

***Trends in Children's Health and  
the Role of Chemicals:  
State of the Science Review***

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## **ECETOC TECHNICAL REPORT No. 96**

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European Centre for Ecotoxicology and Toxicology of Chemicals

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## **SUMMARY**

Children, compared to adults, can be more sensitive, less sensitive, or equally sensitive to changes in health status as a consequence of exposure to chemicals. Broadly based statements indicating that children are generally more sensitive to chemical insults are not supported by existing scientific data.

The susceptibility of children is related to the specific physical, toxicological, and age-related pharmacokinetic characteristics of the particular chemical as well as the stage of a child's development. The likelihood of a specific health outcome following exposure of children is also related to the patterns of exposure that reflect both the presence of chemicals in the environment as well as behavioural characteristics of the developing child. The overall effect of these variations in both susceptibility and exposure is to create specific time frames during which vulnerability to adverse effects is elevated; so called 'windows of vulnerability'.

### ***Trends in Children's Health***

In comparing time trends of disease, a number of factors must be taken into account. These include: improved reporting systems; changes in diagnostic criteria/procedures; a more active approach to early detection of cases to improve prognosis and a better health care system in general. There is clear evidence of increasing rates of asthma in children, although rates in some countries may now have stabilised. There is no convincing evidence of widespread trends in other acute or chronic childhood respiratory diseases. Indoor air quality appears to be related to both asthma and, in some cases, to other respiratory-related diseases (such as otitis media). Interpretation of the available information on asthma and allergies is made difficult by inconsistent application of diagnostic criteria over place and time. Contemporaneous with the increasing frequency of asthma, data also suggest that other atopic disorders such as upper respiratory tract and food allergy may be increasing. Atopic dermatitis remains the most common skin disorder in young children.

Although the frequency of neurodevelopmental disorders such as autism and attention deficit / hyperactivity disorder (ADHD) is commonly believed by the public to be increasing, the limited data available do not support this perception. Besides, diagnostic criteria have changed significantly over time.

Data on reproductive effects are also limited and often suffer from serious data quality issues. Whilst geographic heterogeneity is apparent, broad population trends for these outcomes (e.g. sperm quality, hypospadias, cryptorchidism) are difficult to identify. However, there is clear evidence for decreasing puberty age in females.

There is no evidence for major trends in the frequency of childhood cancer. Data indicate that developed countries tend to have a gradually increasing incidence of leukaemia with a corresponding drop in the incidence of lymphoma. Increases in brain tumour frequency are possibly related to the development of new diagnostic capabilities rather than to a true change in the incidence in the rate of malignant disease. With the increasing number of childhood cancer survivors, secondary cancers following chemotherapy appear to be on the increase.

### ***Role of Environmental Exposure, including Chemicals***

A wide range of environmental factors is thought to have an impact on children's health, extending well beyond industrial chemicals. These factors include nutrition (protein, vitamins, antioxidants), lifestyle and behaviour choices such as tobacco and alcohol use, parental health, socio-economic status, choice of living environment (urban vs. rural, etc.), and parent-sibling behaviour. From the available data, no general conclusions on the contribution of specific chemicals can be drawn across the multiple health outcomes addressed in this report.

There is a need to distinguish between factors causing asthma from those acutely exacerbating an existing state of the disease. It remains difficult to make this distinction in practice, as some non-allergenic chemical agents may modulate the ability of other materials to cause asthma. Overall, genetic propensity and exposure to antigens (mainly proteins) remain the most important determinants of childhood atopic disease.

Primary chemical contributors to exacerbation of childhood asthma appear to be ambient air pollutants (particulates, NO<sub>x</sub>, SO<sub>x</sub>, ozone, etc.) and lifestyle-related indoor air pollutants such as environmental tobacco smoke. Some evidence suggests that volatile organic chemicals can play a role in the exacerbation of childhood asthma, primarily as a result of airway irritant effects. Primary allergens responsible for asthma, and respiratory food allergies, are almost invariably proteins, not environmental industrial chemicals. Nickel is probably the most common allergen for chronic skin sensitisation in the general public.

Early exposure not only to allergens but also to infectious diseases, immunisations, and other environmental immune stimuli appears to reduce the frequency of asthma and atopic diseases ('hygiene hypothesis'). Conversely, lack of environmental exposure to these factors seems to increase the risk of allergic disease. No widespread trend attributable to industrial chemicals is apparent.

Exposure to lead, mercury, and polychlorinated biphenyls (PCBs) is associated with neurodevelopmental disorders. However, the relationship of neurobehavioural changes to these chemicals at general environmental levels has not been well established, except for lead.

Neurodevelopmental disorders in the general population are probably largely the result of genetic, socio-economic, and lifestyle choices (particularly smoking) as well as important gene-environment interactions, for example parents and siblings with ADHD have a profound effect on the child's environment.

The risk for cryptorchidism and hypospadias appears to be related to a variety of non-environmental factors. While it is established that high-level hormonal stimuli can affect reproductive tissues and processes, the actual contribution of lower-level exposure to endocrine agents to reproductive alterations in intact, homeostatic organisms is unclear. The one clearly identified trend in reproductive health in the developed world, the earlier onset of puberty in girls, is probably due to lifestyle changes leading to improved health and nutrition. Sperm quality varies widely over time and place, with no clearly established global trend. Many genetic and lifestyle factors may affect sperm quality from pre-natal to adult life.

Genetic factors are important in childhood cancer. There is no evidence that environmental chemicals other than chemotherapeutic agents used in the treatment of initial malignancies play any significant role in the aetiology of child cancers. A number of factors related to pregnancy and lifestyle (e.g. parental smoking, birth weight, gestational illness such as pre-eclampsia) have been associated with alterations in the frequency of adult cancers as a result of childhood exposure.

### ***Assessing Risks to Children***

The regulatory requirements for safety testing vary between different classes of chemicals. Data from a core set of toxicological studies are not available for all chemicals. It is increasingly recognised that a 'blanket' approach to toxicological testing is not required. A tiered and integrated approach involving use patterns and exposure potential, degree of concern from existing hazard information, together with the use of predictive tools (e.g. QSARs and read-across between chemical categories) are likely to provide adequate hazard data for a risk assessment.

Risk assessment methodology currently in use incorporates significant uncertainty factors, usually based on a No-Observed-Adverse-Effect Level (NOAEL) in animals, to account for potential interspecies and intraspecies differences in susceptibility to chemical toxicity. Additional uncertainty factors can be added for data insufficiency (i.e. lack of a NOAEL). Analyses suggest that the existing uncertainty factors are likely to be adequately protective for children. Future risk assessments will benefit from on-going research into exposures specific to children.

### ***Future Developments***

There are a number of scientific developments that could increase the accuracy of assessing risks to children. Large mother-child and child-youth cohort studies are emerging, but will not yield significant information for many years. Limitations in statistical power for rare disease outcomes mean that studies must be large (with cost and logistic consequences) to make a meaningful contribution. Further, with studies testing multiple hypotheses, findings will need to be externally validated via mechanistic or specifically targeted additional epidemiological research.

The future role of genomics and proteomics as biomarkers for exposure or adverse outcome in assessing risks to children's health is unclear but such techniques could allow for a more rigorous toxicity evaluation as well as provide biological information on the lifestage-specific consequences of environmental exposures. The use of biomarkers for both exposure and disease outcomes offers challenges, especially if incorporated into cohort studies. Biomarkers of either exposure or effect suffer from various limitations, but may offer significant advantages when they improve accuracy of exposure estimation and/or diagnosis of disease.

## 1. INTRODUCTION

The influence of environmental factors on the development of children has gained increasing prominence and has prompted a number of actions from authorities such as the US Environmental Protection Agency pilot programme: VCCEP (Voluntary Children's Chemical Exposure Program) (US EPA, 2000) and the European action plan being developed through SCALE (Science for Children through Awareness, Legislation and continuous Evaluation) (EU Commission, 2004a). Major improvements in the treatment of childhood diseases, nutrition, water quality and many other factors have resulted in significantly improved survival and lowering of childhood mortality over the recent decades. Societies that have seen such benefits have also experienced many lifestyle changes, improved healthcare systems, and have benefited from a rapidly developing industrial base. Nonetheless, there is a growing perception that changes in the daily environment may affect the development of children.

Terms of Reference of the Task Force:

- Review key position papers and scientific reviews, and perform a scientific gap analysis and evaluation of health trends relevant to the chemical industry by taking into consideration:
  - How are / Are children at greater risk than other groups?
  - Are there adverse trends in children's health that are attributable to chemicals?
  - The role of chemical exposure (patterns, frequency and levels) during different stages of development (pre-natal, natal, neo-natal, early childhood, etc.).
  - The contributing physiological and biochemical factors during child development and the critical windows associated with adverse effects.
  - Short- versus long-term (lifetime) effects of children's exposure to hazardous chemicals.
  - Experimental models for child risk assessment.
- Organise an ECETOC / LRI workshop which will address key areas identified by the task force and which will develop critical priority issues for further research.
- Propose a research programme and specific RfPs for LRI to consider.
- Liaise with on-going efforts in other regions and with regulators and scientists outside industry.

The task force addressed all of the Terms of Reference, with the exception of organising a workshop to develop priority issues for further research. Presentations of the findings of the report have been, and will continue to be made, in a number of scientific meetings and workshops.

This report reviews the published literature on how the health status of children is changing and what factors could be identified as possibly causing such changes, if any, in order to provide a factual, balanced assessment on children's health. The review of the scientific and medical literature did not extend into the toxicological hazards of specific chemicals but relied largely on

epidemiological evidence. The report focuses on low-level, long-term environmental exposures, including exposure to chemicals. Information from geographical regions covering mainly Western and so-called developed countries has largely been used in this report.

The task force considered the reports from the technical working groups (TWG) on SCALE set up by the EU Commission in 2003. These reports form the basis for the development of the European Environment & Health Action Plan 2004-2010 (EU Commission 2004b, 2004c). A report from the ILSI workshop to 'Develop a Framework for Assessing Risks to Children from Exposure to Environmental Agents' (Olin and Sonawane, 2003) was also included in the review.

Recently published papers in 'Pediatrics' were brought to the attention of the task force; their conclusions are compatible with those in this report (Brent and Weitzman, 2004). Another paper recently published by Wild and Kleinjans (2003) is worth noting; it concludes: "Initiatives to promote protection of children's health, in relation to what is a rapidly changing environment in many parts of the world, are both admirable and important. There is an almost intuitive recognition that children, as vulnerable members of society, merit special attention and protection with respect to environmental hazards. This position of defending the vulnerable in society can be supported purely on philosophical and moral grounds. However, the apparent scientific corollary to this position is that children are particularly vulnerable to environmental health hazards, i.e. that the consequences of exposure in children are more severe than the consequences in adults. This latter assertion requires careful scientific evaluation. This is important because in the absence of such evidence it is difficult to form a sound rationale for effective public health decisions to protect child health."

Diseases covered in the review of trends on children's health were: asthma, respiratory diseases, allergies, neurodevelopmental and reproductive disorders and cancer. Given the wide range of factors, which could contribute to changes in disease incidence, this report focuses on those environmental chemical agents for which published information is available. Some other factors were also considered, such as socio-economic status, nutrition and electromagnetic fields. However, only few additional factors were covered, because the focus had been to identify any potential environmental chemical exposures, which could contribute to childhood disease. The regulatory framework of risk assessment, including the adequacy of various toxicological tests to identify potential effects in children, is also reviewed. Finally, future developments in the field of children's health were addressed, and a number of areas (albeit not specific projects) identified, where more research needs to be undertaken to understand trends in diseases and causative factors. The task force concluded that experts in the identified areas, such as epidemiology and biomonitoring, should take these recommendations forward to identify tangible research programmes.

A number of stages of human development are covered, and for consistency, the terms used in this report follow those defined by an ILSI workshop (Larsen and Pascal, 1998).

**Table 1-1: Stages in human development** (from: Larsen and Pascal, 1998)

<b>Developmental Stage</b>	<b>Time Period</b>
Embryonic stage	8 days - 8 weeks of pregnancy
Foetal stage	8 weeks of pregnancy - birth
Pre-term birth	24 - 38 weeks of pregnancy
Normal term birth	40 ± 2 weeks of pregnancy
Peri-natal stage	29 weeks of pregnancy - 7 days after birth
Neo-natal stage	Birth - 28 days
Infancy	Birth - 12 months (young: 0-4; older: 4-12)
Childhood	1 year - 12 years (young 1-4; older 4-12)
Adolescence	>12 years - 18 years
Adulthood	>18 years

## 2. SENSITIVITIES AND AGE-RELATED EXPOSURE PATTERNS

Children differ from adults, rendering them more or less susceptible than adults to the effects of chemical exposures. For example, children's chemical exposures and metabolic profiles can be qualitatively and quantitatively different from those of adults, so they may experience higher or lower doses on a body-weight basis for the same exposure levels. Children can be more sensitive to a certain chemical toxicity than adults because they may be more vulnerable to external challenges during critical stages of the developmental process. In other cases, they can be less sensitive to chemical toxicity than adults due to more efficient elimination processes, less mature activating enzymes, and enhanced ability to repair damage.

This chapter describes many of the characteristics that make children unique in terms of their susceptibility to toxicity.

### *2.1 Role of toxicokinetics in sensitivity*

One of the most important determinants of toxicity is dose. The effective internal dose, reflecting blood or tissue levels integrated over time (i.e. the area under the time-concentration curve), is determined both by external exposure and by toxicokinetics, or the body's ability to absorb, distribute, activate, detoxify, and eliminate toxicants following exposure.

Children's and adults' external exposures often differ both qualitatively and quantitatively. For example, children are likely to be exposed to different levels of chemical contaminants in foods than adults because they consume more calories of food per unit of body-weight, fewer types of foods, and more processed foods (USDA, 1999; EEA/WHO, 2002). Normal childhood behaviours such as hand-to-mouth activity and crawling on the floor or ground can increase children's exposures to potential toxicants through ingestion and contact with dusts and residues. Greater risk of lead poisoning from lead-based paint is a well-known example. Children have higher ventilation rates and proportionally more lung alveolar surface area than adults; thus, they may be exposed internally to higher levels of air pollutants. Children consume more water than adults on a body-weight basis, so may be exposed to higher levels of water pollutants. Infants consume breast milk, an important source of nutrition and immunologic protection, which can also introduce a source of fat-soluble materials such as PCBs. Children may not perceive hazards as quickly or effectively as do adults, so may experience some greater exposures by not avoiding them as readily. In contrast, occupational exposures would be greater for adults than children, although there are situations in which parental exposures result in children's exposures when workers return home or in which children, often those of migrant farm workers, work or spend time in an occupational environment.

Once exposure has occurred, age-related differences in toxicokinetics may produce age-related differences in dose. Complex changes in toxicokinetic capabilities occur during development, both before and after birth. Those capabilities develop at different rates, so the body may respond to chemical challenges in different ways at different ages. Table 2-1 summarises the biochemical and physiological changes that occur during growth and development affecting the absorption, distribution, metabolism, and elimination of chemicals and other substances such as nutrients.

Gastrointestinal absorption of chemicals, in general, does not change dramatically with age although several factors that can affect absorption are liable to undergo change (Sreedharan and Mehta, 2004). Gastric acid secretion is low in newborns and gastric pH is correspondingly high: 6-8 compared to 1.5 in adults. The higher pH can result in decreased absorption of weak acids and increased absorption of weak bases. Gastric emptying is also prolonged, peristalsis is irregular, and intestinal motility is reduced in the newborn. In comparison to the adult, newborns also exhibit differences in their intestinal flora, which has been shown to affect absorption. Bile acid metabolism and turnover are not fully developed at birth; primary bile salts exhibit a transient elevation in the first few weeks, then decline steadily for several years while liver function matures, potentially leading to less uptake of fat-soluble contaminants early in life compared to adults. For example, because bile acids play an essential role in the digestion and absorption of dietary lipids, they can affect the absorption of fat-soluble materials such as PCBs.

Absorption also occurs through the skin. The skin of the newborn is thinner than that of infants and adults, potentially permitting greater absorption. However, the *stratum corneum* is fully developed in mature newborns and its barrier function is considered equal to that of adults (Kalia *et al*, 1998). Studies of age-dependence indicate that the barrier function of skin remains virtually constant from infancy to late adulthood (Ghadially *et al*, 1995) and percutaneous absorption of chemicals has been shown to be the same in infants and adults in both *in vivo* and *in vitro* studies (Rasmussen, 1978; Wester and Maibach, 1982; McCormack *et al*, 1982).

**Table 2-1: Biochemical and physiological changes in adults and infants**  
(from: Scheuplein et al, 2002)

<b>Absorption</b>	
Gastric pH	Neutral at birth, pH <4 within 2-4 days, gradually decreasing to adult values
Pepsin	Diminished at birth (3-13%), adult level by 3 <sup>rd</sup> month of life
Lipase	Diminished at birth (3-22%), adult level by 6 <sup>th</sup> month of life
Trypsin	Diminished at birth (13-30%) adult level by 3 <sup>rd</sup> month of life
$\alpha$ -Amylase	Diminished at birth (0.2-3%) adult level by 2-3 years
Gastric acid secretion	Lower in young infants, increases to adult level in 3 months
Gastric emptying time	Lower in neonates; may reach adult level at 6-8 months
GI motility	Slow and irregular motility in infants
GI microflora	Rapidly colonised after birth; high in neonates
<b>Distribution</b>	
Extracellular water	40% body-weight in neonates; 26-30% at one year; 50-60% in adults
Total body fat	Proportionately higher in infants
Total plasma protein	Lower in neonates/infants than in children and adults; composition different in neonates/infants
Volume of distribution	Tends to reflect relatively larger water compartment; adult levels of transport protein present at birth
<b>Metabolism</b>	
Phase 1	Pronounced individual and lifestage related variation
Phase 2	Glucuronidation deficient at birth, reaching adult level by 3-4 weeks; sulfation active in neonates and young children
Biotransformation	Alternate pathways in neonates; rates often lower in neonates
<b>Elimination</b>	
Glomerular filtration rate	Low in neonates, increasing rapidly during first year
Renal tubular secretion	Matures later than glomerular filtration; reaches adult level by one year
Renal function & clearance	Greater in older infants and young children than in older children/adults
<b>Body water</b>	
Total body water	90% body-weight at 2 <sup>nd</sup> trimester, ~75% at birth, decreasing to 60% (adult value) by 3 months
Extracellular	60% body-weight at 2 <sup>nd</sup> trimester, 40-45% at birth, decreasing to ~30% by one year
Intracellular	Increases early in gestation to ~30% body-weight by 2 <sup>nd</sup> trimester; remains near 30% throughout infancy
Plasma water	Unchanged during development; 4-5% body-weight range
<b>Body fat</b>	
Total body fat	Increases from 1-3% body-weight in mid-gestation to ~16% at term, peaking at ~25% by 6-9 months post-natal; decreases as a percent of body-weight by 6-7 years to adult values

Lung alveolar surface area in children is greater than that of adults on a body-weight basis, which together with children's higher ventilation rates, contributes to children's often-greater absorption through inhalation. The number of alveoli continues to increase until about age eight, after which

they increase in size instead. Lung growth continues throughout childhood into early adulthood, reaching a plateau and then declining with increasing age.

The distribution of a xenobiotic is influenced by several factors, including the size of the body water and fat compartments, regional blood flow, and the degree to which drugs bind to plasma and tissue proteins. On a weight adjusted basis, adults have about 20% less body water than neonates, which, together with reduced protein binding, results in an increased apparent volume of distribution for many substances in infants. These differences may, under some circumstances, lead to an increased exposure of infants' tissues to toxicants but often they do not. Reduced protein binding increases the free fraction of toxin available for excretion as well as for distribution. For toxic materials conforming to first-order pharmacokinetics, distribution and excretion cancel each other precisely, and actual tissue exposure remains unaffected by changes in protein binding. Similarly, while a larger volume of distribution can be expected to 'dilute' the toxic material to a greater extent, the material will also be excreted more slowly, resulting in no net change in tissue exposure. The impact of changes in protein binding and volume of distribution thus will be primarily restricted to acute situations involving pharmacologic disequilibria or to materials with non-linear kinetics.

The ability of newborns and infants to detoxify and excrete most chemicals is critically dependent on the relative maturity of enzyme transformation systems. In general, most hepatic biotransformation enzymes do not reach adult levels until after birth, although a few, like placental glutathione transferase, are more active pre-natally. Both Phase I and Phase II biotransformation activities gradually develop during foetal life at varying rates and require additional post-natal maturation. The impact of age on enzyme expression is both species- and strain-dependent. Enzyme development follows different courses, producing different patterns of age-related changes in metabolising capability. Understanding those patterns is one key to predicting age-related differences in susceptibility.

With regard to elimination, both renal and biliary excretion pathways have diminished capacity at birth and compounds eliminated by those routes may accumulate to higher levels in the neonate. Although clearance is generally low in the newborn, it increases rapidly, reaching a maximum at about 6 months, when it is almost twice that of the adult. Once the elimination processes of the infant have matured, substances are often excreted more rapidly than in adults, which can lead to lower internal biologically relevant doses in children compared to adults for the same exposure. Table 2-2 shows three examples of drugs that are cleared much faster by children than adults, with the differences ranging from about 2 to 6 times higher, or faster, for children. The fourth example, lidocaine, is cleared as effectively by infants as by adults but, due to infants' greater volume of distribution, infants need about twice as long as adults to eliminate the drug. Nonetheless, lidocaine is of similar potency in infants and adults because infants' greater volume of distribution reduces its concentration at the site of action and because its decreased metabolism is offset by increased renal clearance (Morselli *et al*, 1980).

**Table 2-2: Age and drug clearance rates** (Adapted from: Renwick, 1998; Morselli *et al*, 1980)

Compound	Age	Clearance Rate
Morphine	Children	20-25 ml/min/kg
	Adults	6-15 ml/min/kg
Methotrexate	Children	0.6 L/hr/kg
	Adults	0.1 L/hr/kg
Thorazine	Children	3.1 L/hr/kg
	Adults	0.6 L/hr/kg
Lidocaine	Newborns	0.30-1.1 L/hr/kg
	Adults	0.30-1.1 L/hr/kg

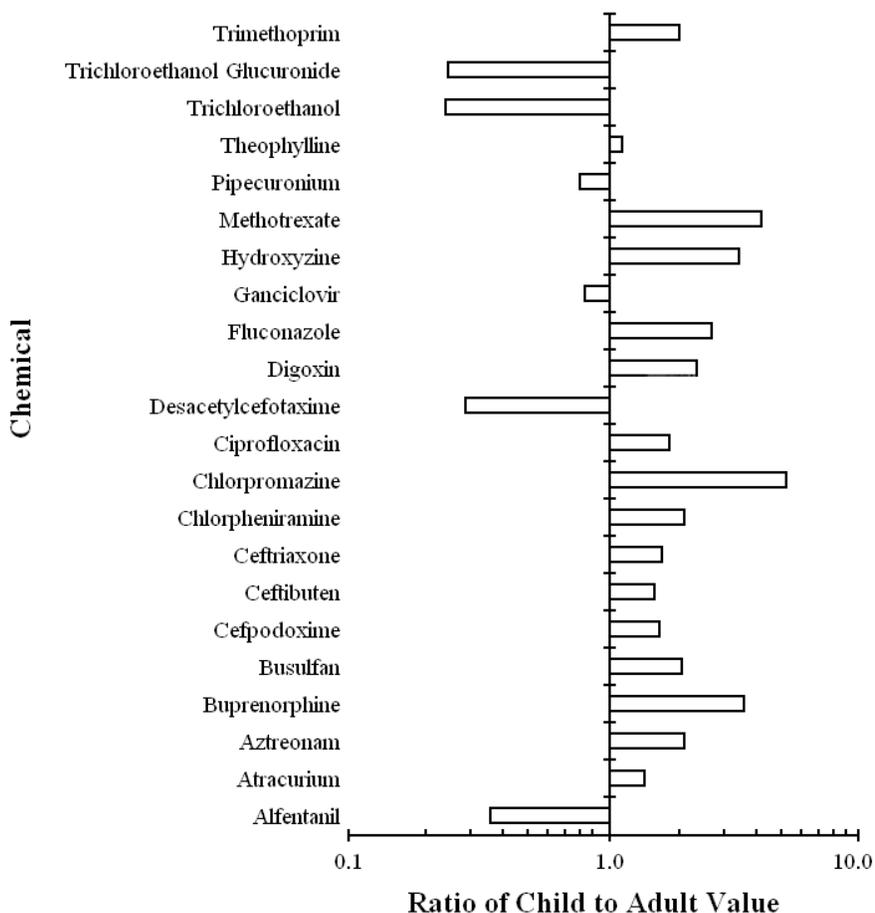
Figure 2-1 is a chart based on data from Renwick (1998) that shows the ratios of child to adult toxicokinetic parameters (clearance and elimination half-times) for 22 drugs. The ratios indicate that, for the examples evaluated, children tend to clear drugs more rapidly than adults in most cases (16/22) but more slowly than adults in others (6/22). This illustrates some of the difficulties inherent in making predictions or generalisations about age-related differences. Figure 2-2 illustrates an evaluation of several pharmacokinetic parameters (elimination half-life  $t_{1/2}$ , area under the plasma-concentration-versus-time curve (AUC), relative volume of distribution  $V_d$ , and total clearance  $CL_{tot}$ ) for 91 drugs with data on children of different ages and adults, compared among age groups (Schneider *et al*, 2002). According to the authors, this comparison indicates that:

- The relative volume of distribution is higher in all age groups of children compared to adults.
- Compared to adults, the elimination half-life is higher in neonates and infants but declines with increasing age and is slightly lower in toddlers and children before it reaches adult levels.
- Clearance shows the reverse trend, being substantially lower in neonates and highest in toddlers and children.
- AUC (area under the plasma-concentration-versus-time curve) data suggest a slightly lower internal load in older children and adolescents compared to adults, although data are limited.
- The variability of the ratios is considerably higher for neonates than other age groups.
- Based on the total clearance data considered, body burdens of drugs were higher in neonates than adults by a factor of approximately 2.
- For more than 5% of drugs considered, clearance was lower in neonates by one order of magnitude.

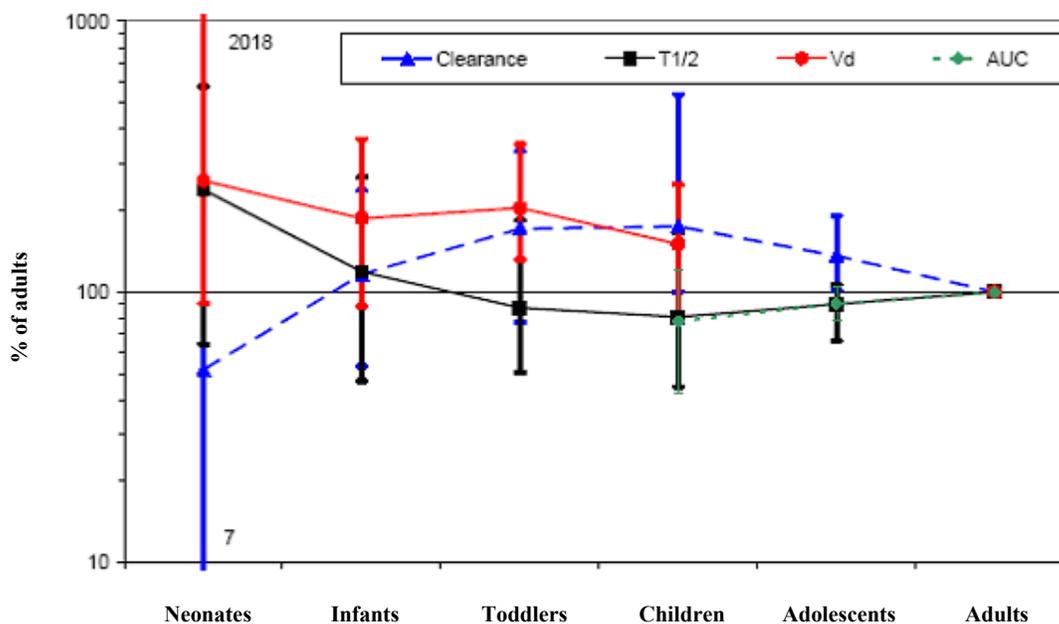
Presumably environmental contaminants would show age-related differences in pharmacokinetic behaviour similar to those found by Renwick (1998) and Schneider *et al* (2002), although most available human data are for drugs.

Current understanding of the rates of maturation of pharmacokinetic capability thus indicates that human infants up to approximately six months of age are typically, but not always, more likely to be subject to higher doses for equivalent exposures than adults, leading to potentially greater toxicity (Scheuplein *et al*, 2002). For most chemicals, the immaturity of infant biotransformation, elimination, and other physiologic systems usually produces higher blood levels for longer periods. The newborn's metabolic capacity rapidly matures and, by about six months of age, children are usually not more sensitive to chemical toxicity than adults due to higher doses. By then, most metabolic systems are reasonably mature, becoming almost completely capable by one year of age. Children over six months of age can be more sensitive to chemical toxicity than adults due to higher doses for equivalent exposures but they usually are not; in many cases they are less sensitive.

