumes, and poisonous animals, plants and fungi are also contributing to a higher exposure in children.

#### 7.1.4 Testing methodology

Well-planned and documented epidemiological studies have a clear advantage over studies in experimental animals in providing the most relevant information on health effects in humans, thus avoiding extrapolation from experimental animals. However, epidemiological studies have limitations too, particularly in determining exposure of chemical substances at environmental levels. In addition, epidemiological data can only be obtained after the substance in question has been introduced into the society.

Owing to the lack of adequate epidemiological data for most chemical substances, toxicological studies in animal species play an important role in hazard identification and risk assessment. For new chemical substances, this is the only way to obtain *in vivo* toxicity data.

Many different experimental methods for investigating toxic effects of chemical substances during the prenatal and postnatal period are in use. Several tests are standardised and guidelines have been issued by various governmental agencies and international organisations. The standardised reproductive toxicity tests can provide information on a number of developmental effects, such as malformations, growth retardation, foetal and postnatal death, fertility, and functional effects on the central nervous system. In a revised proposal for the two-generation reproductive toxicity study (OECD TG 416), assessment of effects on sperm quality and oestrous cyclicity in offspring have been added. It should be noted, however, that the majority of marketed chemical substances have not been tested according to the standardised reproductive toxicity tests and thus, a number of the developmental effects mentioned above have not been examined. In particular, investigations on effects on the developing brain are scarce. The OECD Guideline for Developmental Neurotoxicity Study is still only a draft and therefore not included in the presently used testing strategies.

Repeated dose toxicity testing in adult animals provide information on the potential for systemic toxicity by investigations of growth, clinical symptoms, haematology, biochemistry, organ weights, pathology and histopathology of organs. The investigations of systemic effects in the reproductive toxicity tests are not as comprehensive as the repeated dose toxicity studies in adults. In order to have a sufficient background to determine the sensitivity of the developmental period compared to adulthood there is a need for studies where end-points are investigated similarly for both age groups. Ideally, this would require a two-generation study incorporating developmental neurotoxicity end-points and supplemented with similar investigations of systemic effects in offspring as in repeated dose toxicity studies.

For drugs, there is at present no mandatory requirement in the EU for pharmaceutical companies to investigate new medicinal products in chil-

dren; however, industry is encouraged to investigate the safety and efficacy of a product in children, if it is likely to be of therapeutic benefit to this age group. Guidelines on Clinical Investigation of Medicinal Products in Children have been issued by the European Committee for Proprietary Medicinal Products (CPMP); the guidelines state the scientific data required before medicinal product testing in children and points at juvenile animal safety studies when previous animal data and human safety data are insufficient.

#### **7.1.5 Specific substances, examples of exposure and effects**

For a number of chemical substances, including drugs, differences in biological susceptibility and/or differences in exposure between children and adults have been reported in the literature. The examples mentioned include alcohol, tobacco-smoke, ambient air pollution, pesticides, drugs, PCBs, dioxins, polybrominated flame retardants, phthalates, lead, mercury, copper, boric acid, and nitrate and nitrite. It should be stressed that these examples are illustrative cases and therefore by no means are an exhaustive overview covering all cases and aspects.

#### **7.1.6 Regulations**

In order to assure the best possible protection against adverse effects resulting from exposure to chemical substances, several regulations within different areas have been issued. The most relevant Danish regulations on chemical substances which include the protection of children and pregnant women and their unborn child from exposure to environmental chemical substances have been summarised.

It should be noticed that the regulations of chemical substances are based on the existing knowledge regarding adverse effects and risks related to the use of the substances. For the great majority of chemical substances in use, very little - if any - knowledge exists regarding the relevant exposure scenarios and/or vulnerability of children, including the unborn child.

In 1998, the Danish Government initiated a cross-ministerial overview concerning the administrative measures for protecting children and pregnant women. From this overview, it was concluded that in specific areas an increased effort should be made to increase the protection of children and pregnant women. Several future initiatives in different ministerial sectors were proposed.

Within the field of environmental factors and regulations of chemical substances, it was concluded that more attention should be paid to protection of children and/or pregnant women in relation to:

- the use of chemical substances in cosmetics and toys
- risk assessment procedures in the EU
- risk assessment procedures in relation to the national derivation of limit values for chemical substances in air, soil and water

- the procedures for approval of pesticides (especially pesticides for domestic use) and for risk assessment of food additives and food contaminants.