**Figure 2-1: Mean ratios of child to adult selected kinetic parameters (i.e. drug clearance / elimination half-times)** (from: Dourson *et al*, 2002; based on data from Renwick, 1998)



**Figure 2-2: Geometric means (with 5- and 95-percentiles) for the ratios of kinetic parameters in different age groups compared to adults (from: Schneider et al, 2002)**



## 2.2 Role of toxicodynamics in sensitivity

Far less is known about the role of toxicodynamics in age-related sensitivity to chemical contaminants than about the role of toxicokinetics. It is apparent from studies with drugs that interference with normal development can have lasting impacts throughout adulthood. The developing organism undergoes many complex integrated events involving the regulation of cell growth, differentiation, and morphogenesis. Interfering with those events through mutation or through altered cell division, hormone activity, or enzyme function, for example, can have significant adverse impacts on development. Many environmental factors can have an impact on normal development, including nutritional adequacy such as protein, vitamin, and folic acid availability, maternal smoking and alcohol consumption, prescription drugs, and chemical contaminants such as lead and organic mercury. In general, age-related toxicodynamic effects on susceptibility appear to depend on the chemical of concern, the toxic effect that is observed, and the period of development during which exposure occurred. Infants, children, or the developing foetus are more sensitive than adults in some cases and less sensitive in others.

Table 2-3 illustrates the stages of pre-natal and post-natal development for several organs in humans. The differences in the rates of growth and development have toxicological implications, notably with respect to 'windows of vulnerability', i.e. the critical time periods during which

chemical exposures may produce particular effects. Organs are more susceptible to toxicity, especially mutation and its potential impacts on control of apoptosis, cell division, and gene expression, when they are differentiating and growing rapidly. 'Windows of vulnerability' are more apparent during pre-natal development than post-natal, with exposure during early intra-uterine development often resulting in different effects than exposure during later intra-uterine or post-natal development.

**Table 2-3: Stages of pre-natal and post-natal organ structural development**  
(from: Altshuler *et al*, 2003a)

Organ System	Early Pre-natal Pre-natal	Mid to Late	Post-natal
Central nervous system	3-16 weeks	17-40 weeks	Continues into adulthood
Ear	4-16 weeks	17-20 weeks	—
Heart	3-8 weeks	—	—
Immune system	8-16 weeks	17-40 weeks	Immunocompetence: 0-1+ years Immune memory: 1-18 years
Kidneys	4-16 weeks	17-40 weeks	Nephrons mature in outer cortical region, providing ability to concentrate urine
Limbs	4-8 weeks	—	—
Lungs	3-16 weeks	17-40 weeks	> 80% of alveoli are formed after birth to age 8-10 years
Palate	6-10 weeks	—	—
Reproductive system	7-9 weeks	10-40 weeks	Sexual maturation, breast, and cervix development: 9-16 years
Skeleton	1-12 weeks	—	Ossification continues for 25 years
Teeth	12-16 weeks	17-24+ weeks	Primary dentition: 4 months after conception to 3 years post-natal Permanent dentition: 3 months after birth to 25 years

For example, rubella infection before the 11<sup>th</sup> week of gestation may cause congenital heart defects and deafness, but if it occurs at 13 to 16 weeks, deafness usually occurs without heart defects and if it occurs after 16 weeks, no structural anomalies are seen (Miller *et al*, 1982). 'Windows of vulnerability' for a particular effect can vary between humans and laboratory animals, with most human data obtained as a result of treating pregnant women with drugs. For example, foetal alcohol neurotoxicity coincides with the developmental period of synaptogenesis, also known as the brain growth-spurt period, which in rodents is a post-natal event but in humans extends from the sixth month of gestation to several years after birth (Olney *et al*, 2002).

The pre-implantation period (0–6 days after conception) is characterised by few reports of malformations, as the conceptus will most likely be killed by toxic agents at sufficiently high doses. The period is difficult to study in humans because a high percentage of embryos are lost during this early stage due to genetic or chromosomal anomalies and because specific information on effective dose is difficult to establish (Dencker and Eriksson, 1998).

The organogenic period for humans occurs from approximately 21 to 56 days after conception. This is a sensitive period during which foreign compounds can induce malformations. Known human teratogens active in this period include isotretinoin, thalidomide, diethylstilboestrol, valproic acid, warfarin, ethanol, anti-epileptic drugs, and cytostatics. For example, isotretinoin is a retinoid used as an anti-acne drug that affects the development of the central nervous system during the period of hindbrain segmentation, which starts at about day 20 (Tzimas and Nau, 2001; Means and Gudas, 1995). Thalidomide affects limb development during the period between days 20 to 36 of gestation. Diethylstilboestrol is associated with several types of reproductive changes and dysfunctions in both males and females, including adenosis of the vaginal epithelium, which occurs primarily as a result of exposure before the eighth week of gestation (Mittendorf, 1995).

The foetal period (after 8 weeks) is a period of continuous development. Although most major malformations happen earlier, this period is also vulnerable to pharmacologically induced developmental toxicity. Examples include the effects of drugs such as beta-blockers and anti-inflammatory or anti-rheumatic agents, which can adversely affect the foetus during the third trimester or at parturition. Drugs disturbing the regulation of foetal blood pressure can lead to kidney and skull malformations, and even to mortality (Dencker and Eriksson, 1998).

The neo-natal period is the period of brain development, and this continues throughout the first year of life. Exposure to environmental agents such as PCBs, lead, and mercury has been shown to produce developmental neurotoxic effects, potentially affecting adult brain function (Eriksson, 1997; Dencker and Eriksson, 1998). The immune system also develops extensively during early childhood as immune memory is established (Dietert *et al*, 2000); interference with development during this period can cause allergies or autoimmune diseases.

Normal growth and development are controlled by large networks of regulatory genes and occur as a progression of states of spatially defined regulatory gene expression (Davidson *et al*, 2002). This progression leads to specification, or the process by which cells in each region of the developing animal come to express a given set of genes. In contrast to physiological transcriptional responses, which can vary in response to stimuli such as changes in the levels of nutrients or introduction of toxicants or pathogens and then return to normal, developmental transcriptional systems always move inexorably forward, never reversing direction. This property is a consequence of the complex gene regulatory network that is active during development and has two possible results, again illustrating the difficulty of making generalisations about age and

susceptibility. Perturbations of critical components of the network during development may have consequences that cannot be repaired as development continues to move forward; or the complexity of the system may confer the ability to compensate for perturbations, should they occur.

The genetic variability in growth factor regulators and homeobox genes has shown to modulate susceptibility to the developmental effects of some environmental agents (Faustman *et al*, 2000). For example, an elevated risk of cleft palate has been reported for infants of mothers who smoke and carry an uncommon allele for transforming growth factor alpha (Shaw *et al*, 1996). An increased risk of birth defects in the children of smoking mothers has also been associated with a polymorphism in the homeobox genes (MSX) responsible for vertebrate limb development (Hwang *et al*, 1998).

### **2.3 Conclusions**

- Children may be differentially susceptible to the toxicity of chemicals relative to adults. Specifically, they may be more sensitive, less sensitive, or equally sensitive to various effects that may result from exposure to chemicals over time. Broadly based statements indicating that children are generally more sensitive to chemical insults are not supported by existing scientific data.
- The intrinsic relative susceptibility of children depends on the specific physical, toxicological, and metabolic characteristics of the particular chemical at issue, on the exposure, and on the age (lifestage) of the individual.
- Age (lifestage) affects the response to chemical exposure by creating specific 'windows of vulnerability' during which sensitivity to adverse effects is elevated due to stage of growth and differentiation.
- Pharmacokinetic characteristics also vary with lifestage and can affect sensitivity to chemical toxicity by altering the effective dose of a chemical available to produce toxicity.
- Children over six months of age can be more sensitive to chemical toxicity than adults due to higher doses for equivalent exposures but they usually are not; in many cases they are less sensitive. Infants up to approximately six months of age are typically, but not always, more likely to be subject to higher doses for equivalent exposures than adults, leading to potentially greater sensitivity.

### 3. EVALUATION OF TRENDS IN CHILDREN'S HEALTH

Knowledge and perceptions on trends in children's health are clearly major drivers in the identification of high priority children's health indicators, research gaps, regulatory needs, interventions, and enforcement. As such, these trends require careful assessment. The purpose of this chapter is to review known, suspected, or hypothesised trends in children's health. A wide variety of health outcomes could have been covered in this section. However, the task force has chosen to focus on health trends currently under discussion in the scientific and regulatory community, i.e. respiratory diseases, allergies, neurodevelopmental disorders, effects on the reproductive system and cancer.

It is common knowledge that changing diagnostic criteria/processes, improved health care, changes in health concern of parents and primary health care professionals can have a substantial effect on reported incidence rates. The effect of these factors on time trends should be taken into consideration when studying these trends.

The field of allergy is complex and rapidly developing. Consequently, additional information on allergic mechanisms and the biology of allergic response is presented in the Appendix.

To the extent possible, European data have been identified and utilised in this chapter. In some instances, particularly in the case of neurodevelopmental disorders, pan-European data are limited at this time. Although the task force has been aware of some efforts to compile European data on a number of health outcomes, it has, of necessity rather than choice, relied upon non-European (particularly US) data in a number of cases.

The degree of certainty regarding the existence and magnitude of health trends varies widely among the various outcomes. In some cases, as with asthma, trends are well established on a multi-national basis (Johnson *et al*, 2002; Beasley *et al*, 2000; Hartert and Stokes Peebles, 2000). In other cases, as for cancer, data exist from some areas that show no systematic changes in disease frequency. Finally, as in the case for neurodevelopmental disorders, claims for trends remain unsubstantiated in spite of public debate based upon the perception that these disorders are increasing in the general population.

The disease trends addressed in this chapter will set the stage for further discussion of the role of various environmental and other factors in these disease states (Chapter 4), followed by a specific discussion of the role of environmental chemicals and the potential of the chemicals to contribute to observed trends in children's health (Chapter 5).

The terms 'prevalence' and 'incidence' are used frequently in the following chapters; thus, they are briefly defined here. Prevalence refers to the proportion of individuals in a population with a

disorder or disease at a point in time (includes both existing and new cases). In contrast, incidence refers to the number of newly reported cases in an 'at risk' population during a specified time period (includes only new cases in the numerator and only disease-free individuals, who are at risk of developing the condition in the denominator). As such, incidence is the preferred measure for assessing whether trends in a disorder or disease have truly changed over time since prevalence is affected by changes in prognosis and incidence is not. However, changes over time in diagnostic criteria, level of vigilance about the disease or disorder and related factors can impact both the reported prevalence and the reported incidence measures.

### ***3.1 Respiratory diseases / asthma***

Respiratory diseases remain the leading cause of childhood mortality in the new member states of the EU, and the sixth leading cause of childhood mortality in the rest of the EU (WHO, 2003). Chronic lung and other lower respiratory diseases in children are uncommon in the absence of either a genetic condition (cystic fibrosis, alpha-1-antitrypsin deficiency) or premature birth (broncho-pulmonary dysplasia). Hence, there are few data regarding trends in non-asthmatic lower respiratory disease in childhood, and no known associations of general environmental agents with chronic non-asthmatic lung disease in childhood. As the number of long-term survivors of premature birth, cystic fibrosis, and other conditions increase with advances in medical management, these individuals may represent a population that is particularly susceptible to acute and chronic effects of environmental pollutants. Further, pulmonary function deteriorates gradually during the life of the adult organism. The ultimate outcome of adult lung disease may well be at least in part dependent upon alterations in pulmonary development and/or loss of lung function, which occurs in early childhood.

A number of reviews have concluded that asthma prevalence is likely to be increasing globally (Johnson *et al*, 2002; Beasley *et al*, 2000; Hartert and Stokes Peebles, 2000). According to a WHO report (von Ehrenstein, 2002) a significant increase in prevalence of wheeze and childhood asthma has occurred in affluent Western countries ranging from a slight to a threefold increase. While descriptive European data are available from a number of studies (see Johnson *et al*, 2002), the number of such studies, which allow an evaluation of temporal trends, is limited. For example, in Scotland, asthma is reported to have increased in prevalence from 4.1 percent in 1964 to 10.2 percent in 1989 (Ninan, 1992), with a reported prevalence of 19.6 percent in 1994 (Omran and Russel, 1996). Similarly, the prevalence of asthma in north-Norwegian schoolchildren increased between 1985 and 1995 from 5.1% to 8.6% (Selnes *et al*, 2002).

While evidence suggests increasing frequency, it is important to keep in mind that variation among international centres is substantial as regards the reported prevalence or incidence of asthma and other allergic diseases. The ISAAC study (International Study of Asthma and

Allergies in Childhood) (1998) found “differences of between 20-fold and 60-fold between centres in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema, with a fourfold to 12-fold variation between the 10<sup>th</sup> and 90<sup>th</sup> percentiles for the different disorders. For asthma symptoms, the highest 12-month prevalences were from centres in the UK, Australia, New Zealand, and the Republic of Ireland, followed by most centres in North, Central, and South America; the lowest prevalences were from centres in several Eastern European countries, Indonesia, Greece, China, Taiwan, Uzbekistan, India, and Ethiopia.” Thus, data based upon local trends should be used with caution.

The prevalence of childhood asthma has been increasing in the United States over the past two decades. The precise frequency of asthma is difficult to define, and the change in prevalence may in part be the result of changing diagnostic criteria, but this does not appear likely to account for all of the observed increase in frequency, and the disorder is still believed to be systematically under-reported (MMWR, 2000; Sly, 1999; Eggleston *et al*, 1999). In 1997, some 8.6 million US children are said to have suffered from asthma. This disease, which affects one in thirteen children, is responsible for approximately one third of all emergency room visits, and is the fourth most common reason for paediatric office visits in the US. Clearly, the health and economic impacts of asthma are significant (MMWR, 2000). Further, asthma mortality appears to be rising significantly, with a threefold increase noted between 1977 and 1995 by the US National Center for Health Statistics (US NCHS, 1994).

Recently published longitudinal studies of asthma indicate that in an unselected cohort of children followed from age 9 to 26 years, 51 percent had wheezing on at least one assessment and more than one-in-four children had wheezing which persisted into adulthood (Sears *et al*, 2003). According to these authors, “The factors predicting persistence or relapse were sensitisation to house mites, airway hyperresponsiveness, female sex, smoking, and age of onset. These findings, together with persistently low lung function, suggest that outcomes in adult asthma may be determined primarily in early childhood.”

### **3.2 Allergies**

Allergy is an immunological response after sensitisation has occurred; it describes adverse health effects that may result from a specific immune response. This response is mainly mediated by antibodies, including IgE, although cell-mediated reactions may also be important such as in allergic contact dermatitis. This may take a variety of forms, including hay fever (allergic rhinoconjunctivitis), urticaria, allergic asthma, and allergic conjunctivitis. Allergic diseases may be systemic, as in anaphylaxis, or may be localised to one or more tissues (commonly the skin, gastrointestinal tract, respiratory tract, and other mucosal surfaces, e.g. the eye). Allergic reactions are, by definition, adverse health effects that are associated with tissue damage, tissue remodelling

and inflammation. They can, in the case of anaphylactic shock, be fatal. Consequences of specific immune responses and their impact on the individual as well as on society are considerable in terms of impaired life and societal and economic costs. According to the AAAAI (American Academy of Allergy, Asthma and Immunology) (2003) allergic conditions affect more than 20% of the US population and are the sixth-leading cause of chronic disease in North America. Allergic reactions account for 5-10% of all adverse drug reactions, with skin reactions being the most common form. Acute urticaria (hives) affects 10-20% of the US population at some time in their lives.

The risk of anaphylaxis may affect 1.2-15% of the US population (Neugut *et al*, 2001). Four major subtypes of allergens (food, drugs, latex and insect stings) may induce anaphylaxis. Known estimated occurrences of anaphylaxis in the US population were 0.76% for food, 0.7-10% for penicillin, 0.22-1% for radiocontrast media and 0.32-5% following insect stings.

Prevalence of hay fever is low in pre-school children, increases during school age, has a maximum in young adults and decreases thereafter. During childhood, males are more often affected than females, although this difference disappears with increasing age (Wahn and Wichmann, 2000). The overall prevalence of hay fever in Western countries is approaching 25% in the general population with a clear peak in the 15-24 age group (EU, 2000) though prevalence varies internationally considerably with 1.4-39.7% of 13-14 year olds affected (ISAAC, 1998). In Europe, pollen allergens may account for 10-20% of allergic disease, particularly hay fever (von Ehrenstein, 2002). Prevalence of allergic rhinoconjunctivitis (hay fever) in north-Norwegian schoolchildren increased between 1985 and 1995 from 16.4% to 22.1% (Selnes *et al*, 2002).

The influence of Western lifestyle became evident after the German reunification. There was more bronchitis in Eastern Germany and more asthma, hay fever and rhinitis in Western Germany (lifetime prevalence) (von Mutius *et al*, 1992). Since reunification, the prevalence of hay fever has increased from 2.3% to 5.1% in Eastern Germany, now reflecting the prevalence in Western Germany. This increase happened in a period of decreasing levels of air pollution in Eastern Germany. The prevalence of asthma and bronchial hyperreactivity remained virtually unchanged (von Ehrenstein, 2002; von Mutius *et al*, 1998).

With respect to reproducible adverse reactions to food or food components these reactions comprise either induction of a specific immune response (i.e. true allergy) or food intolerance not related to an immunological response.

Food allergies are most frequently present in early life (first year) and can disappear with increasing age. According to citations of the AAAAI, 8% of children younger than 6 years experience food intolerances, whereas 2-4% appear to have true allergic reactions to food. Among adults, 1-2% are sensitised to food or food additives. In Europe, 1-2% of adults and up to

5% of children show food allergy (Kimber and Dearman, 2002), while approximately 30% of Americans report food allergy or intolerance. A valid diagnosis of food allergy/intolerance can only be made when symptoms are reproducible and immunologically mediated (Wahn and Wichmann, 2000). Thus, the number of self-reported/assumed food allergies is distinctly higher than the actually diagnosed cases. A 1997 report of the European Commission states that true incidence of food allergy is low and usually manifested in less than 1% of the population with the true prevalence of intolerance to food additives between 0.026% and 1%. However, with food allergy being highly dependent on geographic area, prevalence in a specific population might rise to 5% or 6% (EU Scientific Committee for Food, 1997).

More than 90% of food allergies are related to eight common foods: cows' milk, soy, hens' eggs, wheat (likely to resolve) and peanut, tree nut, fish and shellfish (likely to persist) (Kagan, 2003; Kimber and Dearman 2002). An increase in the sensitisation to peanuts from 1989/1990 to 2001 has been reported (prevalence of suspected peanut allergy: 1.1%, prevalence of confirmed peanut allergy: 0.5%; suspected sensitisation to peanut: 3.2%, and confirmed sensitisation to peanut: 1.5%) (Grundy *et al*, 2003).

A review published by the German Federal Statistical Office (Wahn and Wichmann, 2000) showed that cows' milk, besides hens' eggs and peanuts, is one of the most common allergens for suckling babies and toddlers with an incidence rate of 0.3-8%. Prevalence of adverse food reaction in children was 7% in an English cohort study. The same report mentions a Swedish study, where lifetime prevalence of food allergy was 7-8% in school children, and a German study, where prevalence of food allergies was 6% in 5-6 year old children. The prevalence of food allergies in the developing child is reported to be 2% up to the age of 3 months, 3% during the first year of life and 5% during the second year of life. At the age of 6 years, 4.4% of children were allergic to hens' eggs, 5.1% to cows' milk, 3.7% to wheat and 2.3% to soy.

Furthermore, the prevalence of food allergy is higher in children than in adults because some food allergies, commonly found in children, resolve with increasing age (e.g. allergy to cows' milk and hens' eggs).

### **3.2.1 Skin allergy, allergic contact dermatitis, and atopic dermatitis**

Allergic contact dermatitis (ACD) or skin sensitisation, which is clearly caused by chemicals, is not always differentiated from other skin diseases like atopic dermatitis (neurodermatitis) with unclear aetiology. In addition, contact irritation is often erroneously claimed by non-professionals to be an allergic reaction. The reason for this is that the clinical signs of irritant dermatitis and of allergic contact dermatitis are often similar, and sometimes indistinguishable. However, the mechanistic bases are very different. One needs to clearly distinguish ACD from other skin

disorders, which may show similar symptoms. Atopic dermatitis is considered to show increasing trends, whereas this is not the case for ACD.

Atopic dermatitis is an IgE antibody mediated immune response of unclear aetiology. Atopic dermatitis (but not ACD) affects infants and children with an estimated prevalence of 9% in the United States, and frequency appears to be increasing. Wahn and Wichmann (2000) report prevalence of atopic dermatitis in children born before 1960 as 1-3%, for children born between 1960 and 1970 as 4-9%, and for children born after 1970 as 9-20%. The authors do not conclude this effect to be attributable to chemicals. Novak and Bieber (2003, 2004) published comprehensive reviews on the pathophysiology of atopic dermatitis. Chemicals are not mentioned as an aetiological factor in these reviews. Another study of Yura and Shimizu (2001) found that the increase in the prevalence of atopic dermatitis (not ACD) in school children in Osaka observed until 1993 levelled off afterwards.

In contrast to atopic dermatitis, the aetiology of skin sensitisation (ACD) is well understood. ACD develops in a susceptible subject when immunological priming of T lymphocytes is induced following skin contact with an appropriate amount of a chemical allergen. The sensitised individual will mount more aggressive and accelerated immune responses if subsequent contact is made with the same allergen at the same or a different skin site. This results in local cutaneous inflammation that is recognised clinically as allergic contact dermatitis. Symptoms and reactions of the ACD do not occur immediately after the first exposure to an allergen, but weeks or even months following the subsequent re-exposure to the same chemical. Therefore, this type of allergy is also called delayed or (immune-)cell-mediated hypersensitivity. Typical symptoms after repeated exposure to a contact allergen are erythema, oedema and papules at the exposure site. Common allergens include nickel, cobalt, certain preservatives, latex and other plant derived materials (like poison ivy). Estimates of the prevalence of latex allergy in the general US population vary from less than 1% to 6% (AAAAI, 2003).

Data on reported incidence of contact allergy in children are sparse. There is limited information on trends in children for this type of allergy, and there are also conflicting results from these limited data. Sensitisation to nickel and thimerosal was found in 7% and 9% of school children, respectively (Wahn and Wichmann, 2000). In one reference, ACD is claimed to be the most common skin condition in children younger than 11 years of age and the percentage of children affected has increased from about 3% in the 1960s to about 10% in the 1990s. In general, however, it is well accepted, that ACD is rare in early childhood, which might be driven primarily by limited exposure to contact allergens, but is probably also due to reduced responsiveness of the immune system.

With regard to the general incidence of ACD in babies and infants, Wöhrle and colleagues showed that it is highest in children less than 10 years (Wöhrle *et al*, 2003). The lowest incidence was found among patients over 70 years of age. Nickel is the most common sensitiser in children.

However, the above findings have been evaluated on the basis of patch test results of patients reporting to allergy clinics, i.e. in a population already experiencing some sort of dermatological symptoms, which are thereby not reflecting the average population. Therefore, caution should be applied to data that seem to indicate that ACD is higher in infants relative to adults and the elderly. The scientific consensus is that ACD is rare in early childhood (Epstein, 1982).

Neither the elderly nor infants are likely to be at increased risk to ACD as a function of age. This is supported by a report of the American Contact Dermatitis Group, which indicated that there was no age-related pattern to a series of positive responses to allergens (Rietschel *et al*, 1990). For the potent contact allergen dinitrochlorobenzene (DNCB) it has even been shown that newborns and young infants are less responsive to developing ACD as compared to the elderly after being exposed to the same level (concentration) of DNCB (Cassimos *et al*, 1980; see below). This study shows that the immunological disorders are far too complex to be simplified by claiming infants are more susceptible to immunotoxic insults. Overall, it can be concluded that infants are not more susceptible to developing an ACD as compared to adults or to the elderly.

The low number of sensitisation tests done in children and infants confirms the above conclusion that, from a morphological point of view, there is no evidence for an increased susceptibility of children and infants to develop ACD. It has even been shown that skin is less reactive to potent contact sensitisers in early infancy than later in life. In a study reported by Epstein in 1961<sup>a</sup>, 102 children aged 1 month to 8 years were patch tested in an attempt to induce sensitisation with pentadecylcatechol (*Rhus*, or poison ivy allergen). Children under 1 year of age showed an incidence of 30% sensitisation, (with only 8.5% showing severe reactions), while the sensitisation incidence increased to 50% in the age between 1 and 3 years (with 20% severe reactions) and further up to 76% in children between three and eight years (with 38% of them showing severe reactions).

These early findings have been confirmed more recently by Cassimos *et al* (1980)<sup>b</sup>. In their study, Cassimos and colleagues investigated the skin reactivity to 2,4-dinitrochlorobenzene (DNCB). They showed that the sensitisation incidence is exceedingly low in neonates and low in babies up to 3 months. Importantly, the sensitisation incidence in 9-month old infants was shown to be equal to adults but a 30-times higher dose per unit area of DNCB (concentration) was required to achieve equal sensitisation incidences in adults. In detail, a total of 284 infants were evaluated in this study. Data indicated reaction rates of 6.8% in the first 15 days of life, 25.7% in infants 2-4 weeks old, 33.3% in 2-month old infants, 62.9% in 3-month old infants, 76.7% in 4-month old infants, 81.5% in 5-month old infants, 88.5% in 7-month old infants, and 91.3% in 9-month old infants. This supports the conclusion that young infants are not more susceptible to sensitisation

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<sup>a</sup> The authors of the present report recognise that this study raises some significant ethical questions, but it is cited because the results have been published in a peer-reviewed journal.

<sup>b</sup> For this study, the same comment as in footnote <sup>a</sup> applies.

than adults, and might well be less susceptible under conditions of equivalent exposure. This can be explained by the immature immune system of neonates, impaired key elements such as limited immune cell function, reduced cytokine release, limited antibody synthesis, or deficiency of cell-mediated immunity.

In summary, as can be deduced from different sources, a worldwide reported increase in allergic disorders, atopic dermatitis and allergic asthma (see 3.1) has been documented during the last several decades with the majority of allergic reactions provoked by natural substances (e.g. pollen, mites, food). However, with regard to ACD prevalence a trend is either not conclusive or not attributable to chemical exposure. ACD does not show a higher prevalence in babies and infants relative to adults. Overall, the current data are limited and do not provide evidence that infants are at increased risk to develop ACD, nor is there any trend of increased prevalence for ACD in children.

### ***3.3 Developmental disorders***

The term 'developmental disorders' covers many types of problems, which may involve physical anomalies, behavioural abnormalities, metabolic deficits, physiologic alterations, and many other changes. Such damage can be incurred during foetal or childhood development and, depending on its type, severity, or lack of treatment, may be irreversible, having a permanent effect on children's quality of life and socio-economic success. The WHO (2003) reports rates of congenital anomalies among the European Union member states. Overall, there is no discernible trend. While for some of the new member states increases or decreases have been reported, rates appear to be stable for the other states with the exception of Denmark (increasing). This review of developmental disorders focuses only on developmental neurotoxicity and on developmental toxicity that results in reproductive disorders because these areas have been the focus of recent public concern and are highlighted in the European Commission's Environment and Health Strategy (EU Commission, 2004b).

Developmental neurotoxicity and reproductive disorders can include many potential manifestations. Neurobehavioural and other neurological disorders, such as autism, attention deficit / hyperactivity disorder (ADHD), and lowered IQ, are receiving increased attention due to suspected environmental causes and perceived increased prevalence. With regard to neurobehavioural and other neurological disorders, evidence for generally increasing incidence is not available. The absence of national baseline rates or nationwide systems for identifying new or existing cases, changing and inconsistent diagnostic criteria, small sample sizes, and neurological test batteries of uncertain reliability and validity hamper many studies attempting to evaluate potential trends. An evaluation of potential trends in developmental reproductive disorders is similarly hampered by incomplete, incompatible, and conflicting data. Reproductive disorders

currently receiving attention are the most frequent male reproductive tract abnormalities (hypospadias and cryptorchidism), early onset of puberty, and impaired testis function with affected sperm quality in adult life. This chapter evaluates the limited evidence that is available on suspected trends in the prevalence of neurologic and reproductive developmentally related disorders in Europe.

### **3.3.1 Neurodevelopmental disorders**

Current data are insufficient to determine if the incidence of disorders such as learning disabilities, autism, attention deficit and related abnormalities is truly increasing, (Woodruff *et al*, 2004; Schettler, 2001) or whether the apparent rise may be due to factors such as improved vigilance and diagnosis, changing diagnostic criteria, etc.

No population-based registries exist for neurodevelopmental disorders either in Europe or elsewhere in the world. Accordingly, it is difficult, if not impossible, to rigorously assess trends in neurodevelopmental disorders. Estimates of prevalence and incidence in the literature are largely based on clinical and epidemiology studies of select populations. As such, it is unclear how representative these estimates are for the general population. A summary for specific neurodevelopmental disorders follows.

#### **3.3.1.1. Attention deficit / hyperactivity disorder (ADHD)**

There has been a great deal written in the lay literature regarding the prevalence of developmental disabilities and ADHD in the United States. ADHD, which is variously referred to as 'hyperactivity', 'attention deficit disorder', or 'attention deficit syndrome', appears to be present in 3-6% of US school-age children according to the US National Institutes of Mental Health (NIMH, 1999). However, diagnostic criteria have been heterogeneous over time, and as more attention and resources have been turned to the diagnosis and treatment of this area, it appears likely that the frequency of diagnosis has increased as a result of increased public and professional (teacher, physician, psychologist, counsellor, etc.) awareness combined with the ability to access diagnosis-specific funds and programmes. The American Academy of Pediatrics Subcommittee (AAP, 2000) on attention deficit / hyperactivity disorder states that:

“With increasing epidemiologic and clinical research, diagnostic criteria have been revised on multiple occasions over the past 20 years. A recent review of prevalence rates in school age community samples (rather than referred samples) indicates rates varying from 4-12%, with estimated prevalence based on combining these studies of ~8-10%. Prevalence rates vary depending on whether they reflect school samples 6.9% (5.5-8.5%) or community samples 10.3% (8.2-12.7%).”

There appears to be no source of consistent data on which to base an evaluation of ADHD trends in Europe over time. Some national data exist, but suffer from a similar absence of diagnostic consistency across time and place. Trans-national comparisons, while likely to reveal differences in perceived rates, are unlikely to be informative as to whether the differences are reflective of true differences in child development. More likely, such differences reflect varying diagnostic criteria, and/or educational environments, provided that ADHD is, at least in part, a function of the ability to meet behavioural expectations in specific environments.

At this time there is no evidence that the fundamental underlying frequency of ADHD or other learning disabilities has changed systematically over time. While some would argue that 'the absence of evidence is not evidence of absence', it appears unwise to speculate on likely causes of an 'epidemic' that is still completely speculative. Rather, one can legitimately ask what environmental agents may cause or contribute to ADHD, even in the absence of a documented alteration in the overall frequency of this disorder.

### 3.3.1.2 Autism

Fombonne (1999) reviewed 23 epidemiology surveys of autism published in the English language literature between 1966 and 1998. The median prevalence estimate was 5.2 per 10,000 (95% confidence interval = 0.3 - 19.4). Twelve studies were from European countries where prevalence estimates ranged from 1.9 to 9.5 per 10,000, while three studies were from the United States (prevalence estimate range: 0.7-3.26 per 10,000). The 95% confidence limits for virtually all of the study prevalence estimates overlapped. Other reviews have suggested slightly higher prevalence (~10 per 10,000) (Newschaffer *et al*, 2002) but all agree that prevalence has increased over time (Fombonne, 1999; Newschaffer *et al*, 2002). The American Academy of Pediatrics Committee on Children with Disabilities suggests that the recent rise in prevalence of autism may be due to a combination of factors such as changing diagnostic criteria (inclusion of more milder forms of the disorder), higher public and professional recognition of the disorder or a true increase (AAP, 2001).

To determine whether the apparent increase in autism prevalence over time might be due to more efficient case identification and changing diagnostic criteria, Fombonne (1999) examined studies in France and Sweden where repeated surveys of the same population or birth cohort estimates were available. These data provided an assessment of the number of new cases developing in a population at risk (i.e. a measure of incidence); the data did not suggest that the incidence of autism was increasing over time. Other reviewers have reached similar conclusions (Newschaffer *et al*, 2002; Gillberg and Soderstrom, 2003; Barbaresi *et al*, 2005).

### 3.3.1.3 Learning deficits

'Learning disabilities' are operationally defined as difficulty with the learning process as a result of cognitive difficulties, and include a variety of heterogeneous difficulties. There is currently no standardised definition used in the literature for defining 'learning disabilities'. As such, it is difficult to assess trends for learning disabilities. Gillberg and Soderstrom (2003) reviewed the literature on learning disability and, based on the definition of learning disability as IQ less than 70, found that approximately 1-2.5% of the general population in the Western world is affected by learning disabilities. The reported percentage of individuals with IQs less than 50 is lower (0.3-0.5%), but is less variable between populations. In general, however, interpretation of the literature is complicated by differences in definitions, instruments used, and study design. There are no population-level data available on frequency of more subtle decrements in IQ.

As concern about environmental exposure on neurodevelopmental outcomes has increased, IQ scores in many nations have increased 9 to 20 points with each successive generation (Dickens and Flynn, 2001). Interpretation of this trend must consider that intelligence is only one facet of neurological status and function, and that some populations may still face increased neurodevelopment risks due to high dose exposures to agents such as lead, PCBs and methylmercury. Finally, improved survival of pre-term infants has led to increased prevalence of neurodevelopmental dysfunction such as behavioural disorders and learning disabilities in those individuals compared with full-term infants (Aylward and Hayes, 2002).

### 3.3.2 Effects on the reproductive system

#### 3.3.2.1 Male reproductive tract abnormalities: hypospadias, cryptorchidism

Hypospadias is a developmental anomaly in which the urethra opens on the underside of the penis or the perineum. Cryptorchidism refers to a failure of the testes to descend into the scrotum. Some reports have discussed temporal increases in the frequency of these disorders. However, statistics for the prevalence of these disorders vary widely due to differing diagnostics and reporting criteria as well as ethnic/genetic differences. For example, many congenital malformation registers changed the case definition of hypospadias during 1985-1995 to exclude minor forms (EEA/WHO, 2002). Dolk *et al* (2004) conclude in their recent publication that EUROCAT (European network of population-based registries for the epidemiologic surveillance of congenital anomalies) data do not indicate an increasing prevalence of hypospadias in Europe since 1980. They point out, however, that it is doubtful whether systems are in place, worldwide, for the effective surveillance of hypospadias in relation to exposure to potential endocrine disrupting chemicals. Comparison of data for cryptorchidism is particularly problematic, since

prevalence data depend on the age of the child at the time of examination due to possible delayed descent of the testes into the scrotum.

In two recent publications, the rates of occurrence of hypospadias (Pierik *et al*, 2002; Virtanen *et al*, 2001) have been rigorously obtained by prospective structured examination of newborn boys. Pierik *et al* studied 7292 newborns in Rotterdam in a 2-year period and reported a frequency of hypospadias in newborn boys of 0.73%, the rate among live births being 38/10,000. This rate was fourfold higher than expected; the authors argue that this difference is probably due to underreporting in registries. The second report on rigorous prevalence data, where all 5798 male newborns were systematically screened, showed a more than 50% lower rate (17/10,000) (Virtanen *et al*, 2001) than the one reported by Pierik *et al*.

In the same birth cohort as mentioned above, Pierik *et al* also determined the rate of occurrence of cryptorchidism (1.2%), which falls within the range of 0.9-9% reported by previous studies (Pierik *et al*, 2005). Comparison with the prevalence rate in a previous study (3.1%) performed in an area just north of the Rotterdam area may suggest a declining trend. However, data on temporal trends in the incidence of male reproductive tract abnormalities should be interpreted with considerable caution, given the lack of prospective longitudinal studies and the consequent difficulties in comparing data from separate studies.

#### 3.3.2.2 Onset of puberty

Population-based studies show a reduction in the median age of puberty in various countries (IPCS/WHO, 2002). According to Teilmann *et al* (2002) at the turn of the 19<sup>th</sup> century puberty started at the age of 17; then it shifted quickly to an earlier age. Data on pubertal development in boys are limited due to a lack of marked indicators such as age of menarche in girls (de Muinck Keizer-Schrama and Mul, 2001). The age of menarche decreased until the 1980s in most European countries; current mean ages vary between 12.5 and 13.1 years; these are similar in the USA (12.9 in Caucasian, 12.2 in Afro-American girls). However, in Europe the largest decrease was observed until the early 1980s. Thereafter, depending on the country, only a small decrease (Netherlands, Germany, Bulgaria), no significant change (Belgium, Norway) or even an increase (Italy, Croatia) in age of menarche have been observed (de Muinck Keizer-Schrama and Mul, 2001).

In Southern Chinese girls in Hong Kong, where some of the earliest median ages of sexual maturation in the world have been recorded, a significant downward but diminishing trend has been observed when comparing data from 1962 until the early 1990s (Huen *et al*, 1997).

In Puerto Rico, a temporal trend toward premature breast development in girls and gynecomastia (abnormally large breasts in males) in boys has been noted during the early 1980s (IPCS/WHO, 2002; Colón *et al*, 2000).

### 3.3.2.3 Effects on testis function with affected sperm quality in adult life

In 1993, Sharpe and Skakkebaek hypothesised that peri-natal oestrogen exposure could reduce sperm count in adulthood by permanently altering the numbers of Sertoli cells. A relevant impetus for this hypothesis was the publication of data suggesting that sperm counts in certain Western countries might have fallen markedly since 1930-1940 (Carlsen *et al*, 1992). However, this meta-analysis as well as several reanalyses of these retrospective data could not prove or disprove that sperm counts had indeed fallen, but stimulated much further research. As Sharpe (2003) points out, the numerous studies that had been prompted were nearly all retrospective and divided into two camps, those showing evidence of a secular trend related to year of birth and those showing no such trend. It became clear that global analysis of sperm data is not appropriate. A worldwide decline is not supported by the information available to date (IPCS/WHO, 2002).

Beside population characteristics several methodological factors may influence semen parameters: the IPCS Global Assessment of EDCs (IPCS/WHO, 2002) mentions mode of semen collection and analysis methods, number of ejaculations, sexual abstinence delay, intra- and inter-technician variability, season, and statistical methods. In addition, recruitment of study participants can depend on various factors potentially introducing selection bias into the studies.

However, substantial variations in sperm concentrations have been suggested. In the USA for example, higher concentrations have been reported in New York than in California (Fisch *et al*, 1996). One of the European studies which reported large differences in average sperm counts was the study by Jørgensen *et al* (2001); average counts for a 30-year old man with an abstinence period of 4 days have been 34% higher in Finland than in Denmark with Scotland and France intermediate between these two extremes. Seasonal variations were much more pronounced than previously expected with sperm counts being 30% lower in those providing samples in summer compared with winter. A study by Andersen *et al* (2000) in military recruits found particularly low median sperm counts of 41 million/ml, but Sharpe (2003) draws attention to the issue that it is not known whether sperm counts are always low in young men aged 18-20 years, as they are an age group not studied previously in any detail.

Thus, there are probably temporal and geographical variations in human sperm production, but it is not possible to judge whether there is a real decline and to what extent reductions might affect fertility (IPCS/WHO, 2002). Therefore, prospective data using standardised methods of semen analysis and subject recruitment are needed.

### 3.4 Cancer

This section addresses two fundamentally distinct, but related issues: childhood cancers, and the occurrence of adult cancers following childhood exposures. For a variety of biological and statistical reasons, these subjects require different treatment. Childhood cancers are, compared to adult cancers, rare. The overall incidence approaches 14 cases per 100,000 making it exceedingly difficult to perform meaningful epidemiological investigations on even the most common malignancy, i.e. acute lymphocytic leukaemia. Paediatric tumours, particularly those that occur early in life, are often related to inherited genetic disorders and/or embryologic developmental abnormalities and have dramatically shorter latency periods between potential exposure and the development of disease. Finally, the types and relative frequencies of various malignancies in childhood are markedly different from those seen in adult disease.

While trend data have been available in at least some geographies for child cancers, and suggest no major trend in overall disease frequency, the role of environmental factors in childhood cancer has been difficult to define, unless there have been high exposures (i.e. atomic or therapeutic radiation). Recently published European data (Steliarova-Foucher *et al*, 2004) suggest that childhood cancer rates may be increasing. In contrast to the data in children, epidemiologic changes in the frequency of adult cancer are well recognised and numerous studies address occupational and non-occupational contributors to adult cancer risk. Overall, there is a relative paucity of data regarding childhood chemical (i.e. non-radiation) exposures to environmental factors and their impact on adult cancers.

#### 3.4.1 Childhood cancer

Child cancer presents a series of difficult epidemiologic issues. While a variety of isolated epidemiologic associations with parental exposure are reported, the results of these studies are inconsistent and even contradictory. With the exception of high dose radiation, the role of environmental agents in childhood cancers remains essentially unknown. In total, child cancer has an annual incidence of about 1 per 7000 children, and as haematological malignancies (mainly acute lymphocytic leukaemia in younger children and lymphoma in older children) constitute 40% of all childhood tumours, individual non-haematological malignancies are rare (Linet *et al*, 2003). Similar incidence rates of tumours in children were reported for Europe between 1970 and 1999 (Steliarova-Foucher *et al*, 2004). Given the low frequency of childhood cancer, epidemiologic studies are challenging even for multi-organ carcinogens like ionising radiation. Obtaining sufficient numbers of individuals for specific tumour types is difficult. In addition, there is reason to believe that some of the early childhood tumours have a fundamentally different aetiology from adult tumours, being the result of abnormalities of

embryonic development or inherited genetic propensities as seen with retinoblastoma (Altshuler *et al*, 2003a and b).

Data indicate that developed countries have gradually increasing incidences in leukaemia with a corresponding drop in the incidence of lymphoma. However, these data are not consistent for all developed countries. For instance, data from the German Childhood Malignancies Registry reported an incidence of leukaemia that was similar over the whole period reviewed (1980-1992), especially since 1984, with a range between 4.4 and 4.6 per 100,000 (Kaatsch *et al*, 1995). In addition, changes in diagnosis and classification of tumours, as was the case for leukaemia in Sweden, have an impact on determining incidence (Dreifaldt *et al*, 2004).

Another important factor impacting childhood cancer trends is the completeness in case reporting systems (Kaatsch *et al*, 1995). Completeness has a substantial impact on temporal trends. Cancer incidence data are not without limitations, and caution should be taken in deriving trends from cancer registry data in general. With respect to US population-based State cancer registries, Izquierdo pointed out that “users must be aware of diverse issues that influence the collection and interpretation of cancer registry data, such as multiple cancer diagnoses, duplicate reports, reporting delays, misclassification of race/ethnicity and pitfalls in estimates of cancer incidence rates” (Izquierdo and Schoenbach, 2000).

The pan-European child cancer incidence data now becoming available through efforts at IARC (Steliarova-Foucher *et al*, 2004), as mentioned above, may not yet be sufficiently detailed and compatible to allow the accurate assessment of broad trends in childhood cancer within the EU. While available cancer incidence data from the US Surveillance, Epidemiology and End Results (SEER) programme cover approximately 10% of the US population (Ries *et al*, 1999), one should consider whether the US or the EU are sufficiently homogeneous as to environmental exposures to make such an analysis truly meaningful. Certainly, the most striking adult cancer hazards related to contamination of the environment tend to be local or regional in nature, and are unlikely to extend across the entire US or EU in a homogeneous manner.

Regarding long-term trends in child cancer, much has been written suggesting dramatic changes in child cancer rates in the United States. Invariably these figures proved to be based upon total number of cases rather than upon age and gender adjusted incidence rates and simply reflect overall growth in the US population. Review of the US population-adjusted data suggests no major change in the epidemiology of childhood cancers (Linet *et al*, 1999; Linet *et al*, 2003). Rates of leukaemia appear to fluctuate, with an increase in incidence during the 1983-84 time frame followed by a stabilisation in rate and subsequent slight decrease since 1989. These were not statistically significant changes; therefore, no generally accepted explanation for this fluctuation has been postulated. Concomitantly, there has been a modest decline in the rates of lymphoma, primarily due to decreases in Hodgkin disease, in the US and many other developed

nations. In the late 1980s, an abrupt increase in the incidence of central nervous system tumours was noted that is likely to be related to the rapid increase in the availability of CT-scanning. The increase is entirely attributable to low-grade tumours, which may not have been presented as clinical malignancy if not radiographically identified. Rates have subsequently remained stable, and the sudden increase in rates is thus believed to be due to changes in diagnostic technology (Smith *et al*, 1998).

No other significant trends are apparent regarding the relatively more common childhood tumours in the United States. Linet *et al* (1999) did note an increase in two rare tumour types. Non-epithelial skin cancers have shown a gradual increase between 1975 and 1995, rising from approximately 0.17 to 0.27 cases per population of 100,000. This was entirely due to the occurrence of dermatofibrosarcomas. Non-thyroid endocrine tumours increased as well, primarily as a result of an increase between 1983 and 1985 of infant adrenal neuroblastomas.

In short, US data suggest no dramatic increases in major childhood tumours are apparent independent of changes in diagnostic methodology. Non-statistically significant increases in acute leukaemia appear to have stabilised. A few rare paediatric tumour types (dermatofibrosarcoma and adrenal neuroblastoma) appear to be increasing. Alterations in risk factors likely to account for the observed changes (both increases and decreases) in US child cancer rates have not been identified.

The recently published compilation of childhood cancer data from 63 different population-based registries in 19 European countries used data from 1970 through the 1990s (Steliarova-Foucher *et al*, 2004). Twelve registries were specific to childhood cancer, and 14 of the 19 registries contained national data. This analysis indicated increasing cancer rates from the 1970 through the 1990s, with an overall average annual rate of increase of 1.0% per year in children of less than 15 years of age and a 1.5% per year rate in adolescents. Similar rates of increase (average annual rates in percentage per year) over the study interval were reported for a wide variety of tumours including leukaemias (1.4%), lymphomas (1.3%), Hodgkin disease (1.5%), CNS tumours (2.5% in Eastern, 0.8% in Western Europe), neuroblastoma (2%), soft tissue sarcomas (1.86%), germ cell tumours (2.3%), renal tumours (1.1%), hepatic tumours (1%), and retinoblastoma (1.1% in age less than 1 year). Adolescent tumours also increased, including carcinomas (3.9%), lymphomas (2.4%), germ cell tumours (1.7%), soft tissue sarcomas (2.6%), and CNS tumours (1.4%).

The incidence of other or unspecified tumours decreased in both children (4.1% per year) and adolescents (9.7% per year). Although diagnostic reclassification may have played a role in the apparent rate increases, this cannot be a major impact as the category is in fact small, constituting around 2% of tumours in various age groups. As paediatric care has become more centralised, the

effect of improvements in case acquisition and reporting, as well as the effect of possible changes in case ascertainment have not been assessed.

These recently published data clearly need to be subject of much more detailed analysis. As not all paediatric tumours share similar timing and biology, and as it is highly unlikely that any given environmental change will produce all tumour types, the plausibility of a broad increase in tumours across nearly all significant tumour types in all age groups over time suggests that improved reporting or other confounders may be responsible for part or all of the observed changes. The authors suggest that smoking, infectious factors, or increased survival or low birth weight of infants play a role as well.

This initial publication of the IARC pan-European data did not attempt to associate specific environmental causes with particular tumours and has provided guidance for further study to provide more meaningful population-based analyses.

### **3.4.2 Adult cancer as a result of childhood exposure**

There are currently insufficient data to determine whether trends in adult cancers may be related to early life exposures. The issue of adult cancers, in this context, is defined as cancer in people older than 15 years of age, and potentially as a result of pre-natal or early childhood exposure. Valid exposure assessment is difficult, as the exposure would have taken place during pregnancy and several decades prior to the occurrence of tumours. The timing of exposure for the determination of risk is important, and information on time periods of higher risk might provide insights into the mechanism. The effect of pre-natal exposure for carcinogens has to be addressed almost exclusively through animal studies, as specific and accurate exposure information for humans is missing, except for treatment with certain drugs during pregnancy, e.g. DES.

The preponderance of adult cancers occurs after the age of 50, especially smoking related cancers, suggesting a latency period longer than the 20-30 year latency period commonly assumed for most cancers. However, the risk of contracting some types of cancer, e.g. testicular, cervix, CNS tumours, Hodgkin's lymphoma, increases from the age of 20 (IARC, 1992) and, with a suggested latency of 20-30 years, either genetic predisposition or early life exposures may be important. Early life genomic events may be essential for the subsequent occurrence and progression of adult cancers occurring at a younger age. However, testing this hypothesis based on human data is extremely difficult due to the relatively limited number of cases among young adults and the likely poor quality of exposure information. Genomic imprinting, which involves non-permanent DNA modifications during foetal development, has also been hypothesised to contribute to the occurrence of various adult cancers, e.g. prostate and breast cancer (Li *et al*, 2003).

Theoretically, *in utero* and early life exposure to carcinogenic compounds could increase the susceptibility for developing cancer later in life in several ways. These include higher cell division rate, differences in biotransformation activities, the existence of novel developmental processes, which may be altered by exposure, and the longer remaining expected life span following exposure. However, there is the issue of possible enhanced DNA repair during growth and development, which would argue against a permanent impact from early life exposure. Also, some substances have produced fewer tumours when administered to young animals rather than to older animals (Anisimov, 2003) due to the relatively poor function of some of the carcinogen activating enzymes in the foetus in the post-natal period.

Laboratory animal studies have shown that pre-natal exposure to a carcinogen induces genetic changes in genes relevant for the carcinogenic process, including activation of oncogenes in the foetus. A multigenerational effect of pre-natal exposure has been reported for some model carcinogens (IARC, 1989).

Reports of intra-uterine factors impacting hormone related cancers in humans exist, such as birth weight and its association with breast cancer (Michels *et al*, 1996), prostate cancer (Ekbom *et al*, 1996), and testicular cancer in relationship to gestational duration (Richiardi *et al*, 2002). However, these findings are based on only one major study and on some smaller ones.

Birth weight is significantly associated with breast cancer risk later in life, which suggests that intra-uterine factors or processes affect the risk of breast cancer in the offspring. High concentrations of pregnancy oestrogens may have an important role in breast cancer, but other pregnancy hormones or intra-uterine factors may be involved (Michels *et al*, 1996). Indirect support for the hormone hypotheses is provided by the observation that women who had been exposed to pre-eclampsia *in utero* had a striking reduction in breast cancer risk compared to women whose mothers had an uncomplicated pregnancy. As above, these findings are based on this single study only.

Recently, the US EPA has extensively evaluated the issue of relative susceptibility of children to carcinogens leading to adulthood cancer (US EPA, 2003b). Exposure issues unique to children are not addressed in this report; this review rather focuses on alterations of intrinsic susceptibility. Human data are essentially limited to the atomic radiation data. It has been shown that childhood exposure to atomic bomb radiation appears to produce a higher dose-adjusted risk of cancer than similar exposures in adults for at least some selected tissue types. The US EPA study concluded that when evaluating the incremental lifetime risk of mutagenic carcinogens, an increase in the estimated upper-bound slope factor (based on adult studies in animals) by a factor of 10 was indicated for ages from birth up to 2 years, and by a factor of 3 for ages 2-15. The overall finding for genotoxic carcinogens was that developing individuals might be more susceptible to the carcinogenic effects of these compounds. It is worth mentioning, though, that the animal data

reviewed by the US EPA were generated in studies not specifically designed to address this issue, and they suffer from a number of significant methodological deficiencies.

Further epidemiological research is needed to assess accurately the relative susceptibility of children to the induction of cancers in adulthood.

### **3.5 Conclusions**

- Identification of health trends in children can be difficult due to changes in diagnostic criteria, differences in reporting, and improved early detection of disease. Data are sparse, particularly in Europe, and evaluation of trends alone may fail to adequately identify specific environmental causes for those trends or, indeed, even identify those health outcomes to which environmental cases are strongly contributory.
- Commonly held perceptions regarding health trends are not always supported by available data and, in some cases, are refuted by existing data (see specific conclusions below).
- There is clear evidence of increasing rates of asthma in childhood, although rates in some countries may have stabilised. There is no convincing evidence of widespread trends in other acute or chronic childhood respiratory diseases.
- Coexistent with the increasing frequency of asthma, data also suggest that other disorders such as upper respiratory allergies, food allergy and atopic dermatitis may be increasing. However, an increase of allergic contact dermatitis in children is not confirmed by existing data. Scientific data also indicate that babies and children are not at an increased risk of developing allergic contact dermatitis relative to adults at given concentrations of skin sensitisers. As with the asthma data, interpretation of the available information is made difficult by inconsistent application of diagnostic criteria over place and time.
- Neurodevelopmental disorders such as autism and attention deficit disorder are perceived to be increasing in frequency, but supporting data are not available. Diagnostic criteria have changed significantly over time, making it impossible to ascertain trends in actual disease frequency even in the limited geographies for which data distributed over time are available.
- There are limited data on reproductive effects, often suffering from serious data quality issues. While geographic heterogeneity is apparent, broad population trends for these outcomes (sperm quality, hypospadias, cryptorchidism) are difficult to identify except for puberty age in females. The earlier onset of puberty may well result from lifestyle changes such as nutritional factors.
- With regards to childhood cancer, there is no evidence for significant increasing trends in its incidence. Data indicate that developed countries tend to have a higher incidence of leukaemia with a corresponding drop in the incidence of lymphoma. Increases in brain tumour frequency are related to the development of new diagnostic capabilities rather than a true change in the incidence in the rate of malignant disease.

- With a better prognosis of childhood cancer survival, secondary cancers following chemotherapy appear to be increasing. It is not clear whether this is due to the relative contribution of chemotherapy (many agents are themselves mutagens) versus an underlying propensity for the development of malignancy. Besides, it likely varies with tumour type. Outside of this limited population and a few other populations with high-level exposure to carcinogenic agents (i.e. radiation), the low incidence of childhood cancer makes epidemiologic determination of cause difficult.
- It is hypothesised that the biological processes leading to adult cancers can occur during childhood, and may result in the occurrence of, or earlier occurrence of, adult cancers. While there are a number of biological reasons to believe that immature and/or rapidly growing organisms may be more susceptible to pro-carcinogenic effects, there are insufficient data on the issue of relative susceptibility to carcinogens during childhood.
- Animal data, recently reviewed by the US EPA, support the contention that children may be more susceptible to the effects of direct or indirect mutagenic carcinogens, which may lead to cancer in adult life. However, these data are the result of studies not designed specifically to address this issue and suffer from a number of significant methodological deficiencies. Further epidemiological research is needed in order to accurately assess the relative susceptibility of children to the induction of cancers appearing in adulthood.

## 4. CONTRIBUTING FACTORS

This chapter reviews the various factors, environmental and otherwise, which contribute to the health effects and health effect trends described in Chapter 3. Genetic or other trans-generational propensities to disease states are described, and environmental factors are considered on a broad scope, including both chemical and non-chemical factors. The specific contribution of industrial chemicals to these health effects is then reviewed with a sharper focus in Chapter 5.

A complete review of the extensive literature on ambient air pollutants (ozone, SO<sub>x</sub>, NO<sub>x</sub>, photochemical species, particulates, etc.) and acute or chronic respiratory disease is beyond the intended scope of this document. While the task force was aware of these issues and had been provided with a set of references, no attempt has been made to be exhaustive in this area. An assessment of the contribution of environmental factors (and injuries) on disease in children concluded that outdoor and indoor air pollution, inadequate water quality and sanitation and lead exposure were the largest contributors to the disease burden of children (Valent *et al*, 2004).

### 4.1 Respiratory diseases / asthma

Asthma, allergy, and atopy are well known to run in families, and the genetic influence in regards to asthma is well recognised, but environmental factors are also clearly important (Sly, 1999; Sarafino and Goldfedder, 1995). Premature birth and low birth weight (Brooks *et al*, 2001) are risk factors for asthma in childhood, as is infection with respiratory syncytial virus (RSV) (Stein *et al*, 1999). However, viral agents in general, while exacerbating asthma, may not determine long term asthma status (Martinez, 1995). While increasing age (in childhood), race, and lower socio-economic status (comparison within the US, not globally) have been reported to be associated with asthma (Sarpong *et al*, 1996), these factors are, at best, difficult to separate from various aspects of antigen exposure and other immuno-modulatory factors (Call *et al*, 1992). Even body-weight (obesity, mass index) has been associated with the development of symptomatic asthma in girls (Castro-Rodriguez *et al*, 2001) and among military populations in the United States (Young *et al*, 2001).

Environmental factors may influence the incidence of asthma symptoms by a variety of mechanisms including (Altshuler *et al*, 2003b; Kimber, 1998):

- Triggering asthmatic reactions in airways, which are already hyperreactive due to one or more underlying processes or conditions (genetic or otherwise).
- Inducing or enhancing airway inflammation and/or hyperresponsiveness.
- Exerting a direct toxic effect on the respiratory mucosa resulting in inflammation and/or hyperresponsiveness.

- Altering immune responses to other inhaled antigens via local or systemic immunomodulating effects.

While there are few circumstances in which the precise mechanisms for a particular toxic material have been fully characterised, it is important to recognise that one or more of these various mechanisms can occur with any given toxic material and that interactions between various influences upon airway reactivity are possibly the rule rather than the exception (Lindfors *et al*, 1999; Koenig *et al*, 1990; Pierson *et al*, 1984; Leaderer *et al*, 2002).