## 7.2 Conclusions

Much of the current knowledge on adverse health effects from exposure to environmental chemical substances comes from studies on adults. Similarly, many of the present regulations are based on data that do not specifically include risks to children. However, children have different physical, biological and social environments and these factors may all contribute to differences observed between children and adults in response to chemical substances. Children also have more years of life ahead of them than adults and therefore have more time to develop those chronic diseases that take several decades to appear, and which may be triggered by early environmental exposure. This leads to a particular concern for children in relation to exposure to some carcinogenic substances and for substances that accumulate in the organism.

### 7.2.1 Biological susceptibility

In a number of cases, the unborn child and the infant have been reported more vulnerable to toxic effects than adults. Periods of rapid growth and development render them susceptible to specific toxic endpoints. In addition to such toxicodynamic factors, differences in toxicokinetics such as absorption, elimination and metabolic capacity may be contributing factors to an increased susceptibility during these periods.

#### *Susceptible periods in human development*

Developmental deficits are predominantly induced during the pre- and peri-natal periods, which should be considered the most vulnerable periods. The post-natal period appears to be vulnerable with respect to the physiological development of the nervous, immune, and endocrine/reproductive systems as these systems continue to develop until adolescence. There is now increasing awareness that insults to developmental processes, which may not be morphologically apparent, may none the less cause functional disturbances later in life. Based on the current experimental database, concern in this respect has mostly been related to the risk of behavioural and reproductive disturbances.

The present knowledge, however, does not allow an overall generalisation concerning increased susceptibility of foetuses/infants compared to adults, as only a limited number of studies exist in which the susceptibility in foetuses/infants have been systematically compared to adults. Several examples, however, show that serious effects can be found in foetuses/infants at dose levels not causing effects in adults. For foetuses, it is generally recognised that marked toxic effects in the pregnant animal normally also leads to effects in the foetuses, i.e. it is rarely seen that the susceptibility of the foetus is lower than that of adults.

#### *Toxicokinetics*

Regarding toxicokinetic differences between children and adults, examples have shown that during the developmental and maturational periods, the susceptibility to chemical exposure in children may be higher, equal

or even lower than in adults. Children do not seem to represent a special group from a toxicokinetic viewpoint regarding variability among children as the toxicokinetic variability among children generally appears to be of a similar magnitude as the variability among adults.

### *Toxicodynamics*

Age-dependent differences in toxicodynamics are primarily related to the specific and unique effects that chemical substances may have on the development of the embryo, foetus, and child. However, there are insufficient data to evaluate the toxicodynamic variability among children as well as the differences between children, including the unborn child, and adults.

Overall, age-related differences in the biological susceptibility of a specific chemical substance can only be assessed if adequate data regarding adverse health effects during the different periods of life (foetal, neonatal, childhood, adolescence, and adulthood) are available.

### 7.2.2 Exposure

Under certain circumstances, infants and children are exposed to chemical substances to a greater extent than adults because of their higher intakes (on a body weight basis) of diet and beverages, drinking water, and air and also due to their behavioural patterns. Infants and children are curious by nature, have different activity patterns (e.g., hand/object-to-mouth activities, playing close to the ground), and show differences in food preferences (e.g., drink much more milk and soft drinks). Furthermore, substantial dermal exposure of infants and children may occur from the use of cosmetics and child care products.

The unborn child may have special exposures resulting from exposures of the pregnant woman e.g., from her working environment and during her leisure activities.

For most chemical substances, only limited data are available concerning exposure of children as well as of adults. In the risk assessment process it is thus difficult specifically to assess differences in exposures of children compared to adults.

### 7.3 Recommendations

The regulations of chemical substances are generally based on the present knowledge regarding adverse health effects and risks related to the use of the substances. However, the available data on a specific chemical substance are seldom sufficient to evaluate whether children or the unborn child should be considered more vulnerable than adults. Concerning specific end-points such as fertility and teratogenicity, data exist only for 20 to 30% of the 2500 high production volume chemicals, and for thousands of chemicals produced in smaller quantities, this percentage is expected to be considerably lower. Furthermore, for most chemical substances, limited data are available concerning exposure of children as well as of adults.