The development of the immune system may also play a role in the occurrence of asthma. Environmental influences such as infection may direct immunological activity away from responses to environmental allergens. Early life exposures by various routes may influence the degree of tolerance to environmental allergens. Studies of Scandinavian and Eastern European populations have suggested, for example, that 'Western lifestyle' is associated with wheezing, allergic rhinoconjunctivitis and atopic illness in children, although the study provides no insight into what factors may specifically be responsible for this phenomenon (Björkstén *et al*, 1998).

These and other observations have led to the development of the so-called 'hygiene hypothesis', which states that the reduced exposures to certain microbial and infectious agents early in life may in fact increase the risk of asthma (Varga *et al*, 2003; de Sousa Mucida *et al*, 2003; Prescott, 2003; Yazdanbakhsh *et al*, 2002). Associations supporting this hypothesis are reported both within and outside the US and include:

- Relationships to socio-economic status (da Costa Lima *et al*, 2003; Matricardi *et al*, 2002a and 2002b);
- crowded living conditions / family size (da Costa Lima *et al*, 2003; Bodner *et al*, 1998; Leaderer *et al*, 2002);
- prolonged breast feeding (da Costa Lima *et al*, 2003);
- exposure to endotoxin and microbial agents (e.g. livestock farming) (Braun-Fahrlander, 2003; Prescott, 2003; Kalliomäki and Isolauri, 2002; Matricardi *et al*, 2002a and 2002b; Matricardi and Ronchetti, 2001; Message and Johnston, 2002; von Hertzen, 2002; von Mutius, 2001);
- immunisation (Illi *et al*, 2001; Grüber *et al*, 2003);
- helminthic infections (Palmas *et al*, 2003);
- pet exposure (Hesselmar *et al*, 1999).

While it is believed that this may be due to immune modulation toward Th1 lymphocyte mediated immune responses and away from Th2 (atopic) responses by multiple factors, the hypothesis remains the subject of considerable debate (Prescott, 2003; Palmas *et al*, 2003; Kemp and Björkstén, 2003; Liu and Murphy, 2003; Kalliomäki and Isolauri, 2002; Yazdanbakhsh *et al*, 2002; Tantisira and Weiss 2001). Finally, some scientists believe that the increase in asthma

mortality may represent an adverse effect of therapy either as a direct pharmacologic effect or via a delay in seeking hospital-based treatment and supportive care (Mann *et al*, 2003; Shore and Drazen, 2003).

An important issue still open for debate is whether environmental factors (including a lack of exposure in early childhood to certain allergens or infectious agents), which cause the development of underlying asthmatic processes, are necessarily the same factors, which acutely exacerbate existing asthma. The US National Academy of Sciences Institute of Medicine (IOM, 2003) extensively reviewed this subject as it relates to indoor exposures. Regarding specific causes for developing asthma, the IOM determined that causal relationships were firmly established only for house mites. Sufficient evidence exists for a role of environmental tobacco smoke in causing childhood asthma. Limited evidence supports a causal role for respiratory syncytial virus and cockroach antigens, but the evidence for all other investigated causes was considered insufficient. Regarding acute exacerbation of existing asthma, causal relationships were considered to exist for cockroach, cat, and mite antigens. Sufficient evidence was noted for rhinoviral infections, dog antigens, and moulds or fungi, as well as for nitrogen oxides and for tobacco smoke in older children. Limited or suggestive evidence was considered to exist for domestic birds, a variety of infectious agents, for environmental tobacco smoke in pre-school children, and for formaldehyde and some fragrances.

Limited data suggest that indoor and outdoor air pollution may influence the rate of lung development, but the ultimate impact of this is not yet understood, and the subject will not be further discussed here. Upper respiratory tract conditions, in the form of otitis media, are known to be associated with air quality issues, specifically smoking (Ey *et al*, 1995), but no strong connections have been made with other specific environmental factors. Limited research suggests environmental associations for upper airway allergic and other atopic conditions, which may also be increasing in prevalence (Sly, 1999). In some cases, the proposed causes are similar to those invoked for asthma (Matricardi *et al*, 2002a and 2002b; Koenig *et al*, 1985; Hesselmar *et al*, 1999). Due to the limited evidence in these other areas, this review has focused upon the issue of childhood asthma and environmental pollutants. The related issue of other atopic diseases is discussed in detail below (see 4.2).

Associations between short- or long-term exposures to ambient air pollution have repeatedly and consistently been shown to be associated with adverse respiratory health effects (von Ehrenstein, 2002). However, due to the high correlation of ambient air concentrations among the various pollutants it is difficult to assess the specific contribution of a single pollutant. Although a clear adverse impact of outdoor pollutants on respiratory health in asthmatic and non-asthmatic children has been found in many studies from several countries, the risk attributable to outdoor air pollution with regard to respiratory morbidity and mortality remains to be elucidated. While the evidence for the contribution of air pollution to the exacerbation of symptoms in children with

underlying asthma is compelling, it is less clear whether such exposures make any contribution to the cause of asthma. Probably, the most relevant indoor air pollutant is environmental tobacco smoke (Morkjaroenpong *et al*, 2002; Koenig, 1999; Chilmonczyk *et al*, 1993), as well as the use of gas cooking appliances in the home (Jarvis *et al*, 1996; Dekker *et al*, 1991), forced air ventilation (Åberg *et al*, 1996), damp living environments and humidifier use (Dekker *et al*, 1991) (presumably due to mould).

Exposure to biological agents, and even pollen (Ordaz *et al*, 1998) or fungal spores (Delfino *et al*, 1996), is also important in asthma (IOM, 2003). Data demonstrate a role for dust mites (Sporik *et al*, 1999; Call *et al*, 1992), animal dander or other animal derived allergens (Roost *et al*, 1999), cockroach allergens (Sarpong *et al*, 1996; Rosenstreich *et al*, 1997), moulds (especially *alternaria*) (Halonen *et al*, 1997), fungi, bacterial endotoxins (Braun-Fahrlander, 2003; Michel *et al*, 1996), pollen (Ordaz *et al*, 1998; Newson *et al*, 1997), and even lightning (Newson *et al*, 1997). The role of these various factors regarding asthma prevalence versus asthma exacerbation is not entirely defined.

#### **4.2 Allergies**

Exposures to allergenic substances together with genetic predisposition of atopic traits are the most important risk factors for development of atopic allergy. In particular, mite, cat and cockroach allergens are potent sensitizers. Children whose parents are atopic have a higher risk of becoming allergic, especially when the mother is affected (Wright, 2004). It has been proposed that the association between allergen exposure and risk of allergic disease is more related to the general capacity to mount an allergic response rather than to levels of exposure to any specific allergen (Wright, 2004). Socio-economic factors, the number of infections of the upper respiratory tract, early social contacts with other children, number of siblings, pets, frequency of the use of soap and household cleaning agents, i.e. hygienic conditions, and diet play an important role. European and American children of higher social classes and a Western (prosperous) lifestyle are probably more at risk to develop skin and respiratory allergies while children with a poor socio-economic background are more likely to develop asthma (Wahn and Wichmann, 2000).

Exposure of the unborn to xenobiotics during intra-uterine development depends on maternal exposure and pregnancy-related alterations of absorption, metabolism and excretion. Thereafter, adults and children are generally exposed to allergens by the same routes, though contact to allergens and magnitude of exposure is distinctly influenced by differences in physiology, behaviour, environment and relative amount of food and water ingested. Allergic sensitisation (e.g. for cockroach allergen) may occur *in utero*, although it is still under debate whether allergen exposure of the mother is related to the foetal response (Hamelmann and Wahn, 2002; Perera *et al*, 2002a).

Exposure to allergens may occur by inhalation (e.g. pollen, wheat, mould spores, feathers, saliva/sweat/urine/faeces proteins, epithelia of animal origin e.g. furred pets, mite dust, cockroaches, wood and flour dust, indoor and outdoor air pollutants, traffic exhaust, gases, formaldehyde, agrochemicals, industrial chemicals); oral ingestion (mainly food allergens e.g. peanuts, soy, hens' eggs, cows' milk, wheat, fish, shellfish, glutamate; drugs, man-made chemicals); skin contact (e.g. latex, nickel, pharmaceuticals, some proteins); and even by injection (e.g. insect stings, jellyfish, corals, pharmaceuticals). It has been noted that the route of exposure does not necessarily predict the type of clinical symptoms. For example, inhalation of an allergen may well provoke a skin reaction. This finding allows, for example, the use of skin testing (prick test) for sensitivity testing towards specific allergens and most probably reflects the fact that an allergic response is systemic in nature. Allergy assessment is made more complex by the fact that (a) allergies are often triggered by a combination of factors, (b) incidence can vary in the course of a lifetime through acquired tolerance or other mechanisms, and (c) cross-reactivity among allergens, both recognised and unrecognised, makes clinical assessment difficult. Furthermore, children sensitised to indoor allergens with milder allergic symptoms (e.g. allergic rhinoconjunctivitis) may, over time, develop a severe disease requiring constant control and medication. However, the precise nature of this relationship is not yet fully understood (Wahn, 2000).

It is increasingly clear that immunologically mediated diseases in adulthood are influenced by events in early post-natal life (Holt and Jones, 2000). During this period, the immune system is fine-tuning a variety of key functions in the face of direct stimulation from environmental signals not encountered during foetal life, and patterns 'learned' during this period persist into adulthood. The immune system of the foetus and neonate is characterised by a predominant Th2 (allergic/atopic - mediated by interleukins and IgE) immune phenotype. In the early post-natal period, the immune system matures to a more balanced Th1/Th2 status as uncommitted Th0 cells develop from immature dendritic cells. Infectious stimuli (viral illness, immunisation, etc.) may alter this balance in a manner minimising allergic responses ('hygiene hypothesis'), while exposure to allergenic substances may alter this balance toward atopic response, resulting in post-natal disease. A modification of the hypothesis claims that priming of Th regulator cells is responsible for development of a type Th1 or Th2 phenotype (Rook, 2000; Rook *et al*, 2001; Rook *et al*, 2003). It is also suggested (Holt and Jones, 2000) that variations in capacity (polymorphism of CD14 gene encoding the high-affinity receptor for bacterial polysaccharide) to recognise and/or respond to Th1 inducing signals may be the underlying cause. As outlined in 4.1, this 'hygiene hypothesis' remains the subject of considerable debate. Table 4-1 lists possible risk factors discussed as being associated with the development of atopic/allergic diseases.

**Table 4-1: Possible risk factors associated with atopic diseases**

Possible Risk Factor	Comment on Significance/Evidence	Reference
Parental history of allergy	<i>Increasing risk / triggers</i>	Wahn and Wichmann, 2000
Obesity	<i>Increasing risk / triggers</i>	Castro-Rodriguez <i>et al</i> , 2001; Wahn, 2000; Young <i>et al</i> , 2001
Low birth weight	<i>Ambivalent findings; effect depends on type of disease</i>	Brooks <i>et al</i> , 2001; Wahn 2000
Vermin (mites, cockroaches etc. in the home environment)	<i>Increasing risk / triggers</i>	Halmerbauer <i>et al</i> , 2002 ; Leaderer <i>et al</i> , 2002 ; von Ehrenstein, 2002
Dietary factors (e.g. cows' milk, breast feeding, omega-3 fatty acids)	<i>Cows' milk possibly increases risk, breast feeding protective effect, protective effect of fatty acids under discussion</i>	van Odijk <i>et al</i> , 2003; von Ehrenstein, 2002; Wahn, 2000; Schwartz, 2000
Pets	<i>Controversially discussed, should be avoided in atopic children</i>	Leaderer <i>et al</i> , 2002; Svanes <i>et al</i> , 2003; Lau and Wahn, 2003
Smoking during intra-uterine development	<i>Increasing risk / triggers (for some but not all forms of allergy)</i>	Gilliland <i>et al</i> , 2001
Climatic changes (pollen production by allergenic plants)	<i>Increasing risk / triggers</i>	D'Amato <i>et al</i> , 2000
Socio-economic factors	<i>Poor as well as affluent living conditions increase / trigger risk for specific atopic diseases</i>	Strachan, 2000; Heinrich <i>et al</i> , 2000; Lewis <i>et al</i> , 2001
Infectious burden during childhood (e.g. RSV*, respiratory infections)	<i>Increase or decrease of risk; depends on type of infectious agent, time of infection and affected organ</i>	Illi <i>et al</i> , 2001; Karol, 2002; Johnston and Openshaw, 2001
Number of siblings / attendance of day care	<i>No increased risk / reduced risk</i>	Von Ehrenstein, 2002; Wahn, 2000
Early / intra-uterine antibiotic treatment	<i>Increasing risk / triggers</i>	Wright, 2004
Vaccinations	<i>No increased risk / reduced risk</i>	Grüber <i>et al</i> 2001, 2003
Man-made chemicals and gases, indoor and outdoor air pollution (e.g. tobacco smoke, formaldehyde, ozone, nitric oxides, particulate matter, volatile organic chemicals, fragrances, food additives, antibiotics)	<i>For some factors relation to respiratory health / allergies established (tobacco smoke) while others are under discussion / no relation established</i>	Brasch and Geier, 1997; D'Amato <i>et al</i> , 2000; Kulig <i>et al</i> , 1999b; Dietert <i>et al</i> , 2000; EU Scientific Committee for Food, 1997; Holladay and Smialowicz 2000; Wahn and Wichmann, 2000; Lehmann <i>et al</i> , 2001; Rastogi <i>et al</i> , 1999; Yura and Shimizu, 2001; von Mutius, 2000

\* RSV: respiratory syncytial virus

Migrant studies indicate both a genetic and environmental influence on the prevalence of atopic diseases (Grüber *et al*, 2002; Williams, 1995). The prevalence of the different conditions varies with child age, and allergic conditions are inter-related. Sensitisation to food allergens decreases

with increasing age (Kulig *et al*, 1999a) and children showing sensitivity to hens' egg protein during the first year of life are more likely to develop atopic disease (asthma) later on (Wahn, 2000; Wright, 2004). In contrast, newborns and young babies less than 3 months old seem to be less susceptible for the induction of contact allergy as compared to the elderly (see below, allergic contact dermatitis). Aetiological factors discussed in relation to atopic dermatitis are genetic background, low prevalence of bacterial and viral infections ('hygiene hypothesis'), environmental factors (Western lifestyle), food and aeroallergens (triggering effect), reduced skin barrier function possibly due to an intrinsic defect of keratinocytes and immunological mechanisms (Novak and Bieber, 2004). It has to be noted that the concept of the 'hygiene hypothesis' and the Western lifestyle are largely overlapping factors.

In summary, the following factors are currently being investigated in association with allergic disease: genetic atopic predisposition, altered cytokine profiles at birth, selective Th phenotype, early childhood allergen exposure and sensitisation, microbial exposure early in life (viral respiratory infections in young children, hygienic status of living environment), maternal smoking during pregnancy, poor dietary factors, lack of breast-feeding, childhood obesity, air pollution (e.g. heavy traffic) and frequent immunisations in childhood (see also 4.1). The assumed relation of frequent immunisations with increased occurrence of atopic disease was contradicted in a recent study (Grüber *et al*, 2003), where incidence of atopic disease in children (at risk for atopic disease due to an atopic parent) was inversely correlated with overall number of vaccinations.

The underlying cause for development of allergies/atopic diseases and a shift to more type Th2 like immune reactions is under investigation. The development of asthma and allergic disease is understood as a complex interaction between environmental influences, genotype (polygenic inheritance pattern) and the immune system, with the early life environment modulating immune responses. Increased exposure of children to indoor pollutants like tobacco smoke, cooking fumes, etc. may be associated with adverse respiratory and allergic health outcomes (von Ehrenstein, 2002).

Thus, from the data presently available, it is most likely that occurrence of allergic disease is multifactorial (Wahn, 2000; O'Connell, 2003; Wright, 2004).

#### **4.2.1 Allergic contact dermatitis**

ACD is an immunological reaction of the epidermis to small molecular weight chemicals being exposed to and absorbed by the skin. Beyond chemicals (including all types of chemical molecules up to a size of approximately 500 Da) no other agents are known to cause ACD. Provision for inducing an ACD is the direct contact of a chemical with the skin, followed by

penetration of the chemical through the *stratum corneum* (i.e. the upper layer of the epidermis consisting of dead cells), towards the viable epidermis, where the immunological cells responsible for triggering the ACD reside; see also Appendix). The role of mechanisms and of specific chemicals is discussed in more detail in Chapter 5.

Physiological factors such as robustness of the *stratum corneum* and the related skin barrier function play a contributing role in the susceptibility of the individual. Little is known about the immunological predisposition specifically for ACD; most of the contributing factors to allergies described earlier apply to ACD. In addition, skin conditions, in particular of the *stratum corneum* (primarily impacting the penetration potential of suspected contact allergens), are a contributing factor to ACD. It is widely accepted that the *stratum corneum* provides an efficient barrier within the skin towards exposure to chemicals. All healthy individuals feature a more or less robust *stratum corneum*. This also applies to mature newborns; their skin is fully developed and features an intact *stratum corneum*, which may be thinner than that in adults, but features a skin barrier function equal to that of adults (Kalia *et al*, 1998; Cunico *et al*, 1977; West *et al*, 1981). Importantly, structural and functional cellular constitution required to build up a mature *stratum corneum* is already present in late pregnancy (Hammarlund and Sedin, 1979; Harpin and Rutter, 1983). Age-dependent studies have shown clear evidence that skin barrier remains virtually constant from infancy to late adulthood (Lévêque *et al*, 1984; Ghadially *et al*, 1995). Trans-epidermal waterloss (TEWL), an indicator for the skin barrier function, is almost equal in babies and infants at the respective body sites. Hence, from a morphological point of view, there is no evidence of increased skin permeability in babies and young infants.

This assumption is confirmed by various *in vivo* as well as *in vitro* studies, which have shown that percutaneous absorption of chemicals was equal in infants relative to adults (Rasmussen, 1978; Wester and Maibach, 1982; McCormack *et al*, 1982).

The reduced overall thickness of neo-natal relative to adult skin (particular of the dermis, including smaller collagen fibre bundles and immature elastic fibres) is often claimed as evidence for an increased ACD risk of infants. However, the reduced thickness of the overall skin is irrelevant for the induction or elicitation of a sensitisation since sensitisation processes are initiated in the upper epidermis and not the dermis. Consequently, the key barrier function lies with the *stratum corneum*, which is functionally equal between babies, infants and adults.

The above considerations apply to intact skin only. Compromised skin may occur at various body sites as a consequence of certain habits and practices of the individual infant, where special attention needs to be given when assessing the exposure of contact allergens to compromised skin or any other skin type with impaired skin barrier function. Additionally, premature babies born before the 36<sup>th</sup> week of gestation have an impaired skin barrier function due to a not yet fully developed *stratum corneum*.

Beyond the skin physiology, relatively little is known about unique predisposition of the immune system of babies and infants and its impact on ACD. Overall, babies and young infants show a lower susceptibility and responsiveness to contact allergens as compared to adults (see 3.2). Although the mechanisms causing this lower responsiveness have not been investigated, some authors believe that the immature immune system may be an explanation for this observation (Felter *et al*, 2002).

### **4.3 Developmental disorders**

#### **4.3.1 Neurodevelopmental disorders**

There are several known causes of adverse neurodevelopmental effects in children. Genetics plays a key role in the aetiology of many neurodevelopmental disorders such as autism (Newschaffer *et al*, 2002) and ADHD (Public Health Policy Advisory Board, 2003). Other factors strongly associated with adverse neurodevelopmental effects include low socio-economic status, low birth weight, maternal alcohol intake, maternal smoking, and maternal therapeutic and illicit drug use (Thadani, 2002; Weiss and Lambert, 2000). Additionally, there is growing evidence that the low serum thyroid hormone, a critical hormone in brain development, during pregnancy may adversely impact the developing nervous system (Zoeller, 2003). Finally, exposure to noise has been suggested as a possible risk factor, (Goehl, 2000) although scant data are available.

##### 4.3.1.1 Attention deficit / hyperactivity disorder (ADHD)

The causes of ADHD are generally regarded as unknown. Although the genetics of ADHD are not clearly defined and appear to be polygenic, family history of ADHD is clearly an important predictor of risk (Faraone and Doyle, 2000). Its neurochemical basis is not fully defined, but it has been suggested that genetic variation in a number of receptor sites for dopaminergic, serotonin, or other neurotransmitter transmission may be a significant contributing factor (Galili-Weisstub and Stegman, 2003; ADDA, 2003).

There is limited information to suggest a chemical aetiology for ADHD, and in the few instances where associations have been suggested, chemical agents have generally been shown to produce one or more neuro-behavioural manifestations associated with ADHD, but not with the complete ADHD presentation as defined by current criteria. The mechanisms of ADHD are not well defined and exacerbation of one or more manifestations of ADHD by environmental factors may well increase the likelihood of obtaining an ADHD diagnosis. Consequently, it is difficult to know whether these agents cause or contribute to the full ADHD spectrum of dysfunction via a common pathophysiology or simply result in exacerbation of selected manifestations of ADHD by an entirely unrelated mechanism.

A wide variety of other factors have been associated with ADHD, although none begin to compare to the significance of family history. This is likely to be the result of genetic factors, but it has been pointed out that the presence of ADHD in the parent(s) or sibling(s) of a child may significantly alter the child's home environment in a variety of ways which may further predispose to attentional or behavioural difficulties. Thus, genetics and environment may be strongly interactive for this complex, multifactorial condition (Schmitz and Mrazek, 2001; Sonuga-Barke *et al*, 2002; Rietveld *et al*, 2003).

Associated factors include various aspects of maternal behaviour and stress, which have recently been reviewed (Linnet *et al*, 2003). These authors state that:

“Twenty-four studies on nicotine (tobacco smoking), nine on alcohol, one on caffeine, and five on psychosocial stress were identified. All were published between 1973 and 2002. In spite of inconsistencies, the studies on nicotine indicated a greater risk of ADHD-related disorders among children whose mothers smoked during pregnancy. Contradictory findings were reported in the alcohol studies, and no conclusion could be reached on the basis of the caffeine study. Results from studies on psychological stress during pregnancy were inconsistent but indicated a possible modest contribution to ADHD symptoms in the offspring. Many studies suffered from methodological shortcomings, such as recall bias, crude or inaccurate exposure assessments, low statistical power, and lack of or insufficient control of confounders. A general lack of information on familial psychopathology also limited the interpretations.”

Additional environmental factors include sibling interactions (Rietveld *et al*, 2003) and infectious conditions such as tuberculous meningitis (Wait *et al*, 2002) or group-B *streptococcus* (Waldrep, 2002). The latter associations are not surprising given the extent of brain injury, which may occur following infection with these organisms.

#### 4.3.1.2 Autism

Heritability (i.e. genetics) is the only known, established risk factor for autism-related disorders (Newschaffer *et al*, 2002). More speculative risk factors include ‘suboptimal’ pregnancy and delivery (e.g. higher maternal age, maternal diabetes, newborn slow to cry), maternal infection in pregnancy, pre-natal medication exposure, early childhood infection, and measles-mumps-rubella (MMR) vaccine. The issue of autism and a possible link with MMR vaccine and the mercury-based vaccine preservative thimerosal has received media attention in the United States and Britain. The Institute of Medicine has examined the evidence regarding autism, the MMR vaccine, and thimerosal-containing vaccines. They concluded that the data did not show an association between MMR vaccine and autism (IOM, 2001a), and neither was there sufficient evidence to determine whether thimerosal was associated with neurodevelopmental disorders such as autism (IOM, 2001b). The IOM evaluations were recently updated to include biological

mechanism studies and several large epidemiological investigations of thimerosal-containing vaccines completed since 2001. Based on this most recent comprehensive review, the IOM concluded that neither the mercury-based vaccine preservative thimerosal nor the MMR vaccine is associated with autism (IOM, 2004). Moreover, the committee concluded that the hypotheses regarding how MMR vaccine and thimerosal could cause autism lack supporting evidence, and that further autism research should be directed toward other lines of inquiry. Overall, there is little evidence to support the role of non-heritable risk factors in the aetiology of autism and related disorders, although many studies have had methodological weaknesses (e.g. small size, not population-based) and have not focused on potentially susceptible subgroups (e.g. genetically predisposed individuals) (Newschaffer *et al*, 2002).

#### 4.3.1.3 Learning deficits

Exposure to lead *in utero* and in early life has been shown to cause a decreased intelligence quotient (IQ), reduced attention, learning disabilities, and behavioural problems (Landrigan *et al*, 2004; Needleman, 1988). However, debate continues regarding the cognitive impact of low blood lead levels (e.g. < 10 µg/dL). Some investigators believe the lead data suggest no threshold (Lanphear *et al*, 2000; Schwartz, 1994) while others express scepticism about the purported impact of lower blood levels due to study limitations (e.g. failure to adequately control for variables such as home environment, poor measurement of parental and child IQ, analytical issues) and interpretation issues (Kaufman, 2001a; Kaufman, 2001b).

High dose poisoning incidents in Japan and Iraq demonstrate the neurotoxic potential of pre-natal exposure to high doses of methylmercury (Landrigan *et al*, 2004; Rice *et al*, 2003; Rogan, 1995). However, the impact of chronic lower level methylmercury exposures is unclear. Three large prospective studies of populations in the Faroe Islands, Seychelles Islands, and New Zealand have examined neurodevelopmental impacts of pre-natal exposure to methylmercury from dietary sources. Findings from the Faroe Islands and New Zealand cohorts suggest subtle adverse neurodevelopmental changes in intelligence, memory, attention and visuospatial deficits, while results from the Seychelles Islands cohort do not (Rice *et al*, 2003). Reasons for the apparent discrepancy in findings are unclear, although investigators are assessing the role of potentially important factors such as PCB exposure in the Faroe Islands cohort (Longnecker *et al*, 2003).

### 4.3.2 Effects on the reproductive system

#### 4.3.2.1 Male reproductive tract abnormalities: hypospadias, cryptorchidism

An IPCS report (IPCS/WHO, 2002) mentions several known risk factors associated with cryptorchidism including ethnicity, positive family history, use of analgesics during pregnancy, birth order and maternal obesity. Several of these are also risk factors for hypospadias. A seasonal effect with peaks for cryptorchidism at different times of the year in various studies has also been reported. Sharpe (2003) reports about increased risks for both disorders in children with low birth weight / intra-uterine growth restriction. Genetic defects are probably relevant for a small proportion of hypospadias, while the role of assisted reproduction technologies for hypospadias is unclear. In a study in three health districts in the UK, North *et al* (2000) found a higher proportion of boys with hypospadias born to mothers, who were vegetarians during pregnancy, as compared to omnivores. When omnivorous pregnant mothers received iron supplementation, the hypospadias risk for the boys was higher than without the supplement. An episode of influenza of the mother in the first trimester of pregnancy was also associated with a higher hypospadias risk in the boys. Sharpe (2003) suggested a link between hypospadias and cryptorchidism, as well as with testis cancer and low sperm counts. Whether this link really exists, is currently unclear. In their study of a large birth cohort in the general population of Rotterdam, Pierik *et al* (2004) observed co-occurrence of hypospadias and cryptorchidism in 2 out of 7292 boys, not significantly higher than expected, but despite the small total number of cases a link cannot be definitely excluded. Pierik *et al* (2004) also reported results of a case-control study nested within this birth cohort on paternal and maternal risk factors for cryptorchidism and hypospadias: associations of suboptimal maternal health, lower maternal education, fathers with both hypospadias and cryptorchidism, of paternal smoking and small-for-gestational age with hypospadias, and of paternal pesticide exposure and pre-term birth with cryptorchidism have been described.

#### 4.3.2.2 Onset of puberty

Among the factors considered responsible for the trend of earlier onset of puberty are improved general health, socio-economic status, and nutrition (Teilmann *et al*, 2002).

The relationship between increased body mass index and earlier onset of puberty in both white and black girls in the US observed by Kaplowitz *et al* (2001) supports the assumption that obesity is an important contributing factor to the earlier onset of puberty in girls.

Persson *et al* (1999) found an association between small (or shortness) for gestational age and earlier puberty in girls, but not in boys.

Karlberg (2002), reviewing the available literature, specifically pointed out three factors that explain much of the variation in the timing of puberty: genetic factors (as implied by studies in twins), nutritional status in childhood, and shortness and thinness at birth.

Recently, Windham *et al* (2004) analysed the age of menarche in relation to maternal use of tobacco, alcohol, coffee, and tea during pregnancy. While maternal pre-natal alcohol and coffee consumption had no relevant effect, daughters of heavy smokers had a reduced mean age at menarche; tea consumption of the mothers was associated with a slight delay of menarche.

#### 4.3.2.3 Effects on testis function with affected sperm quality in adult life

Genetic factors might explain part of the reported geographic variability in sperm parameters of healthy men. According to the IPCS Global Assessment of EDCs (IPCS/WHO, 2002), population characteristics that may influence semen parameters are: occupation, age, previous medications and diseases (e.g. cryptorchidism, sexually transmitted infections), diet (e.g. caffeine, alcohol, soy intake), clothing, smoking habits, stress, fertility, sexual activity, and excessive heat exposure. Ionising radiation and electromagnetic fields as well as environmental chemicals (such as the agrochemical dichlorobromopropane, DCBP) have also been discussed as potential risk factors. Currently, it is not possible to assess the relative contribution of these factors to the reported differences in incidence.

In particular, information on the relevance of exposures during childhood with regard to sperm quality in adult life is lacking. However, if spermatogonial stem cells or Sertoli cells would be irreversibly damaged during childhood, e.g. by radiation or chemotherapy, an effect on adult sperm parameters could be assumed.

Two recent studies emphasise a likely crucial role for maternal smoking: Jensen *et al* (2004) observed a reduction in sperm concentration of 20.1% (95% CI: 6.8, 33.5) and a reduction in total sperm count of 24.5% (95% CI: 9.5, 39.5) in men exposed to smoking *in utero* in comparison with unexposed men. Exposed men also had a smaller testis size (by 1.15 ml; 95% CI: 0.66, 1.64). Interestingly, the effect of *in utero* exposure on semen quality and testis size was also found among non-smoking men; current smoking had no additional effect. Storgaard *et al* (2003) even observed a 48% reduction in semen concentration among sons exposed to maternal smoking *in utero* of more than 10 cigarettes per day. Although authors of both studies could adjust for several confounders, a causal relationship between maternal smoking and sperm quality is not yet proven. However, according to Storgaard *et al* (2003), if this would be confirmed, the global smoking epidemic among women would correspond to a substantial decline in sperm concentration that may contribute to lower fecundity 20 to 30 years later.

## 4.4 Cancer

### 4.4.1 Childhood cancer

As noted in 3.4.1, the relatively low incidence of childhood cancer limits the power of epidemiological investigations to uncover environmental causes. In addition, children generally lack the relatively higher levels of exposure seen in industry, at least in those economies where detailed study of child cancer aetiology is likely to occur.

Childhood cancer in the US was reviewed by the National Cancer Institute SEER programme for the years 1975-1995 (Ries *et al*, 1999). The cause of various cancers was classified as 'known', 'suggestive but not conclusive', 'conflicting evidence', and 'limited evidence'. Additional reviews based upon SEER and other data, have also been published by Linet *et al* (1999, 2003) and by the US EPA Office of Child Health Protection (Altshuler *et al*, 2003b).

With regard to acute lymphocytic leukaemia (ALL), the most common childhood cancer, known risk factors include gender (30% higher in males), race (2-fold higher in white than in black children), socio-economic status (SES) (higher risk in higher SES), pre-natal and post-natal ionising radiation, and a variety of genetic disorders (Down's syndrome, etc.). Given the usual assumption that higher SES reflects access to opportunities for improved environmental conditions, the positive correlation between SES and risk of leukaemia remains perplexing and perhaps suggests that environmental contributions to this condition are limited or non-existent. Suggestive evidence exists for high birth weight, prior maternal history of foetal loss, maternal age >35, and birth order when a mother has several children. Inconsistent or limited evidence exists for maternal smoking, parental occupational exposures, infectious agents, diet, electromagnetic fields (EMF), vitamin K prophylaxis, maternal alcohol use during pregnancy, and post-natal use of chloramphenicol.

Acute myelogenous leukaemia is known to be associated with race (Hispanic), prior chemotherapy, ionising radiation *in utero*, and various genetic conditions. Suggestive relationships exist for maternal alcohol during pregnancy, parental and child exposure to pesticides, and parental benzene exposure. There is limited or inconsistent evidence for maternal use of recreational drugs, exposure to radon, and post-natal chloramphenicol.

Known risk factors for Hodgkin's disease include family history, E-B virus infection (EBV), and SES (higher risk with high SES above age 10, opposite below age 10). More frequent social contacts in early life (more siblings and playmates) are associated with higher risk of young adult lymphoma, again suggesting a possible infectious aetiology. Non-Hodgkin's lymphoma (NHL) is associated with immunodeficiency states. Substantial evidence relates EBV to NHL, while relationship to ionising and non-ionising radiation (including EMF) is inconsistent.

Second to haematologic malignancy in childhood is the occurrence of brain tumours (excluding neuroblastoma). Known risk factors include gender (male are at high risk for some tumour types), therapeutic ionising radiation to the head (for *tinea capitis*), and genetic syndromes (neurofibromatosis, tuberous sclerosis, etc.). Suggestive evidence exists for maternal diet (possibly cured meats), and family history of brain tumour and (particularly in conjunction with syndromic diagnoses) other malignancies. Limited or inconsistent evidence exists for EMF, a variety of chemical compounds or exposures (see 5.4.1), paternal occupation with a variety of materials (see 5.4.1), pest-strips, head injury, and family history of epilepsy or mental retardation. Most of these latter associations have only limited documentation, many have not been found in other studies, and many (especially those dependent upon recall of exposure or family history) may reflect some degree of recall bias.

Neuroblastoma is an embryonal malignancy and displays unique biology, presenting almost exclusively in early life. Only limited evidence exists for causal relationships for this malignancy, including maternal medications, maternal hormone use, birth weight or pre-term delivery, congenital anomalies, and previous spontaneous abortion or foetal death. Weak relationships have been suggested in one study each for alcohol and smoking, but these were not found in later investigations. Paternal exposures to a variety of occupations and exposures have been reported in multiple studies, but the specific associations have not generally been consistent across studies (see 5.4.1).

Retinoblastoma displays a similarly unique biology and early life predominance. This tumour is characterised by a defect in the RB gene, either as a result of parental carriage (familial retinoblastoma) or as a result of new mutation (sporadic), with the latter showing a predominance of transmission from the paternal side. Known risk factors are restricted to family history and deletion of 13q, where the RB gene is located. Limited data suggest relationships to paternal occupations including military, metal manufacturing, welding, machining, or related occupations.

Renal tumours in children consist almost entirely of Wilms' tumour and are known to be associated with a wide variety of syndromic diagnoses, which are known or presumed to reflect a genetic basis. A specific gene locus (WT) has been described. Inconsistent limited evidence exists for high birth weight, parental pesticide exposure (occupational, one study; household insecticides, one study), diagnostic radiation, maternal tea and coffee consumption, maternal hair dye use during pregnancy, maternal medications, and maternal occupation (hairdresser, electronic, clothing, laboratory, dental). Most of these associations have only been reported in one or a few of these studies.

Bone tumours in childhood comprise primarily osteosarcoma and Ewing's sarcoma. Osteosarcoma is known to be associated with prior radiation and chemotherapy for childhood cancers, hereditary conditions, and radium (based on adult data plus known propensity of radium

for bone and known relationship to radiation). Limited or inconsistent evidence suggests growth parameters (height, age of puberty), prior trauma, short birth length, foetal X-ray exposure, parental exposures (see 5.4.1), and fluoride in drinking water. Ewing's sarcoma is almost exclusively a tumour of white children, with a relative risk of 9 for white vs. black children in the US. Limited or inconsistent evidence suggests associations with growth rate, hernia, paternal employment (agriculture), prior poisoning or overdose, and general family history of cancer.

Limited data exist regarding the aetiology of other tumour types in childhood. Hepatoblastoma is known to be associated with genetic disorders, while limited evidence exists for paternal exposures to chemicals (see 5.4.1). Soft tissue sarcoma comprises a group of rare and varied tumours bearing a variety of often-distinct genetic anomalies. Soft tissue sarcoma data are extremely limited beyond the known associations with genetic conditions and an association with congenital anomalies. Limited data suggest SES, *in utero* ionising radiation, and parental recreational drug use may be associated with soft tissue sarcoma. Germ cell tumours (e.g. testicular tumours) tend to occur bi-modally, with tumours occurring either early in life or in later adolescence, often carrying over into early adulthood. Testicular cancer is clearly related to cryptorchidism (undescended testes). Other suggestive but not-conclusive associations include maternal hormone use or conditions believed to reflect high maternal hormone levels (spotting, hyperemesis, etc.), family history of germ cell tumour, hernia, pre-term birth, and trauma. Limited or conflicting evidence exists for infectious agents, high birth weight, pre-natal X-ray exposure, parental occupation (see 5.4.1) and various chromosomal anomalies, especially of the gender chromosomes. Carcinomas and other malignant epithelial tumours in childhood tend to be exceedingly rare and generally occur in adolescence. Risk factors appear to be understood only for thyroid cancer (radiation, female gender) and possibly melanoma (solar radiation), but these risk factors may only explain a small proportion of the cases.

General environmental factors allegedly related to childhood tumours include both EMF (haematological and brain tumours) and automotive traffic patterns. These factors may in fact be inter-related in a complex manner as wiring patterns (EMF exposure) and road traffic patterns may not be independent risk factors. While review of the entire literature on these subjects exceeds the scope of this document, it is fair to say that both issues are controversial and that these factors remain less than fully established as significant contributors to childhood cancer risk.

#### **4.4.2 Adult cancer as a result of childhood exposure**

The difficulties of getting valid information on mothers' exposure during pregnancy and exposure of individuals during childhood combined with any change in exposure during adolescence makes it difficult to evaluate whether environmental causes initiate or modify carcinogenic

processes. Agents definitely related to cancer include exposure to ionising radiation, maternal use of drugs and some lifestyle factors.

An increased risk of thyroid cancer was observed in adolescents who had been exposed to radiation following the Chernobyl accident, especially in children living in areas with iodine deficiencies (Shakhtarin *et al*, 2003). Radiation was also the most important treatment-related risk factor for the development of secondary malignant neoplasms in children treated for cancer. Chemotherapy alone was not associated with an increased risk, but potentiated the carcinogenic effect of radiotherapy (Garwicz *et al*, 2000). Exposure to UV radiation, i.e. excessive exposure to sunlight during childhood, also increases the risk of developing skin cancer, e.g. malignant melanoma (Mancini, 2004).

Maternal smoking during pregnancy has been suggested as a risk factor for testicular cancer in the offspring. But in other retrospective studies, smoking during pregnancy has not been identified as a significant risk factor for this form of cancer (Møller and Skakkebaek, 1996). In a cohort study on sons of women later diagnosed with lung cancer, an increased risk of testicular cancer compared to the background population was observed, the risk being highest in sons of mothers with squamous cell carcinoma. Furthermore, the effect decreased with the interval between the birth of the son and the diagnosis of lung cancer, suggesting that environmental factors rather than genetic factors are the major contributors (Kaijser *et al*, 2003). Pettersson *et al* (2004) found strong geographical and temporal correlations between female smoking and filial testicular cancer in an ecological study in four Scandinavian countries.

There is no clear indication of a link between maternal smoking during pregnancy and increased risk of cancer in their offspring. As these women continue to smoke after delivery it is difficult to define the window of exposure relevant for the disease and, thus, identification of the critical period. An increased level of DNA damage has been observed in foetal tissues from women who smoked during pregnancy (Topinka *et al*, 1997). A slight increase of lung cancer has been reported in the (non-smoking) offspring of smoking mothers (Vineis *et al*, 2004).

Occurrence of cancer at an early age may be an indication of exposure to carcinogenic agents at high doses, but in most studies, there appears to be an effect of genetic components. For example, people with genetic polymorphisms in some xenobiotic metabolising enzymes and/or in DNA repair enzymes, which lead to decreased repair capacity, have an increased risk of developing cancer at an early age. Exposure to smoking at an early age confers a higher risk of lung, bladder, or possibly breast cancer. This could be an effect of exposure during a more sensitive period or just due to an extended length of exposure (dose).

## 4.5 Conclusions

- Childhood health outcomes tend to have multifactorial causes, as far as these causes have been identified, with genetic and family influences often determining health risk to a greater extent than environmental factors. In some instances, genetic-environmental interactions may be extremely important (attention deficit disorder).
- With regards to asthma, it is important to distinguish between primary factors causing asthma (e.g. allergens) and those exacerbating existing asthma. However, this may be difficult. Some chemical agents, even if not allergens, could modulate the ability of other materials to cause asthma. In addition, re-exposure to primary causal factors (i.e. antigens) is recognised to exacerbate established disease.
- Genetic propensity combined with exposure to antigens (mainly proteins) remains the most important determinant of childhood atopic disease.
- Early exposure not only to allergens but also to infectious diseases, immunisations, and other environmental immune stimuli appears to actually reduce the frequency of asthma and atopic diseases ('hygiene hypothesis'). Conversely, lack of environmental exposure to these factors appears to increase the risk of allergic disease.
- Neurodevelopmental disorders in the general population appear to be largely the result of genetic, socio-economic, and lifestyle factors (smoking) as well as important gene-environment interactions (e.g. parents and siblings with ADHD have a profound effect on the child's environment).
- Risk for cryptorchidism appears to be related to a variety of factors, including genetics (i.e. family history), ethnicity, analgesic use during pregnancy, birth order amongst siblings, birth weight, intra-uterine growth retardation, and maternal obesity. Similarly, a variety of factors are associated with hypospadias, including low birth weight, intra-uterine growth retardation, maternal diet and iron consumption, and influenza infection during pregnancy. The relationship between these two outcomes is unclear in spite of some apparent shared associations. To date, evidence for environmental influences independent of gestational issues is extremely limited.
- While earlier onset of puberty, particularly in females, is a recognised trend in the developed world, it is generally believed that this is the result of improved nutrition and health or changes in lifestyle factors.
- Sperm quality varies widely over time and place, with, as noted above, no clearly established global trend. Factors believed to influence sperm quality (count, mobility, morphology) include, in particular, smoking habits of the mothers, but also occupation, age, medications, disease states, dietary factors, clothing, stress, fertility, sexual activity, and heat. While it is clear that high doses of some agents (radiation) may have an effect on sperm quality in adults, at this time it is not possible to establish clear trends or define clear environmental causes of the observed differences, other than smoking habits.
- Genetic factors appear to be highly important in childhood cancer. A role of environmental factors has been established only for radiation (atomic, therapeutic) and chemotherapeutic

agents. It is possible that exceedingly high levels of exposure to electromagnetic fields may also be implicated. Infections may play a role, too, particularly in the occurrence of haematologic malignancy. Childhood exposure to UV radiation from sunlight has been linked to subsequent increased skin cancer in adults.

- A number of factors related to pregnancy and lifestyle (parental smoking, birth weight, gestational illness such as pre-eclampsia) have been associated with alterations in the frequency of adult cancers. Other than the role of therapeutic and atomic radiation exposure, clear data regarding the contribution of other childhood environmental exposures to adult cancer remain extremely limited.

## 5. ROLE OF CHEMICALS

This chapter addresses the possible contributions of specific industrial chemicals to the health effects and health effect trends discussed in the preceding two chapters. The effects of specific industrial chemicals were not integrated into the discussions of Chapter 4, but, where applicable, reviewed in this chapter to provide a specific focus on the role of industrial chemicals with respect to children's health.

As mentioned in Chapter 4, the document does not attempt to address comprehensively the role of ambient air pollutants in acute and chronic respiratory disease. Rather, the document focuses on selected industrial chemical issues for which data are available.

### 5.1 Respiratory diseases / asthma

The literature on asthma and air pollution is voluminous and cannot be reviewed in detail here. Only a few of the many references are provided below, many but not all of which refer to children specifically as opposed to adult asthma. Air pollutant exposure may be indoor or outdoor (or both), and associated pollutants are sulphur oxides (von Mutius *et al*, 1995; Koenig *et al*, 1992; Koenig *et al*, 1990; Koenig *et al*, 1985; Koenig *et al*, 1983), particulates (Sheppard *et al*, 1999; Norris *et al*, 1999; Choudhury *et al*, 1997; Gordian *et al*, 1996; Delfino *et al*, 2004), nitrogen oxides (von Mutius *et al*, 1995), carbon monoxide (Norris *et al*, 1999), and ozone (Koenig *et al*, 1990; Delfino *et al*, 1996). Traffic density and the associated air pollution have also been associated with asthma risk (Zmirou *et al*, 2002) possibly acting via the pollutants mentioned above and/or ultra-fine particles. According to von Ehrenstein (2002), the most consistent associations have been found for particulate matter and ozone. A number of ca. 3350 children with bronchitis symptoms and a number of ca. 4000 children with lung function values below 85% have been estimated as a result of long-term exposure to a PM 2.5 concentration of 10 µg/m<sup>3</sup> above a background level of 10 µg/m<sup>3</sup>. This was calculated within the framework of the Air Quality Guidelines for Europe (WHO, 2000) in a population of 1 million with 200,000 children. Whether ultra-fine particles may be of even higher relevance than PM 10 or PM 2.5 is currently not known. While NO<sub>x</sub> and SO<sub>x</sub> have also been repeatedly associated with upper respiratory effects in children, their impact on asthma and asthma symptoms is much less evident.

The evidence for the contribution of air pollution to exacerbations in children with pre-existing asthma is compelling. However, this does not necessarily mean that such exposures make any contribution to the causation of asthma. An important study (von Mutius *et al*, 1992) compared two genetically similar populations in Eastern and Western Germany (higher industrial pollution (SO<sub>2</sub> / particulates) in Eastern Germany, and higher traffic density (NO<sub>2</sub>) in the Western part). This demonstrated similar lifetime prevalences of asthma, a lower lifetime prevalence of bronchitis and a lower number of allergic disorders in the Eastern German population. These

findings suggest that the factors investigated contribute to exacerbation of disease in the asthmatic population as opposed to serving as a primary cause of asthma. The evidence for the involvement of chemicals other than these gaseous / particulate matter air pollutants in the general environmental setting is rather limited. However, a number of materials, such as isocyanates, are known to be sensitizers. The extent to which most of these agents may contribute to environmentally induced asthma remains unclear.

Volatile organics are known to exist in the environment, particularly in indoor air. An association with asthma has been reported as a result of indoor exposure (Norbäck *et al*, 1995). However, no clear evidence exists which links this general category of agents with exacerbation of asthma, and experiments with asthmatic subjects using chamber exposures at levels higher than those generally expected in the environment failed to demonstrate an effect (Harving *et al*, 1991). Formaldehyde, which may occur in the home environment as a result of off-gassing from construction materials and textiles, has been suggested to increase the risk of respiratory symptoms following childhood exposures (Rumchev *et al*, 2002; Krzyzanowski *et al*, 1990; Norbäck *et al*, 1995). Exacerbations of adult asthma have been found to occur following residential painting and exposure to air concentrations of volatile paint components (2,2,4-trimethyl-1,3-pentanediol-diisobutyrate, aliphatic hydrocarbons, butanols). This was especially noticed after kitchen painting and wood detail painting (Wieslander *et al*, 1997). Limited studies of other air volatiles suggest that elevated volatile organic levels in homes may be associated with infantile eczema (Herbarth *et al*, 2000a) and respiratory infections (bronchitis, which may be difficult to distinguish from asthma) (Herbarth *et al*, 2000b) and that tobacco smoke as well as volatile and other materials associated with household renovation may increase the risk of pulmonary infections in young infants (Diez *et al*, 2000).

While pesticides of various types are commonly used, there is no evidence to date suggesting a role for them in environmental asthma. It has been suggested that DDE exposure (a metabolite of the insecticide DDT) may diminish the asthma-protective effect of breast-feeding (Karmaus *et al*, 2003) and may directly increase asthma risk in childhood (Karmaus *et al*, 2001).

While data are limited, given the role of biological agents such as mice, rats, cockroaches, moulds, and fungi, appropriate use of pesticidal agents in the context of integrated pest management, may well yield reductions in asthma risk (Eggleston, 2001; Gillies *et al*, 1987; Chapman and Wood, 2001; Carswell *et al*, 1996). The use of control acaricides has been shown to reduce mite dust content in homes (Marks *et al*, 1994; Chew *et al*, 1995; Chang *et al*, 1996; Dietemann *et al*, 1993) and to reduce asthmatic manifestations in children (Hide *et al*, 1996; Bahir *et al*, 1997; Geller-Bernstein *et al*, 1995; Ehnert *et al*, 1992) in many, but not all cases (Carswell *et al*, 1996; meta-analysis of Gøtzsche *et al*, 1998). Cockroach antigen reduction has also been reported using pesticides, whereas household cleaning measures were ineffective at reducing antigen levels (Chapman and Wood, 2001).

Odour has also been postulated as a trigger for asthmatic symptoms. Review of the literature, virtually all of which involves adult asthma, indicates that individuals with allergies (hay fever) or asthma commonly report respiratory complaints or symptoms in response to odours (Baldwin *et al*, 1999; Eriksson *et al*, 1987; Shim and Williams, 1986). While some of the stimuli might generally be considered noxious or unpleasant (cigarette odour, paint), other reported stimuli are generally regarded as benign or often pleasant (trees, flowers). There is a single paper (Shim and Williams, 1986), which measures pulmonary response in four asthmatics reporting the occurrence of odour-triggered symptoms. All four individuals tested showed a decline in forced-expiratory volume at one second following exposure to cologne, and a preventive effect was noted with inhaled metoprolol in three out of the four individuals. While the choice of cologne is convenient, symptom reports suggest that a wide variety of stimuli may induce symptoms, and there is no reason to believe that perfumes and colognes are the only, or even the most important, odour stimuli affecting respiratory functions. A critical question is whether these responses are organically based, or psychosomatic, or result from some combination of both these factors. The potential pathophysiological mechanisms for odour triggered respiratory illness have been reviewed in the context of indoor air pollution (Cone and Schusterman, 1991). In many instances, odour stimuli cannot be assessed fully independently of potential airway irritants. To further complicate matters, limited literature suggests an interaction between irritant and odorant stimuli with reduced temporal attenuation of odour sensation, even when odour and irritant stimuli were presented to opposite nostrils (Cone and Schusterman, 1991). While this review acknowledges the various possible mechanisms of odour-triggered illness, it does not assist in determining which mechanisms are important, and under what circumstances, thus the relative impact of odour on children vs. adults remains undefined.

The US National Academy of Sciences Institute of Medicine reviewed at length agents that may be specifically responsible for causing asthma or for exacerbating existing asthma (IOM, 2003). As discussed in 3.1, strong evidence of contribution to the development of asthma is essentially limited to the role of protein antigens (mite, cat, etc.) and environmental tobacco smoke. A variety of evidence, much of it cited above, connects the indoor exposure to industrial chemicals with asthma. However, the IOM considered the evidence related to development of asthma following exposure to nitrogen oxides, formaldehyde, fragrances, plasticisers, VOCs and pesticides to be “inadequate or insufficient... to determine whether or not an association exists.” Similarly, while exacerbation of asthma was associated with protein antigens, tobacco smoke (in young children) and nitrogen oxides, evidence for the role of other chemicals was again ‘inadequate or insufficient’.

Finally, there has been recent attention to the use, particularly indoors, of solid fuels for cooking and heating purposes. A recent World Health Organization document (WHO, 2004) indicates that solid fuel use may be a major contributor to both indoor and outdoor air pollution. It is apparent that the location (indoor vs. outdoor), proximity, and specific combustion conditions will

determine the magnitude and relative contributions to indoor vs. outdoor air. Not surprisingly, indoor use of solid fuels may be particularly problematic. Further, the impact of indoor solid-fuels related air pollution falls disproportionately on women and children, who spend more time in the household and are more directly involved or more proximate to cooking activities. Concerning children and respiratory tract disease, the document concludes that the evidence for a causal relationship is strong for acute lower respiratory infection in children less than five years of age and is moderate for asthma in children age 5-15 years and for asthma, and tuberculosis in all age groups above age 15.

Currently, if expressed as a proportion of the total number of cases, environmental risks for asthma appear to result mainly from exposure to biological agents (i.e. protein antigens), from lifestyle factors within the home (smoking, gas appliances), and from general gaseous air pollutants and particulates in the outdoor environment resulting from local traffic and other sources. Some evidence exists for a contribution from a limited number of volatile organic substances (formaldehyde and fragrances) within the home environment. The role of other chemical substances in the development and exacerbation of asthma or other respiratory diseases remains to be defined: but data strongly suggest that, globally, the contribution of indoor use of solid fuels may be a major cause of respiratory disease, primarily in rural regions or developing nations. Chemical pesticides, properly used as a component of comprehensive pest management and antigen control measures may in fact offer benefits in controlling asthmatic exacerbations and may reduce the prevalence and severity of chronic childhood and adult asthmatic conditions.

## **5.2 Allergies**

The vast majority of allergic reactions in children are provoked by naturally occurring protein-containing substances, e.g. pollen, house dust, mites, moulds, pets, food stuff. The number of children affected by allergies to man-made chemicals is believed to be much lower. In adults these allergies are often related to sensitisation by lifestyle (e.g. nickel allergy), professional contact (e.g. hairdressers, metalworkers, painters, builders, nurses, chemical industry workers), use of cosmetics or drug treatment.

Despite the impressive extent of research conducted, data linking specific chemical exposures with allergies/atopic diseases in children are sparse. Individual cases of allergic reactions to chemicals (e.g. contact allergies) are published in the scientific literature, but convincing evidence for an impact of chemicals for an increase of allergies in the general population or for a special sensitivity of children cannot be deduced from these publications. Most publications on chemicals in this context deal with effects of air pollution in relation to asthma, or with immunotoxicity of chemicals in animals. The number of specific industrial chemicals included in investigations is small and comprises mainly of persistent organic chemicals (Tryphonas, 1998; IPCS/WHO, 2002;

Damstra, 2002; Kimbrough and Krouskas, 2001). Effects of pre-natal / peri-natal exposure to immunotoxic chemicals (e.g. background levels of PCBs / dioxins) might be associated with a greater susceptibility to infectious diseases and, thus, may even speculatively prevent the development of allergic diseases (Weisglas-Kuperus *et al*, 2000; Weisglas-Kuperus, 2001).

Within the frame of the ISAAC study (1998) a positive correlation between self-reported traffic burden of 13-14 year old school children and asthma and allergic rhinoconjunctivitis were reported for the city of Münster in Germany. In Japanese studies, respiratory allergic symptoms were more prominent when affected persons lived in areas with heavy road traffic, though these studies were criticised for lack of appropriate controls of confounding factors. A further German study revealed an increased sensitisation rate towards pollen (tested by radio allergen sorbent test, RAST) in children of a large city being outdoors for more than one hour a day. However, in another study, an increased sensitisation rate in relation to heavily used roads was not evident. Prevalence of hay fever and allergies in 5-14 year old children was significantly increased in air polluted areas of Lower Saxony (Germany) in comparison to control areas (all as cited in Wahn and Wichmann, 2000). Convincing evidence for a link between development of new cases of allergies and air pollutants has, to date, not been shown, except possibly for subgroups of children exposed to truck traffic (Nicolai 2002).

The EU Scientific Committee for Food (SCF) concluded that food additives only rarely cause allergies, which are immunological responses (EU Scientific Committee for Food, 1997). However, food additives are more commonly involved in non-immunologically based food intolerance. Adverse reactions (due to intolerance to food additives) have been recorded with tartrazine and non-azo colours, monosodium glutamate, benzoates and several other additives. The SCF estimates that the true incidence of food allergy is low and usually below 1% of the population.

Sulphite is a special case, now included in the list of food ingredients to be declared in foodstuffs as allergens (within the EU). Sulphite may induce asthma attacks in asthmatic persons and occasionally skin reactions in others (delayed eczema, instant urticaria) (EU Scientific Committee for Food, 1997).

The nature of the relationship between the presence of man-made specific industrial chemicals in the environment and the evidently increased prevalence of allergic diseases and atopic dermatitis remains unclear. The underlying cause for development of allergies/atopic diseases and a shift to more type Th2 like immune reactions is not yet known, and within the context of the 'hygiene hypothesis', IgE-mediated allergies appear to be more closely related to the prosperous lifestyle of the developed world than to exposure to specific man-made chemicals.

### 5.2.1 Allergic contact dermatitis

ACD is caused by low molecular weight chemicals (naturally occurring as well as man-made chemicals), which need to come into direct contact with the skin, followed by penetration of the chemical through the *stratum corneum* into the viable epidermis. Only chemicals, and no other agents, are known to cause ACD.

The most prominent contact allergens are metals (e.g. nickel, cobalt), pesticides and preservatives. Naturally occurring contact allergens include perfume ingredients or plant ingredients, like in poison ivy.

Due to the provision that contact allergens need to penetrate the skin to induce ACD, typical contact allergens need to fulfil certain molecular criteria, like size and lipophilicity, enabling them to penetrate the skin sufficiently fast. It is widely accepted that potent contact allergens are usually smaller than 500 Da (ideally <300 Da) and amphiphilic (ideally with an octanol-water partition coefficient not less than -2 and not greater than +4). Those physico-chemical properties allow the chemical to penetrate into the two dermal layers, i.e. both the more lipophilic *stratum corneum* and the more hydrophilic epidermis. Beyond those physico-chemical properties, the chemical (peptide) reactivity in the epidermis is of key relevance for its overall skin sensitisation potential. Chemicals do not need to be direct allergens; they can also serve as pro-haptens being converted to the actual contact allergen by the skin metabolism.