From a regulatory point of view, different approaches can be applied in order to protect children, including the unborn child, from adverse health effects resulting from exposure to environmental chemical substances. One regulatory approach is to improve the risk assessment of chemical substances by including requirement of data specifically relevant for the protection of children, including the unborn child. Another approach is to pay more attention on children and pregnant women in the risk management process.

Also the use of the precautionary principle should be emphasised. Thus, regulatory measures should be undertaken in cases where potentially adverse health effects are of concern but the scientific evidence is not available due to lack of data.

### 7.3.1 Risk assessment

In relation to human health, a risk assessment consists of an effect assessment (hazard identification, and dose/concentration-response and -effects assessment), an exposure assessment (estimation of the concentrations/doses to which human populations are or may be exposed), and a risk characterisation (estimation of the incidence and severity of the adverse effects likely to occur in a human population due to actual or predicted exposure to a substance). In most cases, the risk assessment is performed for a specific substance and only seldom data are available to account for interactions due to simultaneously exposure to other substances.

For the great majority of chemical substances used, limited data - if any - are available on adverse health effects during the different periods of life as well as on exposures of children, including the unborn child. Increased vulnerability of children, including the unborn child, has been reported for a wide range of different substances, but a clear answer on such differences in vulnerability can only be given for a specific substance if a rather extended data set is available. Therefore, the possibility of increased vulnerability of children, including the unborn child, should always be kept in mind in the risk assessment of chemical substances. Greatest concern, however, exists for the unborn child and neonate (up to the age of 3 to 6 months) because they are in a period of rapid growth and development and have a lower capacity to eliminate toxic substances.

In risk assessment, the exposure assessment is as important as the effect assessment. However, only very little is known concerning specific infant/child exposures. Especially in relation to the increasing use of chemical products marketed for children (cosmetics, child care products, and toys) data regarding exposure are not available.

#### *Recommendation*

In the risk assessment of chemical substances in products and foods intended for children (e.g., in cosmetics, toys, child care products, food additives in preferred foods, and pesticide residues in processed baby foods and infant formulae), it is strongly recommended to perform child-specific risk assessments.

In the risk assessment of chemical substances in other use categories than the above-mentioned, it is recommended specifically to focus on chil-



dren, including the unborn child, if a potential exposure to a given substance may occur to these age-groups.

As children, including the unborn child, are simultaneously exposed to several chemical substances, the possibility of combination effects should also be considered.

Furthermore, it is recommended that the risk assessment should be performed by experts on a case-by-case basis for each substance and for each exposure scenario.

### 7.3.2 Testing of chemical substances

One regulatory approach in relation to the risk assessment of chemical substances is to require a more extensive documentation of health effects as well as of exposures specifically regarding children, including the unborn child. Such documentation could be obtained from epidemiological studies as well as from studies in experimental animals. Different testing strategies have been issued for various regulatory areas.

For new high production volume chemical substances (marketed after 18 September 1981 and produced in amounts above 1000 tonnes/year), food additives, pesticides, and drugs, testing of adverse health effects is required by the competent authorities to an extent which is generally considered sufficient to assure the safety in use, including the intentional use to children. However, the majority of existing chemical substances (marketed before 18 September 1981), including contaminants in foods, have generally not yet been tested adequately to evaluate the risk of inducing adverse effects in children.

Regarding the unborn child and neonate, the standardised reproductive toxicity tests provide information on a number of developmental effects and in the revised proposal for the two-generation reproductive toxicity study (OECD TG 416), assessment of effects on sperm quality and oestrous cyclicity in offspring have been added. However, investigations on effects on the developing brain are not included in the presently used testing strategies because the OECD Guideline for developmental neurotoxicity is at present only a draft. In addition, the investigations of systemic effects in the reproductive toxicity tests are not as comprehensive as in the repeated dose toxicity studies in adults. Also, investigations of endocrine disruption are not included in guidelines at present.

Ideally, a comprehensive testing of end-points specifically relevant for protection of children, including the unborn child, would require a two-generation study (OECD TG 416) incorporating developmental neurotoxicity end-points and supplemented with similar investigations of systemic effects in the offspring as in the repeated dose toxicity studies in adults as well as investigations of endocrine disruption.