It is well accepted that chemicals larger than 500 Da hardly penetrate the skin. Polymers for example are of no concern as skin sensitisers. The exception is exposure to severely compromised skin lacking skin barrier properties (e.g. open wounds).

Much less is known with regard to the molecular substructures driving the skin sensitisation potential. Current chemical structures known to be associated with a contact allergen risk are the so-called Michael acceptor (a carbonyl group to an  $\alpha$ - $\beta$  c-double bond) or 1,2- or 1,3-di-ketones, as well as all other chemicals featuring a particular reactivity (like alkylating agents). Currently, many research groups are working on characterising further chemical reactivity patterns required to become a potent contact allergen.

Not all contact allergens are equally potent, but vary from weak sensitisation potential to strong and very potent skin sensitisers. This quantification is of particular importance for the risk assessment process in order to safely formulate known skin sensitisers in products. Here, an important aspect is that not the total dose, but the dose per unit area (concentration), usually expressed in  $\mu\text{g}/\text{cm}^2$ , is relevant. It is also important to highlight that potency of a skin sensitiser does not necessarily correlate with its risk, i.e. the prevalence of contact allergy to this specific compound in the population. The level and frequency of exposure to the chemical is important.

For example, one of the most potent sensitisers ([5-chloro] 2-methyl-4-isothiazolin-3-one, a preservative) shows limited sensitisation prevalence in the public. In contrast, the contact allergen showing the highest sensitisation prevalence in Western Europe and North America, nickel, has just a very weak sensitisation potential. The high nickel sensitisation incidence is driven by the high exposure to this chemical, particularly by poor quality jewellery releasing high amounts of nickel during wearing.

### **5.3 Developmental disorders**

#### **5.3.1 Neurodevelopmental disorders**

The developing nervous system may be more sensitive to environmental insult than that of adults (National Research Council, 1993). The complexity of the developing nervous system and the critical stages in the development process are often cited for why children should be assumed more vulnerable to neurotoxic agents (Altshuler *et al*, 2003a; Rice and Barone, 2000; Rodier, 1995). Tilson (1995) reviewed the evidence regarding the assumption of increased neurodevelopmental sensitivity of children and concluded that sufficient evidence exists to support it. On the other hand, he also noted that several investigators have challenged this and disagree with that assumption based on the adaptability, resiliency and compensatory mechanisms of the developing nervous system. Additionally, there are certain agents (such as carbon monoxide) where children are neurologically less sensitive to the development of permanent sequellae compared with adults.

Data on potential chemical agents and their influence on neurological development are limited (Altshuler *et al*, 2003a; Weiss and Lambert, 2000). Assessing the independent effect of chemical exposures is particularly challenging because many neurodevelopmental endpoints are impacted by non-chemical factors such as genetic background, socio-economic status, alcohol and drug abuse, and family environment. When chemical and non-chemical exposures occur simultaneously (as they often do) separating effects is difficult (Altshuler *et al*, 2003b). Some investigators believe that future studies should consider genetic background, socio-economic status, etc. as potential effect modifiers rather than confounding factors (Weiss and Lambert, 2000).

Several organisations and investigators have attempted to estimate the percentage of neurodevelopmental disorders potentially related to environmental contaminant exposures. The US National Research Council estimates that approximately 3% of developmental disabilities, which includes structural abnormalities (e.g. neural tube and heart defects), growth retardation (e.g. low birth weight), functional deficits (e.g. mental retardation), and pre- and post-natal deaths, are related to exposure to neurotoxic chemical agents (e.g. lead, mercury) and physical (e.g. radiation) agents in the environment (National Research Council, 2000a). Further, the

combination of genetic susceptibility and exposure to a broad range of environmental factors such as infections, nutritional deficiencies and excesses, lifestyle factors (e.g. alcohol, diet, tobacco, chemicals), radiation, manufactured chemicals (e.g. pharmaceuticals, synthetic chemicals, solvents, pesticides, cosmetics, and food additives) and natural chemicals (e.g. plant and animal toxins) may account for approximately 25% of neurodevelopmental disorders. Landrigan *et al* (2002) estimate that 10% (range 5-20%) of neurodevelopmental disorders in US children are at least partly caused by toxic exposures, not including alcohol, tobacco, or drug abuse; however, the authors provide little detail as to how this estimate was derived other than to say that the estimate is based on the aforementioned NRC analysis.

#### 5.3.1.1. Attention deficit / hyperactivity disorder (ADHD)

There is no known association of any environmental chemical exposure (excluding personal habits like smoking and maternal alcohol consumption during pregnancy) with well-defined ADHD. However, it does appear that a number of chronic environmental exposures may influence attention and behaviour in a manner, which might mimic or exacerbate ADHD, or possibly even cause the full ADHD spectrum of dysfunction.

The best-documented example of such an environmental agent is lead, which is associated in various studies not only with impaired cognitive function, but also with attention deficits and with impulsive or aggressive behaviour (IFCS, 2003). Methylmercury exposure has also been associated with a complex neurological syndrome, which with high exposure (Minimata Bay example) can include overt mental retardation and cerebral palsy (IFCS, 2003). Less severe manifestations appear to include deficits of attention in the context of other cognitive changes. Similarly, PCB exposure has been associated with a variety of neurological deficits at exceedingly high levels of exposure. Effects at lower doses are less well documented, but alterations of attention are reported in some but not all studies, with additional studies suggesting that early childhood effects may not persist (IFCS, 2003).

Evidence for a contribution of general environmental chemicals to ADHD beyond these now-classic examples is extremely limited. Isolated case reports suggest possible associations with manganese in drinking water (Woolf *et al*, 2002) and with DDT exposure (Hardell *et al*, 2002), but given the high prevalence of ADHD, little can be concluded on the basis of these isolated reports. While other case reports or hypothesised associations can undoubtedly be found within the medical literature for ADHD specifically or for various findings associated with ADHD, the authors of this report have been unable to locate evidence of other widely accepted or strongly supported environmental chemical aetiologies for ADHD.

### 5.3.1.2 Autism

There is little epidemiologic evidence to support a relation between specific post-natal chemical exposures and autistic-related disorders (Newschaffer *et al*, 2002). Recent debate has focused on vaccines containing the mercury-based preservative thimerosal and potential increased risk of autistic-related disorders. Thimerosal has been used as a preservative in some vaccines and pharmaceuticals since the 1930s. The Institute of Medicine recently completed a comprehensive review of clinical, biological mechanism and epidemiological investigations of thimerosal-containing vaccines and concluded that the mercury-based vaccine preservative is not associated with autism (IOM, 2004). Moreover, the committee concluded that the hypotheses regarding how thimerosal could cause autism lack supporting evidence, and that further autism research should be directed toward other lines of inquiry.

### 5.3.1.3 Learning deficits

Accidental ingestion of contaminated rice oil in Japan and Taiwan show that high dose PCB exposures adversely impact the developing nervous system (Kimbrough *et al*, 2001; Mendola *et al*, 2002; Rogan, 1995). Studies of cohorts exposed to more environmentally relevant PCB concentrations have reported impaired memory, learning and attention-related deficits associated with pre-natal or early post-natal PCB exposure. Several authors suggest that differences across PCB studies (e.g. exposure, study designs, potential confounders) do not support a causal interpretation (Kimbrough *et al*, 2001; Schell *et al*, 2001; Winneke *et al*, 2002) although others view the data as indicating a relationship (Mendola *et al*, 2002). Similar reports of neurodevelopmental delay and impairment related to peri-natal dioxin exposure has generated the hypothesis that PCBs and dioxins may exert adverse neurodevelopment effects via disruption / alteration of thyroid hormone activity during pregnancy (Winneke *et al*, 2002; Zoeller, 2003). A similar mechanism has also been hypothesised for perchlorate (Zoeller, 2003), although there is little evidence to suggest neurodevelopmental effects at environmentally relevant concentrations. It should be noted that most testing methods are designed to detect alterations in thyroid hormone levels, not interference with thyroid hormone actions at the receptor (Zoeller, 2003).

The role of pesticides in adverse neurodevelopmental effects in children is of considerable media and scientific interest. However, while some existing animal data suggest certain classes of pesticides may be associated with adverse neurodevelopmental effects (Eriksson and Talts, 2000; Slotkin, 1999), there are scant epidemiology data on children peri-natally or post-natally exposed to these chemicals. Pre-natal exposure to polybrominated diphenylethers (PBDEs – flame retardants) has also been suggested to increase risk of cognitive impairment (McDonald, 2002).

One positive trend of note is that body burdens for many of the industrial chemicals mentioned above have been declining over time. For example, significant reductions in levels of organochlorine compounds in breast milk have been observed over the past two decades (Norén and Meironyté, 2000), PCBs in breast milk (Norén *et al*, 1996), and in most environmental media (Gunderson, 1995). Additionally, concentrations of dioxin taken from human tissue samples of residents from Germany, France, the US, and Canada showed that exposure has declined by more than 95% since 1972 (Aylward and Hayes, 2002). Similarly, dioxin concentrations in breast milk have declined approximately 50% since 1980 in Germany, Norway, and the Netherlands. (LaKind *et al*, 2001). In contrast, levels of polybrominated diphenylether flame retardants in breast milk increased in Sweden from 1970 to 1990, but have been decreasing since then (Norén and Meironyté, 2000).

### 5.3.2 Effects on the reproductive system

It has been hypothesised that so-called endocrine disrupters could exert adverse effects on the reproductive system in humans.

The working definition for endocrine disrupters that was adopted by the OECD at a workshop sponsored by the EU, WHO, and OECD in Weybridge, UK in December 1996 is: “An endocrine disrupting chemical (EDC) is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function”.

Existing epidemiological data on the reproductive system are rarely related to specific EDCs. There are, however, some studies in children with effects on the reproductive system that were exposed pre-natally to the therapeutic use of diethylstilbestrol (DES) (see below).

#### 5.3.2.1 Male reproductive tract abnormalities: hypospadias, cryptorchidism

In humans, the induction of reproductive tract abnormalities in sons of DES-exposed mothers has been well documented, but pre-natal DES exposure was not associated with hypospadias (EEA/WHO, 2002). The WHO publication did not take into account a study by Klip *et al* published in 2002, which reported a 20-fold increase in incidence of hypospadias in boys born to mothers, who had also been exposed to DES *in utero*. However, this increase is based on 4 cases only with mothers reporting *in utero* exposure, and information / selection bias could have occurred.

A Mayo Clinic cohort study compared 660 males born to women who had taken DES at some time during pregnancy to 592 men unexposed to DES by this route. There was a slight, but not

significant disparity between the DES-exposed and unexposed men in the percentage of reported cryptorchidism (Strohsnitter *et al*, 2001). Sharpe (2003) comments on the different results with regard to extraordinarily high DES exposure over a longer period of time on the one hand, and brief and single exposure to high levels during hormone administration on the other hand. He suggests that only exposure to exceedingly high levels of oestrogens possibly poses a major risk to male reproductive tract development.

A meta-analysis of 14 well-selected human studies on the influence of exogenous hormones has not produced any convincing evidence of an effect of pre-natal exposure (Raman-Wilms *et al*, 1995).

A recent publication by Vrijheid *et al* (2003) with a crude exposure classification found little evidence for a relation between risk of hypospadias and maternal occupation or occupational exposure to potential endocrine disrupters.

Pierik *et al* (2004) recently reported an association of paternal pesticide exposure and cryptorchidism. However, this observation is based on only 5-6 exposed cases; paternal pesticide exposure was qualitatively assessed by self-reports or job exposure matrix, both based on retrospective information mostly given by the mothers. Selection and response bias cannot be ruled out. The exposure classifications of pesticides were too broad to allow identification of specific (groups of) chemical agents to be held responsible for the increased risk observed.

Thus, the conclusion of the Committee on Hormonally Active Agents in the Environment (National Research Council, 2000b) still holds that the reported increases in the incidence of male reproductive disorders could not be linked to exposures to environmental hormonally active agents at this time.

#### 5.3.2.2 Onset of puberty

Review of the literature has not established a relationship between precocious puberty mediated by the central nervous system and environmental agents (IPCS/WHO, 2002). Associations of precocious puberty mediated by hormonal receptors in peripheral tissues have been discussed for DES, estradiol, and mestranol, DDE, and PBBs exposure with conclusions limited by weak exposure assessment or selection bias.

A Puerto Rican study (Colón *et al*, 2000) reported an association between premature breast development and phthalate esters based on the detection of diesters of phthalates in the serum. However, phthalate diesters are normally metabolised to monoesters before absorption, thus, the detection of phthalate diesters would not be expected (IPCS/WHO, 2002).

### 5.3.2.3 Effects on testis function with affected sperm quality in adult life

An important study assessing the effect of pre-natal exposure to oestrogen on semen quality has been performed by Storgaard *et al* (2002). The investigators studied sperm count in twins and single brothers to see if twin brothers have lower sperm counts and if the lowest values are for dizygotic twins, as the concentration of free oestrogens in plasma is much greater in twin pregnancies from the first week of gestation, and greater for dizygotic than for monozygotic twins. The authors conclude that higher pre-natal concentrations of oestrogens were not related to reduced sperm counts in adulthood and comment that both the concentration and potency of oestrogens during pregnancy with twins are greater than for most environmental oestrogens.

Testis function in men, who have been exposed *in utero* to the oestrogen DES administered to their mothers in early pregnancy, did not show a clear impairment (IPCS/WHO, 2002). This underlines that it is highly unlikely that environmental exposure to substances with a considerably lower oestrogenic potency could affect sperm counts via an oestrogen-related mechanism.

Hauser *et al* (2002) reported data which are suggestive of an association between serum concentrations of PCBs and p,p-DDE and semen parameters, while Duty *et al* (2003) described a relation between urinary levels of some phthalate metabolites and semen parameters. The authors of both cross-sectional studies emphasise the need for further studies on these preliminary associations before any conclusions can be drawn.

However, as already pointed out by the National Research Council (2000b), determining the risk of specific environmental hormonally active agents is difficult, because effects that might be attributed to background concentrations could be complicated by endogenous hormones, pharmacologic oestrogens, and naturally occurring hormonally active agents that are ubiquitous in the environment.

## 5.4 Cancer

### 5.4.1 Childhood cancer

In general, the strongest causal links in childhood cancers beyond demographic factors (age, gender, race, socio-economic status) relate to genetic disorders, hereditary factors, or family history of cancer. A review of the SEER data (Ries *et al*, 1999) and subsequent reviews (Altshuler *et al*, 2003b; Linet *et al*, 1999; Linet *et al*, 2003) demonstrate that only few well-established relationships exist between environmental factors and childhood cancers, the best recognised being ionising radiation.

Causal relationships of chemicals (excluding radioactive materials) with childhood cancers within the SEER data set can be summarised, as in table 5-1:

**Table 5-1: Environmental factors reported to be associated with specific childhood cancers, adapted from SEER data (from: Ries et al, 1999)**

Cancer type(s)	Chemical Agent(s) / Factor	Level of Association	Comment
Acute leukaemia	Smoking	Limited/inconsistent	
	Parental occupation	Limited/inconsistent	Motor vehicle exhaust, hydrocarbons, paints
	Vit. K prophylaxis	Limited/inconsistent	Refuted by later studies
	Alcohol	Limited/inconsistent	(Weak, see AML)
	Chloramphenicol	Limited/inconsistent	
AML (Acute myelogenous leukaemia)	Chemotherapy	Known	Alkylators and epipodophyllotoxins
	Alcohol	Suggestive	Maternal use in pregnancy
	Pesticide (parent)	Limited/inconsistent	
	Pesticide (child)	Limited/inconsistent	
	Benzene (parent)	Limited/inconsistent	
	Recreational drug (parent)	Limited/inconsistent	
	Chloramphenicol	Suggestive	
Hodgkin's disease	None		
NHL (non-Hodgkin's lymphoma)	None		
CNS (central nervous system)	Various products	Limited/inconsistent	Antihistamines, beer, incense, make-up, diuretics, rubber nipples and pacifiers.
	Paternal occupation	Limited/inconsistent	Aircraft, agriculture, electronics, petroleum, painter, paper or pulp mill, printer, metal related occupation, paint, ionising radiation, solvents, EMF.
	Pest strips	Limited/inconsistent	
Neuroblastoma	Maternal medications or hormones	Limited/inconsistent	Amphetamines, diuretics, tranquilisers, muscle relaxants, anti-fungals, phenytoin.
	Alcohol	Limited/inconsistent	
	Tobacco	Limited/inconsistent	
	Paternal exposures	Limited/inconsistent	Electronics, agriculture, packaging and materials handling, EMF, pesticides, hydrocarbons, dusts, rubber, paint, radiation.

**Table 5-1: Environmental factors reported to be associated with specific childhood cancers, adapted from SEER data (from: Ries et al, 1999) (cont'd)**

Cancer type(s)	Chemical Agent(s) / Factor	Level of Association	Comment
Retinoblastoma	Paternal occupation	Limited/inconsistent	Military, metalworking, welder, machinist, or related occupation.
Wilms' tumour	Pesticides (parental)	Limited/inconsistent	Hairdresser, electronic, clothing, laboratory, dental
	Pesticides (child)	Limited/inconsistent	
	Coffee, tea (maternal)	Limited/inconsistent	
	Maternal hair dye	Limited/inconsistent	
	Maternal occupation	Limited/inconsistent	
Hepatoblastoma	Parental exposure	Limited/inconsistent	Metals, petroleum products, paints and pigments.
Osteosarcoma	Prior chemotherapy	Known	Alkylating agents (and radiation)
	Parental exposure	Limited/inconsistent	Chicken farming, fertiliser, herbicides, pesticides.
	Fluoride (water)	Limited/inconsistent	
Ewing's sarcoma	Paternal occupation	Limited/inconsistent	Agriculture
	Prior poisoning or overdose (any)	Limited/inconsistent	
Soft tissue sarcoma	Recreational drugs (parental)	Limited/inconsistent	
Germ cell tumour	Hormonal agents	Known	
	Parental occupation	Limited/inconsistent	Maternal - medical, solvents, plastic and resin fumes. Paternal - service station, aircraft, X-rays.

While the authors of this report are aware that this table neither cites all postulated associations nor includes all studies to date, few if any of the limited/inconsistent associations have risen above this level of certainty in the past several years. The more recent review for US EPA (Altshuler *et al*, 2003b), for example, cites only one additional association with intra-uterine exposure, a single publication linking exposure to trichloroethylene, tetrachloroethylene and styrene-acrylonitrile trimer to ALL (acute lymphocytic leukaemia) and central nervous system tumours in girls below age five.

Certain trends are evident:

- All known chemical associations are with therapeutic use of drugs, not with broader environmental exposure. The drugs include alkylating agents and other known mutagenic

agents whose relationship to cancer risk is certainly unsurprising given that they were administered in doses intended to produce substantial biological effects. The remaining associations are primarily with hormonal agents which were also used at therapeutic doses and which are known to regulate the development of the organs for which tumour outcomes are observed.

- All remaining associations with industrial chemicals and occupational exposures are classified as having limited or inconsistent data by the NCI SEER programme and by other reviewers cited above.
- Associations with childhood exposures (as opposed to parental exposures) are extremely limited. These consist of therapeutic agents used in pharmacologic doses (chemotherapeutics, chloramphenicol) and extremely sparse data on childhood pesticide exposure and fluoride in water.
- The various associations with parental occupation or exposure have been largely the result of multiple-comparison studies looking at a broad range of materials and professions. Findings may be due to multiple-hypothesis testing alone or to recall or other bias (Linet et al, 2003). In most cases, the findings have not been tested in repeat studies or have been refuted in one or more other studies of the same hypothesis.

In short, while many associations with childhood cancers have been reported in individual studies, no convincing, widely accepted examples of causal relationships to environmental, non-pharmacologic exposures have been identified by the authors of this report. Known causal relationships are primarily related to genetic, familial, or demographic factors and ionising radiation. Further research may indeed reveal additional postulated associations or may strengthen existing hypotheses. It can currently be concluded that only individually identifiable environmental chemical exposures play a prominent role in the aetiology of childhood malignancy in the broad population studied.

Finally, it is worthwhile noting that while there seems to be a general perception that food-borne residues of pesticides and other agricultural chemicals are making a major contribution to the risk of childhood cancer, such beliefs remain unsubstantiated by either the highly conservative risk assessments underlying pesticide regulation or by the existence of any supporting epidemiologic data, despite the many studies carried out so far.

#### **5.4.2 Adult cancer as a result of childhood exposure**

Due to the lack of complete information it is difficult to determine whether chemical exposure during childhood is associated with increased cancer risk in adulthood. Most of the information on potential associations has been attributed to lifestyle factors, smoking and treatment with drugs during pregnancy or youth, or has been obtained from laboratory animal studies.

The classical example of peri-natal carcinogenesis in humans is the development of clear-cell adenocarcinoma of the vagina in the female offspring of women treated with diethylstilbestrol (DES) during pregnancy (Herbst *et al*, 1971). To date, men exposed to DES *in utero* do not appear to have an increased risk of most cancers, and it remains uncertain whether pre-natal DES exposure is associated with testicular cancer (Strohsnitter *et al*, 2001).

Parental occupational exposure has occasionally but inconsistently been associated with an increased risk of testicular cancer in young men, but no specific compounds have been implicated in the aetiology. In one study, mothers of men, who developed testicular cancer, were reported to have a significantly increased concentration of the sum of PCBs and HCB, and the sum of chlordanes, both compared to the control mothers (Hardell *et al*, 2003). In another study, an excess risk of seminomas was seen in young men born to fathers employed in the manufacturing industries and mothers in health related occupations (Kardaun *et al*, 1991).

Consequently, there is limited information on pre-natal events in man that could contribute to the occurrence of cancer as a consequence of either the direct exposure of embryonic or foetal cells to a carcinogenic agent or a prezygotic exposure of the germ cells of one or both parents to a carcinogen before mating. However, multigeneration effects of carcinogens have been reported in laboratory animal studies. The types of observed tumours were diverse and depended on the carcinogen used and the animal species. Treatment was mainly on the pregnant mother, but for some carcinogens an effect was also seen when treatment took place before mating (Anderson *et al*, 2000).

## 5.5 Conclusions

- Primary chemical contributors to exacerbation of childhood asthma appear to be ambient air pollutants (particulates, NO<sub>x</sub>, SO<sub>x</sub>, ozone, etc.) and lifestyle related indoor air pollutants such as environmental tobacco smoke. Some evidence suggests that volatile organic chemicals may play a role in the exacerbation of childhood asthma, primarily as a result of airway irritant effects.
- Almost invariably, proteins, not environmental industrial chemicals, are the primary allergens responsible for asthma, aero-allergy, and food allergy. Nickel is probably the most common allergen for chronic skin sensitisation in the general public, and often occurs in jewellery or other accessories designed to have repeated and/or prolonged skin contact.
- An association with neurodevelopmental disorders has been reported for exposure to lead, mercury, and PCBs. The relationship of these chemicals to neurobehavioural changes at typical environmental levels has not been well established, except for lead exposure. These materials produce various neuropsychological effects, which are also seen with other neurodevelopmental conditions, but have not been clearly shown to contribute to classically defined neurodevelopmental conditions such as ADHD and autism.

- Estimates of the overall contribution of environmental chemical exposures (including drugs, alcohol, and tobacco) to neurodevelopmental disabilities vary widely, from 3 - 25% of total disabilities, the latter estimate incorporating assumptions regarding gene-environment interactions, which are not substantiated by scientific research. Such estimates are based on multiple assumptions and cannot be regarded as accurate, nor do they help to identify specific causal agents.
- There is no documented evidence about the role of industrial chemical exposure in producing alterations in reproductive and endocrine function in children, despite the extensive research that has been done in this area. The only clear trend identified, earlier onset of puberty, appears to be due to improved health and nutrition or changes in lifestyle choices. While the ability of reproductive tissues and processes to be affected by high-level hormonal stimuli is established, the actual contribution of lower-level exposure to endocrine agents to reproductive alterations in intact, homeostatic organisms is unclear.
- There is no evidence that environmental chemicals other than chemotherapeutic agents used in the treatment of initial malignancies play any significant role in the aetiology of child cancers.
- The role of juvenile exposures to industrial chemicals in the causation of adult onset of cancers over and above the risk apparent in adult exposures has not been established. Biological reasoning and animal data suggest that children may have a higher susceptibility to some types of carcinogens, but the actual impact will depend on both patterns of exposure and on the specific biological properties of the chemical in question.

## 6. ASSESSING RISKS TO CHILDREN

Over the past decade, an enormous effort has been invested in updating toxicity testing guideline protocols, revising current testing strategies, and developing new models for hazard and exposure assessment in order to ensure that children's health is adequately addressed in the risk assessment process. This work continues today as new guideline protocols to evaluate potential data gaps, such as developmental neurotoxicity and endocrine effects, are subject to international review and/or multi-laboratory validation programmes and as the potential of new technologies such as toxicogenomics and proteomics becomes apparent (US EPA, 2002; Kimmel and Makris, 2001; Daston *et al*, 2003; MacGregor, 2003).

The regulatory requirements for safety testing vary between different classes of chemicals. Data from a core set of toxicological studies are not available for all chemicals. It is increasingly recognised that a 'blanket' approach to toxicological testing is not required. A tiered and integrated approach involving use patterns and exposure potential, degree of concern from existing hazard information, together with the use of predictive tools (e.g. QSARs) and read-across between chemical categories are likely to provide adequate hazard data for a risk assessment (ECETOC, 2005).

Understanding the trends in diseases of children and linking these to likely causative factors relies heavily on human data as opposed to results generated in animal studies. The numerous factors influencing children's health can only be assessed from human population studies, usually in the form of case-control or cohort epidemiology studies. In contrast, toxicological evaluation of chemicals allows a more accurate prediction of the potential hazards that may arise as a consequence of exposure to a specific chemical, but must generally be extrapolated from animals to humans. Current procedures for evaluating growth, survival, and morphological changes due to gestational exposures are generally adequate. However, functional consequences of gestational exposure and post-natal exposure are not as well studied. Challenges associated with extrapolating from animal models to humans for post-natal toxicity evaluation include divergent differentiation of structure, function, and physiology across species, lack of understanding of species differences in functional ontogeny, and lack of common endpoints and milestones across species (Morford *et al*, 2004).

The use of various toxicology tests for hazard identification and the particular considerations involved in assessing chemical risks to developing progeny are discussed in this chapter. Tools currently used for assessing hazard and exposure to children are summarised, the adequacy of safety factors is discussed, and some perspective on developments in the short and medium term are provided. Guidance on the interpretation of data from toxicological studies covering developmental and reproductive effects is provided in ECETOC Monograph 31 (ECETOC, 2002).

A review of the adequacy of animal studies in the hazard assessment of effects on children has been undertaken (Morford *et al*, 2004). The majority of animal studies are intended to relate to adults and, with the possible exception of gestational / morphologically related developmental studies, there is a possible lower coverage of a number of functional and post-natal development aspects. A number of weaknesses were identified, such as lack of knowledge on post-natal differentiation of structure, organ functionality and physiology and relevant common endpoints to address cross-species extrapolation.

### **6.1 Regulatory testing strategies for hazard assessment**

Testing requirements vary significantly according to the programmes under which the chemicals are regulated. They range from tiered approaches, e.g. for new industrial and commercial chemicals in the EU in which testing requirements are linked to production volumes, to the extensive data package necessary for the (re-)registration of food-use pesticides. Each authority has a system of rules and criteria by which the hazard of each chemical is evaluated and then indicated by classification listings or labels. These regulatory requirements continue to evolve and the European Commission has recently proposed a new regulatory system for chemicals to be known under the acronym REACH (Registration, Evaluation, Authorisation and Restrictions of Chemicals) (EU Commission, 2003).

The core data set of studies for a new high tonnage production chemical or pesticide in Europe covers a broad range of endpoints including acute, subchronic, and chronic toxicity, genotoxicity, carcinogenicity, and developmental and reproductive toxicity. Toxicity may also be evaluated via a number of different routes of exposure (oral, dermal, inhalation etc.). These studies are all relevant for hazard identification and characterisation for both adults and children but it is the studies in which animals are exposed during embryonic, foetal, neo-natal and/or juvenile lifestages, such as the pre-natal developmental toxicity and reproduction toxicity studies, which have particular relevance for the assessment of potential effects on children's health.

**Toxicity studies in adult animal models.** Subchronic and chronic toxicity studies in rodents and other species tend to be conducted in sexually mature, adult animals. Nevertheless, these studies provide valuable information for evaluating potential hazards to children by identifying both target organs and tissues and by generating dose-response information. The results of such studies also help set priorities for higher tier testing. Modifications to enhance some of the standard guideline protocols so that they might better predict effects of relevance to children's health (e.g. for immunotoxic and endocrine active substances) are currently being validated (Kennel *et al*, 2003; Vohr and Rühl-Fehlert, 2001).