#### *Recommendation*

For chemical substances, it is specifically recommended to consider whether there is a need for additional testing of reproductive toxicity. The evaluation should be performed on a case-by-case basis for each substance based on the available data on adverse health effects, on potential exposure of children, including the unborn child, as well as on other properties of the substance. If concern for children and/or the unborn child arises, a comprehensive testing of end-points specifically relevant for protection of

children, including the unborn child, should be required. The relevant study is a two-generation study (OECD TG 416) incorporating developmental neurotoxicity end-points and supplemented with similar investigations of systemic effects in the offspring as in the repeated dose toxicity studies in adults as well as investigations of endocrine disruption. This recommendation clearly implies that updated and new testing guidelines should be incorporated into the presently used testing strategies concurrently with their endorsement.

### 7.3.3 Risk management

For many chemical substances, a risk assessment specifically endeavoured to children, including the unborn child, cannot be performed due to lack of data on health effects as well as exposure. In such cases, the risk management process should more specifically focus on the protection of children and pregnant women. Furthermore, the need for regulatory action has to be balanced against the need for a comprehensive documentation, which would require extensive resources and time. Different regulatory approaches can be applied.

#### *Additional safety measures*

In the establishment of health based limit values for chemical substances in ambient air, soil, and drinking water, three safety factors (uncertainty factors) are used in the derivation of the tolerable daily concentration or intake to account for interspecies variation in susceptibility, differences in intraspecies susceptibility, and for the quality and relevance of the available data.

The safety factor for differences in intraspecies susceptibility is used to account for the general variability of the population including susceptible subgroups such as children, pregnant women and their unborn child, and elderly or sick people; a default value of 10 is generally used. The possibility of increased susceptibility of children, including the unborn child, should always be kept in mind in the risk assessment of chemical substances. Greatest concern, however, exists for the unborn child and neonate (up to the age of 3 to 6 months) because they are in a period of rapid growth and development and have a lower capacity to eliminate toxic substances.

The safety factor for quality and relevance of the available data is used to account for all other relevant aspects than the inter- and intraindividual variability. Examples include testing on only a few animals or at just one or a limited number of doses/concentrations, the exposure route is not relevant for the human exposure situation (injections), cases where a NOAEL cannot be established, extrapolation from a short exposure duration to lifetime-exposure, and severity of effects. Lack of the relevant data are accounted for in the magnitude for this safety factor.

Having obtained the tolerable daily concentration or intake, the health based limit value is derived by taking into account a default value for the daily exposure from the various media. For the derivation of the limit value for ambient air and drinking water, default values for exposure of an adult person is used; however, for soil exposure, a default value for exposure of children is used.

#### *recommendation*

In case that the available data are insufficient to evaluate the susceptibility of children, including the unborn child, it is strongly recommended

that additional safety measures (choice of safety factors) should be considered when acceptable or tolerable daily intakes (ADIs or TDIs) or health based limit values for chemical substances in products and foods intended for children (e.g., in cosmetics, toys, child care products, food additives in preferred foods, and pesticide residues in processed baby foods and infant formulae) are established.

In the derivation of health based limit values in ambient air, soil, and drinking water, it is recommended to use default values for exposure of children for all three environmental media.

*Group-evaluation*

For the majority of chemical substances, very little, if any, knowledge exist regarding toxicity from pre- and perinatal exposure. Classification and risk assessment of chemical substances are based on available data. Although having different properties, the majority of chemical substances can be assigned to groups defined on the basis of their chemical structure, physico-chemical properties, biochemical effects, or other resemblance or relationship, all of which may be significant for the toxicological effects of a specific substance.

*recommendation*

In order to make the best possible use of the available data on chemical substances, it is recommended to use the knowledge and models concerning structure activity relationships (SAR) as a tool in risk assessment, in prioritising for further testing, and in regulation of chemical substances.

*Positive or negative list*

Infants and children are often exposed to a higher degree than adults to certain chemical substances e.g., in cosmetics, toys, childcare products, food additives in preferred foods, and pesticide residues in processed baby foods and infant formulae. However, for most chemical substances, limited data are available concerning exposure of children from these products as well as additional exposure from various other media (other foods, drinking water, contaminated soil). Food additives are listed in the Positive List and special regulation on food additive use in infant formulae is in force in the EU. Certain substances are considered undesired in products and foods intended for children, particularly for younger children (0 to 3 years old). An example is the EU prohibition of the use of phthalates in toys and childcare products to children at the age of 0 to 3 years. Another example is that in the EU, some specific pesticides must not be used in agricultural products intended for the production of baby foods and infant formulae.