**Pre-natal developmental toxicity study.** Formerly known as a teratology study, it is most often conducted in rats and rabbits. Groups of pregnant females are exposed to varying dose levels of the test substance from implantation through to the end of gestation. Approximately one day prior to parturition, the dams are sacrificed and the foetuses are weighed, sexed, and examined for external, visceral, and skeletal effects. The updated test guidelines have increased the group sizes for rabbits and extended the maternal dosing period for both rats and rabbits until the end of gestation. They also recommend that, in addition to visualisation and study of the ossified structures, assessment of the cartilage should be performed. These studies therefore permit a full evaluation of effects on pregnancy and embryo-foetal development following *in utero* exposure during the sensitive periods of histogenesis and organogenesis (OECD, 2001a).

Developmental toxicity studies are considered capable of predicting the majority of human teratogens. The significance of developmental toxicity that occurs only in the presence of maternal toxicity remains problematic, however, leading in some cases to over-attribution of a potential hazard where, in practice, none would exist (Barlow *et al.*, 2002; ECETOC, 2004).

**One or two-generation reproduction study in rats.** Parental animals ( $P_0$ ) are exposed for ten weeks prior to and during mating. Males are then sacrificed while  $P_0$  females continue to be exposed throughout gestation, parturition and lactation (OECD, 1983). In the two-generation study, selected  $F_1$  males and females are exposed after weaning and then through mating until an  $F_2$  generation is produced (OECD, 2001b). This study thus involves a variety of exposure scenarios during different lifestages:  $F_1$  pups exposed *in utero*, during lactation, and directly after weaning and  $F_2$  pups exposed similarly in addition to having their parents exposed during pregnancy and lactation.

The reproduction study is a powerful and comprehensive tool for evaluating effects on reproductive performance and fertility. Guidelines have been updated in recent years to include measures of oestrous cyclicity, semen quality (including sperm number, motility, and morphology), developmental landmarks (including a number related to sexual maturation) including organ weight, and histopathological examination of reproductive and other potential target organs in the  $F_1$  and  $F_2$  pups (Kimmel and Makris, 2001). Although this complex study is already extremely resource-intensive, additional modifications have been proposed to target oestrogen, androgen, and thyroid endpoints so as to maximise the ability of the study to detect endocrine disruptors.

The comprehensive nature and experience in the use of the rat reproduction study enables reasonably sound extrapolation to humans. It is important that the timing of human exposure is considered in relation to the timing of certain developmental events for which differences exist between rats and humans (ILSI, 2001).

**Reproduction/developmental toxicity screening test.** This study provides limited information on effects on male and female reproductive performance, including gonadal function, mating behaviour, conception, development of the conceptus, and parturition. Male and female rats are dosed for two weeks prior to mating and during mating. Exposure of the males is continued for about two weeks post-mating until necropsy, while females are dosed after mating for the duration of pregnancy and at least 4 days after parturition (OECD, 1995a). The test can be combined with a repeated-dose toxicity study (OECD, 1996).

Neither screening test is able to provide complete information on reproductive performance and should not be considered a replacement for the one- or two-generation reproduction studies in rats.

**Developmental neurotoxicity study.** The US EPA has published specific guidelines for developmental neurotoxicity (DNT) testing for pesticides and industrial chemicals while the OECD has drafted a proposed guideline that is currently being reviewed by member countries. Pregnant animals (generally rats) are administered varying levels of the test substance from implantation through to the end of lactation (post-natal day 21). Selected offspring are evaluated for gross neurologic and behavioural abnormalities, including developmental landmarks related to sexual maturation, physical development, reflex ontogeny, motor activity, motor and sensory function, and learning and memory. Evaluations of brain weights and neurohistopathology during post-natal development and adulthood are also performed (US EPA, 1998; OECD, 2003). One feature of the protocol that is yet to be resolved is under which conditions direct dosing of the offspring should be considered. This point is based on the differences in the timing of the structural development of the brain between humans and rats and the concern that compounds not transferred via the milk may not be fully evaluated in the DNT study. The OECD draft guideline requires that direct dosing of pups be considered if direct exposure of infants through means other than milk is expected (OECD, 2003). However, direct dosing of pre-weaning rats could introduce confounding effects that are not specific to the test chemical (e.g. effects secondary to stress or injury) or relevant to human exposure (e.g. gavage dosing). A number of additional issues remain, including optimisation of the logistics of conducting such a large and complex study, and the scientific and regulatory interpretation of the data generated (Kaufmann, 2003). In addition, the possibility that DNT testing could be applied to a large number of industrial chemicals means that alternative approaches, including more optimal screening methods should be developed.

The DNT study is a higher-tier test that should only be conducted under certain circumstances, such as when the chemical of interest has been observed to have neurotoxicologic effects in adult animals or to induce significant effects on endocrine systems (particularly those involving thyroid, pituitary, or reproductive function) (Kimmel and Makris, 2001; OECD, 2003).

**Immunotoxicity testing.** Over the past several years, the US EPA has indicated that specific investigations of immunotoxicity in adult animals will become part of the core data set for certain types of pesticides (Kimmel and Makris, 2001). These investigations will supplement the information on immunotoxic potential that can already be garnered from the routine repeated dose studies. For example, the OECD guideline for a 28-day rodent study includes spleen and thymus weight measurements and histopathological examination of spleen, Peyer's patches, thymus, lymph nodes, and bone marrow. It provides a useful screening tool for detecting immunotoxic agents (OECD, 1995a). Further modifications to this guideline are under consideration that will enhance the ability to predict effects of this study (Vohr and Rühl-Fehlert, 2001).

Recent efforts to identify a protocol for assessing toxicity to the developing immune system (*in utero*, neo-natal, and during sexual development) have been initiated by research that has linked exposure to environmental chemicals with developmental immunotoxicity in laboratory animals (e.g. Smialowicz *et al*, 2001). At this stage, the question as to whether it is necessary to develop such a protocol remains controversial. However, it is clear that developmental immunotoxicity is an emerging topic of concern in the regulatory arena (Dietert *et al*, 2002).

**Endocrine activity testing.** The concern that hormonally active chemicals in the environment may have significant impact on human health has led to significant efforts by the US EPA (Endocrine Disruptor Screening and Testing Advisory Committee, EDSTAC) and the OECD (Task Force on Endocrine Disruptors and Assessment, EDTA) to develop protocols to assess endocrine disruptor potential. There is particular concern that effects may be more pronounced in vulnerable populations such as infants and children. A number of protocols are currently being validated that would allow the detection of compounds that interfere with oestrogen, androgen, and thyroid function. These include the Hershberger assay and uterotrophic assays, which are designed to detect androgenic and oestrogenic effects, respectively. The results of these and other screening studies will determine whether higher-tier studies, such as multi-generation reproduction studies in rats, should be conducted on specific compounds (OECD, 2001c; Daston *et al*, 2003). As part of the OECD initiative, an enhanced version of the 28-day rodent study (OECD, 1995b) is being subjected to a multi-laboratory validation. The additional endpoints added to the standard protocol involve a more in-depth analysis of effects on sex organs, accessory tissues and other organs under the control of androgens, oestrogens or thyroid hormones, spermatology, oestrous cycling, and hormone levels (Kennel *et al*, 2003; OECD, 2001d).

***In utero* and peri-natal carcinogenicity testing.** In standard rodent carcinogenicity assays, the administration of test substances begins in young adulthood (approximately 6-8 week old) and continues until senescence. The animals are not exposed during development, which raises the question of whether a peri-natal study may be more valuable in evaluating carcinogenicity. Following a comparison of the available standard and peri-natal carcinogenicity studies conducted on the same chemicals, US EPA (1997) concluded that the routine application of

peri-natal carcinogenicity testing is not necessary. This decision was based on the findings that, qualitatively, similar types of tumours are produced and, quantitatively, there is no clear evidence of enhanced sensitivity following peri-natal exposure.

**Toxicogenomic and proteomic technologies.** While these tools will undoubtedly revolutionise toxicology and risk assessment over the coming decades, it is difficult to predict how and when they will be applied. The development of new screens and regulatory guidelines and the elucidation of mechanisms of toxicity are clearly areas in which these technologies are likely to make significant contributions. Identification of effects in sensitive sub-populations at the level of key genes or proteins is a potential application, especially if one considers the gross nature of the changes currently evaluated in pre-natal developmental toxicity studies or the difficulty in predicting neurobehavioural changes in humans by studying juvenile rats. (Cunningham *et al*, 2003; MacGregor, 2003). (Further discussion of this emerging topic is presented in Chapter 7).

## 6.2 Assessing childhood exposures

Estimates of childhood exposure to environmental agents are increasing in accuracy, although standardised approaches are not yet readily available. Nonetheless, models incorporating factors to account for child-specific demographics, physiology, activity patterns, transfer coefficients, and exposure frequency and duration have been developed for a wide range of dietary, drinking water, and residential exposure scenarios. Some exposures are unique to infants and children while others may exceed those of adults (on a body-weight basis), given the same contaminant concentrations due, for example, to children's greater breathing rates and water consumption.

Infants are exposed to substances and materials to which adults usually are not, such as breast milk. Dewey and Lönnerdal (1983) reported an average breast milk intake of about 670g per day for 1-month-old infants, increasing to about 900g for 6-month-old, exclusively breast-fed infants. Another study (Butte *et al*, 1984) reported breast milk consumption of about 750g per day. Thus, infants can consume up to 16% of their body-weight on a daily basis through breast milk, which can accumulate fat-soluble contaminants. Breast milk substitutes may also serve as a source of contaminant exposures.

Due to their unique behaviour, other infant-specific exposure scenarios arise. For example, intense mouthing serves as a key-sensing tool for many infants. Special attention to toys and devices intended for mouthing (like pacifiers or teething rings), or for which mouthing is a foreseeable (mis-)use, is needed. However, assessing exposure to chemicals that can be released during mouthing is problematic. This was illustrated by the complexity of estimating phthalate exposures from soft PVC mouthing toys and pacifiers (EU, 1999; US CPSC, 2003; Babich *et al*, 2004).

Because of their unique crawling behaviour, infants may experience greater exposure to chemicals released from carpets or floors than adults, such as volatile compounds or particles. Other exposure scenarios of particular relevance to children include direct contact with pets, dermal contact with outdoor surfaces (soil, lawns, other vegetation), and contact with non-toy, child-specific products, such as arts and crafts supplies (Armstrong *et al*, 2000, 2002). The possibility of unique or differential exposures associated with children's environments, such as schools and day care centres, should also be considered.

Infants and children require more energy than adults and therefore consume more food, water, and air on a body-weight basis. If those media are contaminated, higher exposures compared to adults may result. Children's greater surface area/body-weight ratios may also lead to greater exposure to contaminants from dermally applied consumer products like ointments, creams, or soap, and from bathing water.

The following examples illustrate the extent to which children's contaminant exposures can exceed those of adults on a body-weight basis given equivalent contaminant concentrations in environmental media.

*Drinking water.* The community drinking water intake recommendations in EPA's Child Specific Exposure Factors Handbook (US EPA, 2002) suggest a mean value of 0.34 litre water per day for infants less than 1 year old, which translates to 46 ml/kg/day. For 11-19 year old adolescents, a mean value of 0.68 litre per day is suggested, which translates to 12 ml/kg/day. Thus, infants less than one year old consume almost 4 times more water on a body-weight basis compared to adolescents.

*Food.* For fruit intake, EPA's Child Specific Exposure Factors Handbook recommends a default value for 1-2 year old children of 19.3 g/kg/day (mean), which decreases to 2.8 g/kg/day for the 12-19 year-old adolescent. Babies thus consume almost 7 times more fruit on a body-weight basis than do adolescents. EPA's age-specific recommended consumption values for vegetables and grains have similar ratios (9.5 g vegetable/kg/day for the 1-2 year-old vs. 4 g/kg/day for the 12-19 year-old and 11.2 g grain/kg/day for the 1-2 year-old vs. 4.4 g/kg/day for the 12-19 year-old, respectively).

*Air.* Similar higher exposure rates in the infants can be obtained for air contaminants. For infants less than 1 year old, EPA gives a breathing rate of 4.5 m<sup>3</sup>/day. Assuming an average body-weight of about 7.6 kg, that rate translates to 0.59 m<sup>3</sup>/kg/day. For 15-18 year old males (66 kg) the daily breathing rate is 0.25 m<sup>3</sup>/kg and for females (56 kg), 0.21 m<sup>3</sup>/kg/day. Infants thus have about a 2-3 times higher inhalation rate than adolescents.

The examples above are based on information found in the US EPA's Child Specific Exposure Factors Handbook, which is the most complete reference resource for children's exposure factors to date. Limited child-specific exposure factors data for European populations are found in the Exposure Factors Sourcebook for European populations (with focus on UK data) (ECETOC, 2001b). A public database of European exposure factors data, which will include additional child-specific data, is being developed under sponsorship of Cefic's Long Range Research Initiative (ExpoFacts project website: [www.ktl.fi/expofacts/](http://www.ktl.fi/expofacts/)).

More detailed descriptions of approaches, methods, and data sources for conducting child-specific exposure assessments are found in several documents developed for use as part of the US Voluntary Children's Chemical Evaluation Program (VCCEP) (Reiss *et al*, 2003; Armstrong *et al*, 2000, 2002). Examples of child-specific exposure assessments submitted to date under the VCCEP are found in the peer consultation VCCEP section of the website [www.tera.org](http://www.tera.org).

### **6.3 Uncertainty and risk characterisation**

Traditionally, risk characterisation has been performed by comparing a measured or estimated human dose to a dose associated with a toxicity endpoint, such as a no-observed-adverse-effect level (NOAEL) or a benchmark dose, after adjustment by adequate uncertainty and/or safety factors. Adjusting for uncertainty generally involves dividing a NOAEL or benchmark dose derived from human data by 10 to yield a level of exposure that would be protective of individuals who might be more sensitive than those tested or observed. If no human data are available, a NOAEL or benchmark dose identified using laboratory animals is divided by 100. This comprises of an intraspecies factor of 10 (to protect sensitive individuals), and an interspecies factor of 10 (to account for the possibility that humans could be more sensitive than the species tested). The resulting exposure level is considered likely to be without adverse effects in humans, including sensitive subgroups or lifestages, because the intraspecies uncertainty factor is meant to protect sensitive groups such as children or the elderly. Further discussion on appropriate factors is presented in a related ECETOC report (ECETOC, 2003).

A number of scientists have attempted to investigate quantitatively whether the intraspecies uncertainty factor is adequate to account for the variability of susceptibility to chemical toxicity between the overall human population and its potentially more sensitive groups, including children. Dourson *et al* (2002) reviewed 17 studies that performed quantitative analysis of the extent of toxicodynamic and pharmacokinetic variability using different data and different starting points, some specifically evaluating age effects in both humans and animals. That analysis suggests that a high percentage of the population, including children, is protected by using a 10-fold uncertainty factor for human variability. Studies indicating that in some cases the young would not be protected by the standard uncertainty factor were those that evaluated acute

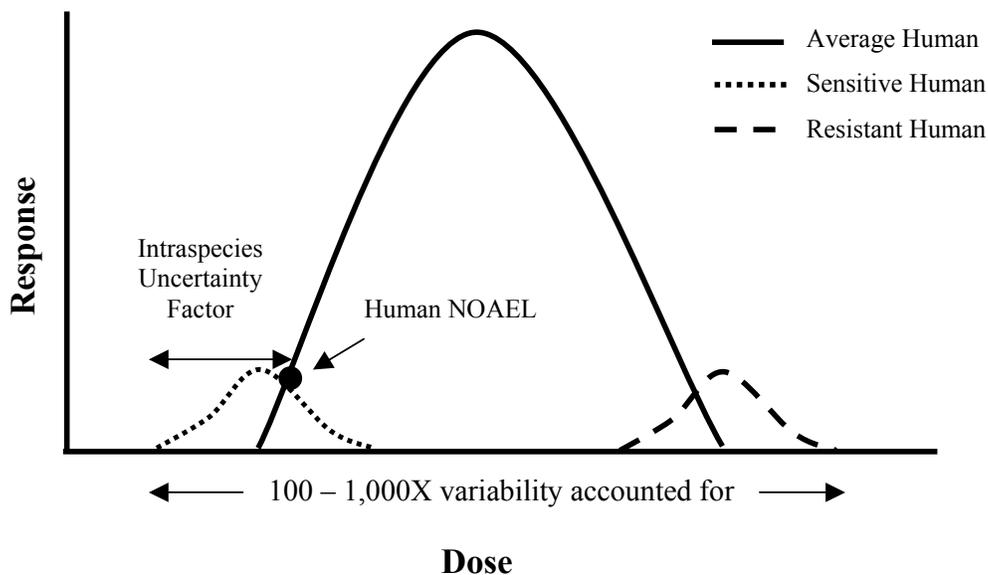
lethality ( $LD_{50}$ ) in laboratory animals and are therefore less relevant to evaluating risks from environmental exposures. Based on specific comparisons for newborns, infants, children, and adults, the range of the population protected is between 67% and 100%. Studies using larger populations that include sensitive individuals (Hattis *et al*, 1999a; Hattis *et al*, 1999b; Renwick and Lazarus, 1998) suggest that the value is closer to 100%.

Other evaluations concur with those of Dourson *et al* (2002). For example, the German Research and Advisory Institute for Toxic Chemicals concluded that, based on toxicokinetic differences, the most susceptible group of neonates is protected by a 10-fold intraspecies uncertainty factor in most cases (Schneider *et al*, 2002). The authors also conclude that the protection of neonates and infants may require consideration of their lower xenobiotic clearance rates.

Conclusions about the adequacy of the 10-fold intraspecies uncertainty factor do not mean that inter-individual sensitivity varies 10-fold, as is often thought. Its application to a value in the low end of the distribution of human sensitivities, such as a NOAEL, and its use in conjunction with other uncertainty factors and conservative assumptions, actually cover total human sensitivity variations of 100 to 1,000 times (see Figure 6-1).

**Figure 6-1: Response as a function of dose for humans of different sensitivities**

(from: Dourson *et al*, 2002)



The interspecies uncertainty factor can also be modified when appropriate data are available. If pharmacokinetic data are available to demonstrate that a substance's active metabolite is generated to a different extent in laboratory animals than in humans, the standard 10-fold uncertainty factor can be replaced with an interspecies dose-response extrapolation. For example, a pharmacokinetic model for isopropanol has been developed to extrapolate the dose-response relationship for isopropanol-induced neurobehavioural effects observed in rats to humans (Gentry *et al*, 2002). The interspecies uncertainty factor can be removed in cases where human data support the animal data upon which a safety assessment is based. The interspecies uncertainty factor was removed for acephate by the US EPA because, although the acephate reference dose was based on rat data, supporting human *in vivo* and *in vitro* data indicated that there were no species differences in adult sensitivity (US EPA, 2005a). In some cases, the interspecies uncertainty factor can be modified, as is the case for boron, because the chemical is not metabolised so there are no metabolic differences among species (US EPA, 2005b). The interspecies uncertainty factor is not used at all if a safety assessment is based on human data instead of animal data.

The intraspecies uncertainty factor also can be modified or removed if the toxicity endpoint of concern is derived from the most sensitive human subgroup. The US EPA's reference dose for nitrite has an intraspecies uncertainty factor of 1 because it is based on methaemoglobinemia observed in human infants (US EPA, 2005c). The US EPA's reference concentration for beryllium has an intraspecies uncertainty factor of 1 because it is based on human beryllium sensitisation and progression to chronic beryllium disease, to which only a small percentage of the population (1-5%) appears to be susceptible (US EPA, 2005d).

In the US, the 1974 Food Quality Protection Act, amended in 1996, requires that an additional safety factor of 10 be applied when establishing tolerances or exemptions for pesticide residues on food to account for the possibility that children may be more susceptible to a pesticide's toxicity than adults or may be proportionately more exposed to a pesticide than adults (unless 'reliable data' justify a smaller safety margin). Information demonstrating that developing animals or children are not more sensitive than adults or that developmental toxicity is not the most sensitive endpoint can be used to support a safety factor of 3 or 1 instead of 10. For example, when the US EPA established a tolerance for myclobutanil, no extra safety factor was applied because the pre-and post-natal toxicology database was considered complete and the most sensitive endpoint was reproductive toxicity, not developmental toxicity. Some question the need for such an additional safety factor, in view of the likelihood that the intraspecies uncertainty factor already protects children, as described above.

More recently, the US EPA proposed a quantitative approach to modifying cancer potency estimates for genotoxic carcinogens to account for the possibility that exposure during childhood could have a greater impact on lifetime cancer risk than exposures during adulthood (US EPA,

2003b). That proposal is also controversial, with some arguing that the available quantitative information on age-related sensitivity is too limited to support the proposed approach. Evaluating the available data, Wild and Kleijnans (2003) concluded that, "... the available data do suggest that there is a scientific basis to the general concern for special attention to the vulnerability of children to environmental carcinogens and that a greater emphasis on this research question is needed. However, more data are needed to permit informed assessments of cancer risk in children and to be able to establish a scientific basis for strategies to minimize risk in this vulnerable section of society."

The International Life Sciences Institute's (ILSI's) Risk Science Institute convened a workshop in 2001 to develop a framework for assessing children's health risks from exposure to environmental agents. The framework envisioned three major steps (problem formulation, analysis, and risk characterisation) refining each to capture the areas of special emphasis for the lifestages constituting childhood (conception through to adolescence). The framework is presented and described in terms of its toxicokinetic and toxicodynamic components by Daston *et al* (2004).

#### **6.4 Conclusions**

- Regulatory requirements for chemical toxicity testing vary widely across different regulatory programmes.
- Data from a core set of toxicologic studies are not available for all chemicals. The need for a core data set, or even a more extensive data set, should be based on an evaluation of whether a significant number of individuals is likely to be exposed at potentially meaningful levels. A tiered approach to chemical toxicity testing, dependent upon production, use patterns, exposure potential, and the results of prior testing, is the most effective way to produce the required data in a timely manner at a manageable cost.
- The newer areas of neurodevelopmental toxicology, developmental immunotoxicology, and testing for hormonal activity are still under development and discussion. In a tiered testing protocol, such tests most likely belong in a later testing scheme, but optimal requirements remain to be clearly established.
- While it is apparent that the newer forms of developmental toxicity testing may well identify novel toxicologic endpoints compared to more traditional toxicity testing, it is not apparent that they will often identify toxicity at dose levels significantly below those found to affect adult organisms. Thus, the new findings, while of scientific interest, may or may not have an impact on risk assessment or risk management outcomes.
- Children's exposures to chemicals differ qualitatively and quantitatively from those of adults. Estimates of childhood exposures to environmental agents are increasing in accuracy, although standardised approaches are not yet readily available. Continued research directed

at characterising infant- and child-specific exposures and using those characterisations in risk assessment is needed.

- Risk assessment methods currently in use incorporate significant uncertainty factors to account for potential interspecies and intraspecies differences in susceptibility to toxicity. Additional uncertainty factors can be added to compensate for data insufficiency (for example, no identified No-Observed-Adverse-Effect-Level). Analyses suggest that the existing uncertainty factors are likely to be adequately protective of sensitive individuals, including children, under most circumstances.

## 7. FUTURE DEVELOPMENTS

### 7.1 *Epidemiological studies – population based studies*

Well designed and well conducted high quality studies with sufficient statistical power should be capable of detecting risk factors for health effects in children. Case-control and cross-sectional epidemiological studies are most commonly used to elucidate the role of environmental risk factors on birth outcome and adverse health effects during childhood. Some of the problems associated with these types of study design concerning children's health are recall bias concerning exposure, selection bias, selection of a proper control group, and misclassification as cases are more likely to over-estimate the actual exposure. Large, population-based prospective cohort studies are therefore the preferred method for studying the influence of environmental risk factors during the pre-natal and post-natal period and early childhood on children's health and on chronic diseases later in life. The advantage of this type of study is that information on exposure is collected at a time when judgement has not been influenced by the adverse outcome, although these investigations face challenges in terms of cost, logistics, and participation. Additionally, these studies often test multiple hypotheses, which may increase the likelihood of spurious findings. As such, results from prospective studies should be externally validated via mechanistic or additional epidemiological research.

When biological material is collected as part of these studies, it is possible to use biomarkers to assess exposure and to study gene-environment interaction in the development of adverse health effects. Two different approaches have been taken and should be expanded: establishment of mother-child cohorts and establishment of child-youth cohorts.

**Mother-child cohorts.** Childbearing is a high-risk period for the mother as well as the unborn child. 30% of all pregnancies end in spontaneous abortions, 3-5% of newborns have congenital malformations and close to 1% are stillborn (Olsen *et al*, 2001). Foetal growth or factors related to foetal growth have been associated with the development of adverse health effects that manifest themselves later in life. Birth cohorts provide an important tool with which to study the effect of exposure from the time of conception to early childhood on adverse health effects.

A research register of exposure in intra-uterine life and early childhood can be established and can be used in nested case-cohort studies. Information on exposure can be obtained by questionnaires or other means (e.g. direct measurement) during the pregnancy period and at specific times after birth until adolescence. Information on parental occupational exposure, diet and lifestyle factors, as well as other risk factors such as infections and complications during pregnancy, can be collected, together with supporting information on residential history. During pre-natal consultation, blood and urine samples could be collected and, at time of birth, samples

of both the placenta and the umbilical cord could be collected and used for chemical and genetic analysis, as well as other biomarkers.

Several small mother-child cohorts have been established in different countries, mostly European, with specific objectives, such as determining the effect of parental occupation on birth outcome. However, these have been too small to investigate the link between specific exposure and health outcome. Other cohorts include multi-ethnic ones of urban mother-child or those where the mothers are especially predisposed, e.g. atopic for asthma.

More recently, the Nordic countries have started establishing national cohorts. The background for these projects based on large populations required the study of gene/environment interactions. In addition, it is possible to trace cohort members, and good health registers are available. Their objective is to study the long- and short-term consequences of factors that influence foetal growth, such as infections, diet, medicine and lifestyle/environment. In the Danish National Birth Cohort, more than 70,000 mother-child pairs are currently enrolled. The information thus collected could be linked to social registries giving information on family structures, income, occupation and education. *Ad hoc* surveys can likewise be conducted to provide information on, for example, childhood diseases and cognitive functions.

Several problems were encountered when establishing the cohort, such as cost and logistics involved, quality control, and no immediate benefits for the clinicians and researchers establishing the cohort (Olsen *et al*, 2001). A major problem in establishing a representative cohort is that it would require participation of general practitioners and midwives, who are first in the health system to come in contact with pregnant women.

If collection of biological material is involved, then there may be a limit in the use of advanced biomarkers, where the time between collection and processing may influence the quality, e.g. the break down of unstable products like RNA.

It is important that these cohorts are large in order to reliably detect changes. The estimated, theoretical, relative risk of common adverse health effects at different exposure frequencies (1-10%) is as presented in table 7-1 (Olsen *et al*, 2001). It takes into account current incidence rates and a power of 80% to detect the indicated relative risk (or higher) at a testing level of 0.05 per 100,000 mother-child pairs.

**Table 7-1: Estimate of power in the Danish mother-child cohort** (adapted from: Olsen *et al*, 2001)

Exposure prevalence among controls		10%	5%	1%
Expected cases	Outcome	RR	RR	RR
3,400	Congenital malformations	1.1	1.2	1.6
560	Genital malformation	1.5	1.7	2.7
220	All child cancers	1.8	2.2	4.1
55	Leukaemia	3.1	3.9	9.4

RR = relative risk.

These cohorts have so far not been used to study the possible effect of specific industrial chemicals on adverse health effects, but have mostly been used to study socio-economic, occupational and lifestyle factors on birth outcome. The advantage is that the cohort members can be linked to various specific disease registers, e.g. cancer registry, and, when sufficient years at risk have accumulated, nested case-control studies on specific exposures and endpoints can be initiated.

Small mother-child cohorts have been established with specific study objectives and collected biological material, i.e. blood, urine, hair, and nails. Combining these data for a meta-analysis may provide additional information on the effect of exposure for environmental risk factors during the pre- and post-natal periods.

**Child-youth cohorts.** Most of the studies on children's health have been case control studies, eventually combining data from many different studies in a meta-analysis. Many studies on the link between air pollution and health effects in children, e.g. asthma, are ongoing in the EU.

Another approach is to establish representative cohorts of children in different age groups and to collect information on exposure either by questionnaires or by analysis of collected biological material (see above). Information on the health status of these participants can be obtained by regular medical examinations, health status questionnaires or by linkage to hospital admission registers or specific disease registers. Information in the pre-natal period can be obtained by parental questionnaires (Hofman *et al*, 2004).

A project 'Kinder-Jugend-Gesundheit' has been initiated in Germany. The focus on exposure has been on nutrition, drugs, lifestyle and also natural and anthropogenic pollution ([www. Kinder-jugend-gesundheit21.de](http://www.Kinder-jugend-gesundheit21.de)).