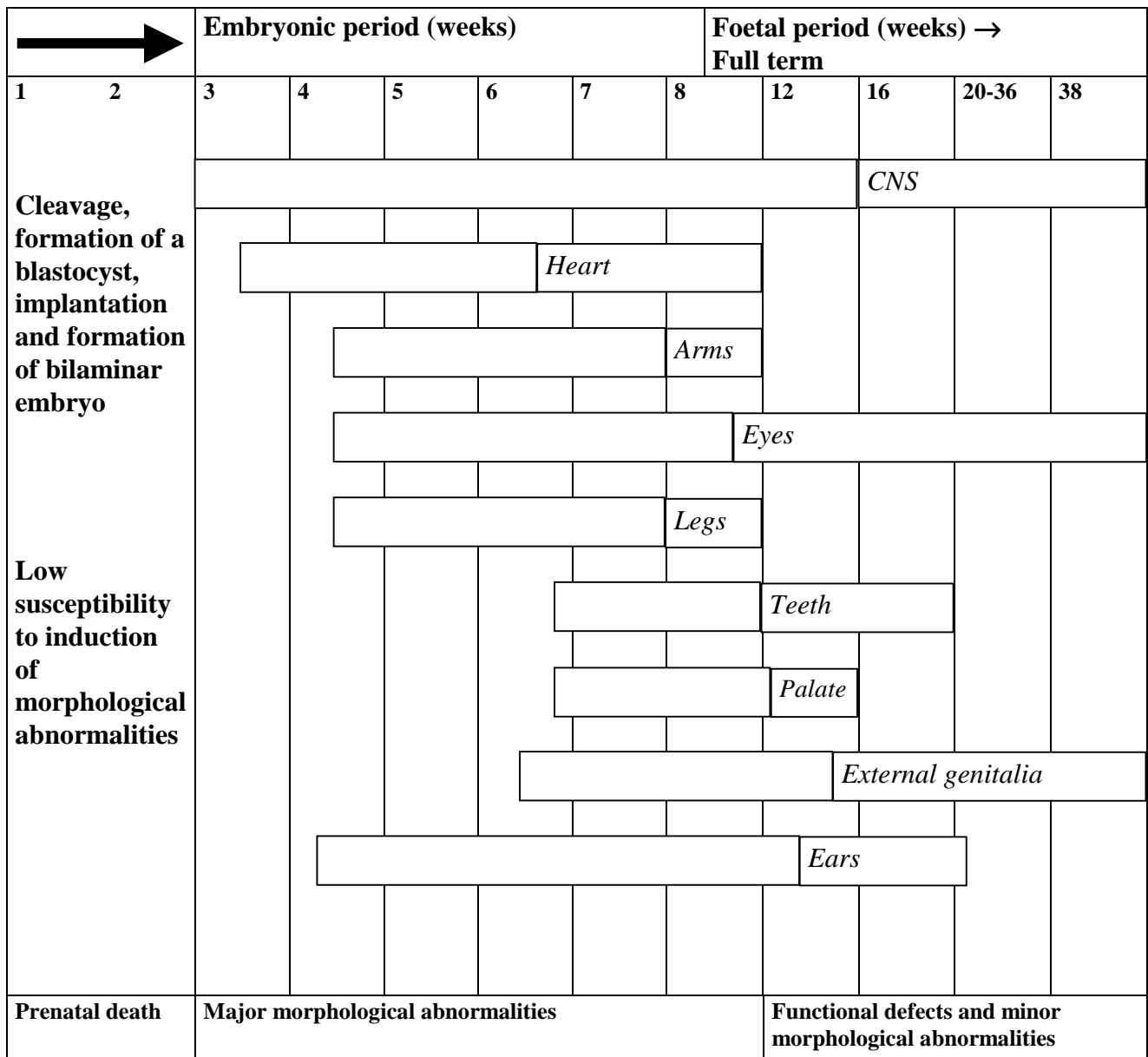
*recommendation*

In order to protect children from exposure to undesirable substances, it is recommended to introduce specific lists (positive or negative) for use of chemical substances in products intended for children such as cosmetics, toys, child care products, and pesticide residues in processed baby foods and infant formulae.



# Annex 1

Figure 2.1 Periods of susceptibility “windows” of susceptibility for persistent malformations.



The open white bars indicate the periods most susceptible for development of major morphological abnormalities while white bars with text show periods prone to functional defects and minor morphological abnormalities.