## 7.2 Use of biomarkers

Children's exposures to certain environmental contaminants can be different, and potentially higher than in adults. Differences in exposure are due in part to differences in physiological function, but also to differences in behaviour, e.g. different time spent in the various microenvironments (see Chapter 2 and 6.2). Different approaches have been used to collect information about child exposure through personal monitoring, activity pattern data and biological monitoring (Hubal *et al*, 2000).

Biomarkers can serve as a useful measure of direct exposure, aggregated over all sources and pathways to provide a measure of integrated exposures from all routes (Wild and Kleijnans, 2003). The use of chemical-specific biomarkers for identifying stages in the procession of development of the toxic effects has the potential for providing important information for risk assessment.

A biomarker is defined as any substance, structure or process that can be measured in the body or its products and can predict the incidence or the outcome of disease. However, the detection of a chemical in the body does not necessarily translate into an adverse effect or disease. Biomarkers can be classified into markers of exposure, biologically active dose, early biological effect and susceptibility (IPCS/WHO, 2001). The optimal biomarker should represent both the exposure and the effect providing a measurable effect exists. However, in reality the optimal biomarker has yet to be identified, and will depend on the endpoint under investigation. Application of bioinformatics and molecular analysis may in the future provide an optimal biomarker. Biomarkers can be measured in easily available biological index media, e.g. blood, urine, hair, nail and deciduous teeth, and for exposure *in utero*, cord-blood and umbilical cord.

Urine appears to be the preferred biological index medium for studies in children based upon ease of collection and analysis. Furthermore, exposure to heavy metals, different pesticides, air pollutants and benzene has been determined by measurements of the compounds or their metabolites in urine. These studies have been conducted mostly in school children and toddlers (US EPA, 2003a). Exposure to tobacco smoke *in utero* has been determined by measuring metabolites in the void urine at time of birth. The levels of these biomarkers have not been related to effects.

Biomarkers of exposure and biologically active doses have been applied in numerous studies. Many compounds require metabolic conversion into biologically active compounds that can then react with the target molecule, e.g. DNA in the case of some genotoxic carcinogens, or with surrogate molecules, e.g. proteins. The levels of these biomarkers represent the biologically active dose. Carcinogen-DNA adducts have been detected in both umbilical cord DNA and in lymphocytes isolated from the umbilical cord, indicating that the ultimate form of the carcinogen

is formed *in utero*, and the level of DNA adducts correlated with birth weight (Perera *et al*, 1998). In adults, a meta-analysis has shown that the level of specific adducts is associated with cancer risk (Veglia *et al*, 2003). Most of these studies have been focused on smoking-related damage.

A biomarker of effects is defined as a measurable biochemical, physiological or other alteration within an organism that, depending on magnitude, indicates an established or a potential health impairment or disease (National Research Council, 1989). A requirement for an optimal biomarker is to be relevant for its effect, i.e. it is important to investigate molecular and biochemical changes relevant to the adverse health effect endpoint, e.g. neurotoxicity, immunotoxicity, kidney toxicity, liver toxicity or cancer.

A biomarker of effects can either be measured in the target organ or in surrogate tissues. These markers include chromosomal aberrations, mutations and carcinogen-DNA adducts to estimate exposure for carcinogenic agents. Chromosomal damage, another potential marker for cancer risk, and specific mutations in 'reporter' genes have been reported in the lymphocytes of newborns (Perera *et al*, 2002b). The same study also remarks that the use of biomarkers has demonstrated that foetuses are exposed to a greater level of damage than their mothers, e.g. carcinogen-DNA adducts. Induction of various interleukines has been investigated in relationship to exposure for allergens, although most of these studies have been conducted in adults, especially in relationship with air pollution (Holgate *et al*, 2003).

Genomics, transcript profiling (transcriptomics), proteomics and metabonomics are rapidly developing technologies that may be used to develop compound and effects specific biomarkers (ECETOC, 2001a). Changes in specific genes/gene clusters have proved to be promising indicators for toxicity. In the case of chemically induced cancer, the expression cluster profile in the tumours depended on the carcinogen used to induce the tumour. Exposure to toxic metals or metal compounds results in the induced expression of many genes. Some are specific for a given metal, but there are also genes expressed by two or more metals. Furthermore, the expression profile depended on the dose used (Andrew *et al*, 2003).

Although the 'omics' methodologies have a great potential in toxicological investigations, their application as biomarkers has currently not been explored in any systematic way. Another concern is the sensitivity of the assays at levels relevant to the exposure in the general population, and the interactions of compounds in complex mixtures.

However, increasing information on the mechanism of the toxic response will lead to the development of many new biological markers. These may be more specific for the effect rather than the exposure, as many compounds mediate their toxic effects through specific pathways; but there is interchange between some of these pathways, e.g. signal transduction.

Susceptibility biomarkers are becoming increasingly important. A susceptibility biomarker can be defined as a marker of individual variability in metabolic, detoxification or repair capabilities resulting in increased or decreased susceptibility to the effects occurring as a result of exposure to a xenobiotic. Genetic polymorphisms in genes coding for uptake and biotransformation of xenobiotics and in the repair of damage induced by the compounds may be important determinants for toxic response. Some of these polymorphisms are linked to altered substrate specificity and altered expression. Several reports show that genetic polymorphisms are associated with an increased risk of cancer and asthma (Autrup, 2004; Kabesch *et al*, 2004). There is an indication that some of these polymorphisms are more important in the case of low dose exposure situations. The polymorphisms also influence the level of biomarkers for exposure, biologically active dose and early biological effects, e.g. carcinogen-DNA adducts, chromosomal aberrations (Autrup, 2004).

### **7.3 Genomic imprinting**

Genomic imprinting describes a phenomenon whereby the expression of an allele (of DNA) differs depending upon its parent of origin. Chemical groups (e.g. methyl-, acetyl groups) are bound to an allele of a gene (epigenetic modifications) that allows controlled expression of one parental allele only (Surani, 2001; Reik *et al*, 2001; Recillas-Targa, 2002; Kierszenbaum, 2002). Allele-specific epigenetic modifications (epigenetic marks) comprise e.g. methylation of DNA at cytosine residues, phosphorylation, acetylation and methylation of histone proteins, and modification and assembly of regulatory protein complexes on DNA. Generally, acetylation is usually associated with transcriptionally active chromatin (i.e. the gene product will be produced), whereas methylation appears to promote a transcriptionally inactive state (gene silencing). In germ cells, these marks are erased, subsequently newly established in a parent-specific manner (re-programming), and maintained after fertilisation (Meehan, 2003; Walter and Paulsen, 2003).

With respect to function, imprinted genes are associated with reproduction, placentation, energy homeostasis, lactation, brain function and behaviour (Surani, 2001; Walter and Paulsen, 2003).

Impaired genomic imprinting has been observed in human diseases, which are characterised by a non-Mendelian inheritance pattern that exhibits parental-origin effects (Beckwith-Wiedemann Syndrome; Prader-Willi/Angelman syndrome) (Walter and Paulsen, 2003) and genomic imprinting is discussed besides many others as one reason for the parent-of-origin effect in immunological diseases like atopy, type I diabetes or rheumatoid arthritis (Cookson, 2002). Furthermore, there is growing evidence that deregulation of imprinted genes is related to tumour formation (e.g. Wilms' tumour, colorectal cancer predisposition) (Recillas-Targa, 2002).

A field of on-going research for some years now is the relationship between genomic imprinting and environmentally induced disease. Genetic mutations (i.e. alteration of base sequence of DNA) or epigenetic mutations/variations (e.g. altered methylation pattern of DNA, chromatin modifications) may affect expression of imprinted genes. It is assumed that an environmental factor, which causes epigenetic changes in DNA, may alter expression of imprinted genes, thus leading to disease: e.g. an allele supposed to be expressed might be silenced by DNA-methylation/chromatin condensation or, to the contrary, loss of repression/gain of expression might lead to uncontrolled cell growth (Jirtle *et al*, 2000). Modification of epigenetic mechanisms by environmental factors and perhaps nutrition may play a role in the intra-uterine programming of adult disease (Reik *et al*, 2003). It is speculated that factors such as lifestyle and diet leave an imprint that is passed through to the next generation, and thus affect the following generations by surviving the process of re-programming in the germ cells (Dennis, 2003).

The question whether genomic imprinting is exclusively controlled on a genetic basis or is also influenced as a response to factors such as nutrition (food supply), nursing and others is currently under investigation, and the impact of this type of research is expected to grow in future (Walter and Paulsen, 2003).

#### **7.4 Toxicogenomics**

Toxicogenomics is an emerging science that studies the interactions among environmental exposures, gene expression, and disease outcomes. Now that the human genome has been mapped, scientists have been able to develop gene chips, or micro-arrays that can contain numerous DNA sequences at specific locations on the chip. These chips can then be used to test indirectly which genes are expressed (i.e. turned on or off) by exposure to particular hazardous agents. Micro arrays are more sensitive markers of effects than more conventional toxicological endpoints like tumours or lesions, which generally require exposing laboratory animals to high doses. Also, the changes in gene expression indicated by micro arrays tend to be chemical-specific, whereas the more obvious physical changes traditionally used in toxicology may be produced by several different agents.

Scientists hope that toxicogenomics data will play an important role in improving the scientific basis for human health risk assessment and expediting research of various stages in the process. Of particular relevance to children's risks is the hope that toxicogenomics could help identify characteristics that are associated with variations in an individual's susceptibility to toxicity, including those that may be related to life stage. Toxicants' modes of action may become better understood through toxicogenomics, improving the relevance of hazard identification by improving risk assessors' abilities to extrapolate from laboratory animals to humans and to identify effects relevant to development. Toxicogenomics data could also increase the accuracy of

children's exposure assessments by helping to identify and characterise their unique exposures. Improved understanding of children's susceptibility, hazards, and exposures through toxicogenomics might permit improved targeted risk assessments and risk management actions.

Drawing causal associations between a particular pattern of altered gene expression and adverse outcomes (or protective effects) is a long-term hope, but knowing that a particular pattern of altered gene expression is associated with certain effects will not necessarily tell us what was responsible for those alterations. Most diseases and conditions of public health concern (cancer, birth defects, asthma, and respiratory and pulmonary disorders) are produced as a result of complex interactions among environmental and genetic factors. Without parallel information about environmental factors such as diet, lifestyle, behaviour, and chemical and radiation exposures, potential associations might be misinterpreted and risk management actions misdirected. As a consequence of such uncertainties, the US EPA has established a policy of considering toxicogenomics information with caution and on a case-by-case basis (US EPA, 2004).

### 7.5 Conclusions

- Large mother-child and child-youth cohort studies are emerging today, but will not deliver information within the next few years. Due to statistical power limitations for rare disease outcomes, studies must be large to make a meaningful contribution. Such studies face challenges in terms of cost, logistics, and participation. Further, as studies are testing multiple hypotheses, findings will need to be externally validated via mechanistic or additional epidemiological research. It is strongly recommended that these investigations are guided by specifically *a priori* described hypotheses and not become broad 'fishing expeditions'.
- The use of biomarkers for both exposure and disease outcomes offers new promises and challenges, especially if incorporated into the cohort studies discussed above. Biomarkers of either exposure or effect suffer from various limitations depending upon the specific disorder, chemical, and disease outcome under investigation, but may offer significant advantages when they improve the accuracy of exposure estimation and/or diagnosis of disease.
- While 'omics' technologies are topical, the role of genomics and proteomics in toxicity testing, exposure assessment, and health outcome assessment remains to be defined.
- The impact of environmental factors on genomic imprinting has yet to be clarified.
- Toxicogenomics might improve the understanding of children's susceptibility, hazards and exposure. But there are still considerable uncertainties on causal associations of altered gene expressions and disease outcomes. Thus, toxicogenomic information needs to be treated with caution and on a case-by-case basis only.

## 8. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions are drawn from each of the technical chapters comprising this document. They review trends, contributing factors, and role of chemicals for specific health conditions, as well as provide an overview of the sensitivity of children, risk assessment and emerging topics.

### *Age Related Sensitivity and General Comments*

The susceptibility of children depends on the nature of the chemical and the age (lifestage) of the individual. Children may be differentially susceptible to chemicals relative to adults. They may be more sensitive, less sensitive, or equally sensitive to various endpoints resulting from various chemicals over time. Generalisations indicating that children are more sensitive to chemical insults cannot be supported by existing scientific data.

The likelihood of a specific health outcome following chemical exposure in children is also affected by patterns of exposure. These, in turn, reflect both the presence of chemicals in the environment and the physical and behavioural characteristics of the child. It is possible that these variations in both susceptibility and exposure patterns can lead to elevated vulnerability to adverse effects, so called 'windows of vulnerability'.

Childhood health outcomes tend to have multiple, complex causes. Genetic and family influences often determine health risk to a much greater extent than environmental factors. In some instances (e.g. attention deficit disorder), genetic-environmental interactions may be extremely important. The environmental factors affecting children's health extend well beyond the realm of industrial chemicals to include nutritional factors (protein, vitamins, antioxidants), lifestyle choices such as tobacco and alcohol use, parental health, socio-economic status, choice of living environment (e.g. urban vs. rural), and even parent and sibling behaviour.

Given the diversity of health outcomes, the diversity of chemicals, and the numerous factors that determine potential 'windows of vulnerability', no broad conclusions can be drawn regarding the effect of chemicals on children's health. The potential contribution of chemicals to individual disease outcomes is discussed below.

### *Asthma, Respiratory Disease, and Allergic Disorders*

There is clear evidence of increasing rates of asthma in childhood, although rates in some countries may now have stabilised. There is no convincing evidence of widespread trends in other

acute or chronic childhood respiratory disease. However, evidence does suggest that changes in local socio-economic and environmental conditions are associated with changes in the frequency of asthma and other disorders related to respiratory disease, such as otitis media. Interpretation of the available information on asthma and allergies is hampered by changing diagnostic criteria over place and time. Data also suggest that other atopic disorders such as upper respiratory tract and food allergies may be increasing. While atopic dermatitis remains the leading skin disorder in young children, no widespread trend attributable to chemicals is apparent.

There is a need to distinguish between factors causing asthma and those serving to acutely exacerbate the condition. However, it remains difficult to distinguish primary causes such as allergens from exacerbating factors, because non-allergenic chemical agents may modulate the ability of other materials to cause asthma. Furthermore, re-exposure to primary causal factors (antigens) is recognised to exacerbate established disease.

Overall, genetic status and exposure to antigens (mainly proteins) remain the most important determinants of childhood atopic disease, including asthma. Early exposure to infectious agents, immunisations, and other environmental immune stimuli, appears to reduce the frequency of asthma and atopic diseases. Conversely, lack of environmental exposure to these factors seems to increase the risk of allergic disease ('hygiene hypothesis').

Primary chemical contributors to exacerbation of childhood asthma are most likely to be ambient air pollutants (such as particulates, NO<sub>x</sub>, SO<sub>x</sub>, ozone) and lifestyle related indoor air pollutants such as environmental tobacco smoke. Some evidence suggests that volatile organic chemicals may play a role in the exacerbation of childhood asthma, primarily as a result of airway irritant effects. Primary allergens responsible for asthma, respiratory allergies, and food allergy are almost invariably antigens of biological origin, including proteins. Nickel is probably the most common allergen for skin sensitisation in the general public.

### ***Neurodevelopmental and Reproductive Disorders***

Although the frequency of neurodevelopmental disorders such as autism and attention deficit disorder is perceived to be increasing, the limited data available do not support this perception. Diagnostic criteria have been inconsistent over time, and there are no population-based surveillance data available for neurodevelopmental disorders. This makes it impossible to ascertain trends in actual disease frequency.

An association with neurodevelopmental disorders has been reported for exposure to lead, mercury, and PCBs. The relationship of these chemicals to neurobehavioural changes at general environmental levels has not been well established except for exposure to lead. These chemicals

are thought to produce various neuropsychological effects, which are also seen with other neurodevelopmental conditions, but have not been shown to clearly contribute to classically defined neurodevelopmental conditions such as ADHD and autism.

There are limited data on reproductive effects and, when available, they are of poor quality. While geographic heterogeneity is apparent, broad population trends for these outcomes (sperm quality, hypospadias, and cryptorchidism) are difficult to identify.

The risk for cryptorchidism appears to be related to a variety of factors, including genetics, ethnicity, analgesic use during pregnancy, birth order, birth weight, intra-uterine growth retardation, and maternal obesity. Similarly, a variety of factors are associated with hypospadias, including low birth weight, intra-uterine growth retardation, maternal diet and iron consumption, and influenza infection during pregnancy.

Sperm quality varies widely over time and place, with, as noted above, no clearly established global trend. Factors believed to influence sperm quality include, in particular, smoking habits of the mothers, but also occupation, age, medications, disease states, dietary factors, clothing, stress, fertility, sexual activity, and heat.

The role of industrial chemical exposure in producing alterations in reproductive and endocrine function in children has not been documented, despite the many studies that have been done. The only clear trend identified, earlier onset of puberty, appears to be due to improved health and nutrition or changes in lifestyle choices. The ability of reproductive tissues and processes to be affected by high-level hormonal stimuli is established. Effects on human reproductive health have not been demonstrated as a result of hormonally active agents at levels occurring in the environment.

### ***Cancer***

Data indicate that developed countries have a gradually increasing incidence of leukaemia with a corresponding drop in the incidence of lymphoma. The reason for this is not apparent. Increases in brain tumour frequency may be related to the development of new diagnostic capabilities and a wide application of these tests, rather than a true change in the incidence of malignant disease. With a better prognosis of childhood cancer survival, secondary cancers following chemotherapy appear to be increasing. Apart from this limited population and a few other populations with high-level exposure to carcinogenic agents (i.e. radiation), the low frequency of childhood cancer makes epidemiologic determination of cause difficult.

For childhood cancer, genetic factors appear to be important. A role for environmental factors has been established only for radiation (atomic and therapeutic) and for chemotherapeutic agents. There is no clear and consistent evidence that environmental chemicals play a significant role in the aetiology of childhood cancers.

The role of juvenile exposures in adult cancers has not been established. A number of factors related to pregnancy and lifestyle have been associated with alterations in the frequency of adult cancers. There are a number of biological reasons to believe that growing organisms may be more susceptible to carcinogens. Animal data suggest that young organisms may be more susceptible to the effects of some carcinogens, but these data suffer from significant deficiencies. Further research is needed to accurately assess the relative susceptibility of children to exposures, which induce adult cancers.

### ***Risk Assessment***

Requirements for chemical toxicity testing vary widely across different regulatory programmes. A 'blanket' approach to testing for risk assessment is increasingly recognised as counter-productive. An integrated and tiered approach to toxicity testing, dependent on use patterns, exposure potential, (Q)SAR and other supporting approaches as well as the level of concern about existing hazard information is likely to be more effective than an approach that subjects all chemicals to a full testing battery.

Tests for neurodevelopmental toxicity, immunotoxicity, and endocrine activity are still under development. While these newer forms of testing may well find novel toxicological endpoints relative to more traditional testing, it is not yet clear that they will often find toxicological endpoints in children occurring at dose levels significantly below those determined for adult organisms.

Risk assessment methodology for limiting chemical exposures incorporates uncertainty factors. These account for potential interspecies and intraspecies differences in sensitivity to toxicity. Additional uncertainty factors may be considered for data insufficiency (e.g. lack of a No-Observed-Adverse-Effect-Level). The need for an additional safety factor that is intended to ensure the protection of children cannot be supported by current evidence.

### ***Future Developments***

There are currently a number of developments in technology, experimental tools, mechanistic information and risk assessment that may alter the understanding of impact of chemical agents on children's health.

Large mother-child and child-youth cohort studies are emerging today, but the results of these will not be available for many years. Due to statistical power limitations for rare disease outcomes, studies must be large to make a meaningful contribution. However, for more common diseases, they may provide important information of the role of environmental risk factors on disease pathogenesis. Such studies face challenges in terms of cost, logistics, and participation. Further, with studies testing multiple hypotheses, findings will need to be externally validated via mechanistic or additional epidemiological research.

The use of biomarkers for both exposure and disease outcomes offers new opportunities, especially as they are incorporated into the above mentioned cohort studies.

While 'omics' technologies are rapidly developing, the role of genomics and proteomics in toxicity testing, exposure assessment, and health outcome assessment remains to be defined. The role of genomic imprinting (e.g. in eliciting immune responses, hormone dependent cancers) requires further study. The impact of environmental factors on genomic imprinting has yet to be clarified.

The emerging science of toxicogenomics is expected to produce large sets of data. Their causal association with disease outcomes needs to be considered with caution.

### ***Recommendations for Future Research***

#### **Biomarkers**

- Compile and analyse existing biomarker data to investigate the relationships between environmental exposures and children's health.
- Identify and validate specific biomarkers for the assessment of the relationships between exposures and effects on children's health, and use those to evaluate biobank samples on a population basis.
- Investigate how / if 'omics' can be used as biomarkers of children's exposure to environmental hazards.

#### **Databases**

- Facilitate the standardisation of pan-European diagnostic criteria and compilation of a database for health effects in children.

### **Disease / Health Effects**

- Facilitate investigations of contributing factors that may be influencing a trend in increasing childhood leukaemia and asthma.
- Establish whether there are relationships between respiratory allergy, respiratory disorders and chemical pollution.
- Develop standardised methods to study the effect of chemicals on neurodevelopmental outcomes, taking advantage of established mother-child birth cohorts.
- Determine the relative contribution of environmental and societal factors on children's health.

### **Hazard and Exposure Assessment**

- Review the experience with 'newer' toxicological studies, in particular for developmental neurotoxicity (DNT), and their utility in hazard assessment. Develop alternative approaches for DNT studies.
- Determine which exposure models are applicable to children, using new or existing exposure measures (including biomarkers).
- Establish consequences of early life exposure to carcinogens to cover *in utero* (and pre-conception), neo-natal and post-natal exposures using animal models to establish any common mechanisms.

**ABBREVIATIONS**

AAAAI	American Academy of Allergy, Asthma and Immunology
AAP	American Academy of Pediatrics
ACD	Allergic contact dermatitis
ADDA	Attention Deficit Disorder Association
ADHD	Attention deficit / hyperactivity disorder
ALL	Acute lymphocytic leukaemia
AML	Acute myelogenous leukaemia
AUC	Area under the plasma-concentration-versus-time curve
CL <sub>TOT</sub>	Total clearance
CNS	Central nervous system
CPSC	US Consumer Product Safety Commission
Da	Dalton(s) (molecular weight)
DCBP	Dichlorobromopropane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
DNA	Deoxyribonucleic acid
DNCB	Dinitrochlorobenzene
DNT	Developmental neurotoxicity
EBV	EB virus
EDC	Endocrine disrupting chemical
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Endocrine disruptors testing and assessment
EEA	European Environment Agency
EMF	Electromagnetic fields
EUROCAT	European Network of Population-based Registries for the Epidemiologic Surveillance of Congenital Anomalies
HCB	Hexachlorobenzene
IARC	International Agency for Research on Cancer
IFCS	International Federation of Classification Societies
Ig	Immunoglobulin
ILSI	International Life Sciences Institute
IOM	US Institute of Medicine of the National Academies
IPCS	International Programme on Chemical Safety
ISAAC	International Study of Asthma and Allergies in Childhood
LRI	Long-range Research Initiative
MAS	Multicentre Allergy Study
MMR	Measles-Mumps-Rubella

**ABBREVIATIONS (CONT'D)**

MMWR	Morbidity and Mortality Weekly Report
MSX	Homeobox gene
NAS	US National Academy of Sciences
NCHS	US National Center for Health Statistics
NHL	Non-Hodgkin's lymphoma
NOAEL	No-Observed-Adverse-Effect-Level
NO <sub>x</sub>	Nitrogen oxide(s) (oxides of nitrogen)
NRC	US National Research Council
OECD	Organisation for Economic Co-operation and Development
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenylether
PCB	Polychlorinated biphenyl
PM	Particulate matter
PM <sub>2.5</sub>	Particles 2.5 µm in diameter or smaller
PM <sub>10</sub>	Particles 10 µm in diameter or smaller
QSAR	Quantitative structure activity relationship
RAST	Radio allergen sorbent test
RB	Retinoblastoma
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfP	Request for Proposal
RNA	Ribonucleic acid
RR	Relative risk
RSV	Respiratory syncytial virus
SCALE	Science for Children through Awareness, Legislation and continuous Evaluation
SCF	EU Scientific Committee for Food
SEER	Surveillance, Epidemiology and End-Results
SES	Socio-economic status
SO <sub>x</sub>	Sulphur oxide(s) (oxides of sulphur)
t <sub>1/2</sub>	Half-life
TEWL	Trans-epidermal waterloss
Th	T helper cell
TWG	Technical working group
US EPA	US Environmental Protection Agency
VCCEP	Voluntary Children's Chemical Evaluation Program
V <sub>d</sub>	Volume of distribution
VOC	Volatile organic compounds
WHO	World Health Organization
WT	Wilms' tumour

## APPENDIX

### *Additional information on mechanisms of allergy and the biology of allergic response*

In humans, immunocompetence starts to develop *in utero* at approximately gestation week 5 and is largely completed in early life. Qualified reviews describing evolution of the immune system and gaps of knowledge are available from a Workshop to 'Identify Critical Windows of Exposure for Children's Health' organised by the EPA in 1999 (Holladay and Smialowicz, 2000; Dietert *et al*, 2000) and a recent article from Holsapple *et al* (2003).

Imbalances of the normal immune response may lead to immunosuppression, hypersensitivity or autoimmune disease.

Allergy means hyperreactivity of the immune system towards normally well tolerated harmless xenobiotics, which induce a specific immune response during the first contact (sensitisation or induction) and elicit allergic symptoms during further contacts (elicitation). Allergen usually means a protein (or polypeptide) that at extremely low concentrations can cause an IgE-mediated sensitisation or a low molecular weight chemical that after binding to serum albumin or other self-protein ('altered self') can cause a lymphocyte-mediated sensitisation (von Ehrenstein, 2002). Atopy refers to those allergic conditions that tend to cluster in families, including hay fever, asthma, and eczema, and which are associated with an innate disposition for the production of specific IgE antibodies to common environmental allergens. The process of sensitisation may or may not result in the induction of clinical symptoms characterised by inflammation, as a result of hyperresponsiveness of the skin or mucous membranes (Wahn, 2000). However, in the intrinsic (non-allergic) form of atopic dermatitis sensitisation is not necessarily required for manifestations of disease. Autoimmune disease will develop if the immune system turns towards constituents of its own body. The risk of autoimmune diseases may be increased or decreased by factors, which alter the reactivity of the immune system, but little clinically relevant data exist in this area.

Pseudoallergy means diseases that clinically resemble an allergic reaction but where an immunological cause cannot be proven (e.g. intolerance towards acetylsalicylic acid). A pseudoallergic reaction may result from e.g. overload of macrophages with sooty particles, which induce unspecific inflammatory reactions. This type of 'asthma-like' reaction is hard to differentiate from a specific allergic reaction. Non-allergic food intolerances (e.g. lactose or fructose intolerance, metabolic disorders, antipathy against certain foods) may also be mistakenly regarded as being allergies.

Different types of allergic reactions are distinguished by the mechanisms of the immune response and the onset of symptoms. The allergic response is either mediated within seconds to hours (fast

to immediate type reaction) by humoral factors (membrane based IgE antibodies and specific mediators) or by sensitised immune cells (T-lymphocytes) with a more delayed reaction time (hours to days). The most common type of allergy is an IgE-mediated fast type response.

Haptenation (where a low molecular weight chemical binds to a high molecular weight chemical, mostly a protein) is the most common mechanism by which low molecular weight chemicals (e.g. metals) cause allergic and autoimmune reactions (contact eczema).

Allergic contact dermatitis is a T-cell-mediated immune response to small molecular weight chemicals that penetrate the skin. The first step in developing a contact allergy is the penetration of haptens (usually small molecules of less than 500 Dalton molecular weight) into the skin, and further into the viable epidermis. Here, they react with proteins and are processed and presented by Langerhans cells, the principal antigen presenting dendritic cell in the skin (Stingl, 1993). During the antigen processing, the Langerhans cells migrate into the next draining lymph node. Here, an antigen-specific encounter between these Langerhans cells and naïve lymph node T-cells occur. The naïve T-cells become activated and undergo division. This results in the production of a population of lymphocytes responsive to the specific allergen. This primary clonal expansion of lymphocytes within the draining lymph nodes during sensitisation is the basis for future allergic reactions after additional skin exposure to the same allergen. The above-described reactions are called the induction phase, during which clinical signs like erythema or oedema occur rarely and weakly, if at all.

The initial clinical adverse skin reactions occur after re-exposure to the contact allergen. After the allergen enters the epidermis, the allergen-specific population of lymphocytes, which are now circulating in the body, will react immediately and directly with the allergen, with resulting clinical signs like erythema, oedema, vesiculation and puritis.

This allergic and immunological reaction after re-exposure is called elicitation of an existing skin sensitisation. Usually, at least 2-6 weeks need to elapse between the induction (i.e. first contact to the contact allergen) and elicitation.

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