Lactational Transfer of Cadmium in Rodents – CNS Effects in the Offspring

Kierstin Petersson Grawé

Department of Pharmacology and Toxicology

Uppsala

Doctoral thesis

Swedish University of Agricultural Sciences

Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Veterinaria 150

ISSN: 1401-6257
ISBN: 91 576 6371 8

Tryck: SLU Service/Repro, Uppsala 2003

Abstract

Petersson Grawé, K. 2003. Lactational transfer of cadmium in rodents – CNS effects in the offspring. Doctor's dissertation.
ISSN 1401-6257, ISBN 91 576 6371 8

This thesis comprises studies on the transfer of cadmium (Cd) from the lactating dam to the suckling and CNS effects of Cd during development, using rodents as a model. The purpose has been to conduct low–level exposure studies relevant for risk assessment of Cd. Cadmium is recognised as a toxic environmental contaminant with renal dysfunction considered as the critical effect after long-term exposure. For the non-smoking, non-occupationally exposed population, food, especially of vegetable origin, is the main source of cadmium exposure.

Cd concentration in suckling pup kidney was strongly correlated with Cd in milk and kidney of the Cd exposed lactating rats, showing that cadmium is transferred to the pup via milk and absorbed in the suckling. This indicates that Cd in kidney can be used as a biomarker of the Cd dose in pups. A prominent uptake and retention of Cd was demonstrated in the mammary tissue, where Cd binding to metallothionein was indicated. Cd in milk was present mainly in the fat and casein fractions with a smaller part in the whey fraction.

The serotonergic system in the developing brain was found to be susceptible to Cd. Hippocampal and cortical levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid, were markedly reduced in animals exposed via milk. The exposure did not cause any detectable levels of Cd in the brain. A positive linear correlation was revealed between spontaneous locomotor activity and kidney cadmium concentrations in pups exposed via milk. There were no effects on learning, memory or anxiety due to treatment.

Effects on the fatty acid composition after Cd exposure were found in the liver and milk of the dams, and a minor modification was detected in the brain of the pups. The long-chain polyunsaturated fatty acids, which are important for normal development of the CNS, were unaltered, as were zinc levels in the brain. Probably, the observed CNS effects are due to a direct effect of very low levels of Cd in the developing brain.

The results indicate that neurochemical and neurobehavioral effects during development may be a more sensitive endpoint for cadmium toxicity than renal dysfunction.

Keywords: neurobehaviour, neurochemical, neurotransmitter, neurotoxicity, neonate, postnatal, pollutant, heavy metal, toxic metal

Author's address: Kierstin Petersson Grawé, Department of Pharmacology and Toxicology, SLU, Box 573, SE-751 23 UPPSALA, Sweden.

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numeral.

I. Petersson Grawé K, Oskarsson A. 2000. Cadmium in milk and mammary gland in rats and mice. *Archives of Toxicology* 73, 519-527.

II. Andersson, H., Petersson Grawé, K., Lindqvist, E., Luthman, J., Oskarsson, A., Olson, L. 1997. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. *Neurotoxicology and Teratology* 19, 105-115.

III. Petersson Grawé, K. Teiling-Gårdlund, A., Jalkesten, E., Oskarsson, A. Neurobehavioral effects in rats postnatally exposed to cadmium via milk. *Manuscript*.

IV. Petersson Grawé K, Pickova, J., Dutta, P., Oskarsson A. Fatty acid composition in liver and milk of cadmium exposed lactating rats and in brain of their offspring. *Manuscript*.

Introduction

This thesis comprises studies on the transfer of cadmium from the lactating dam to the suckling, and CNS effects of cadmium during development, using rodents as a model. Cadmium (Cd) is recognised as a toxic environmental contaminant (WHO, 1992). For the non-smoking general population, food, especially of vegetable origin, is the main source of cadmium exposure (WHO, 2001).

Normally, the main source of exposure to toxic compounds during the neonatal and first part of the infancy period is breast milk. This period is characterised by rapid growth and development of the nervous, immune, and endocrine/reproductive systems, which render the newborn more vulnerable than adults to harmful substances (Rice & Barone Jr, 2000). Also, kinetics during the neonatal period differs from that in adults, which may put the neonate at special risk (WHO, 1986). In risk assessment of chemical hazards, it is widely recognised that pregnant women and their foetuses are risk groups that need special attention. Compared to the well motivated interest in these established risk groups, very little attention has been paid to the susceptibility of the newborn.

Lactation

The lactating mammary gland

The ability to feed the newborn with endogenously produced milk is an essential characteristic of mammals, and the development and structure of the mammary gland has been conserved during evolution (Gould, 1983). The secretory cells in the mammary gland are arranged in a single cell layer in alveoli surrounding an interior lumen, in which the synthesised milk is emptied. These cells are referred to as mammary epithelial, alveolar, or acinar cells. A network of blood vessels and myoepithelial cells surround each alveolus, the latter being contractile and responsible for the ejection of milk from the secretory cells into the lumen. The alveoli are arranged in clusters forming lobuli, each lobulus having a lactiferous duct in which the alveolar ductules empty the secreted milk. The lactiferous ducts all end up in the nipple where the milk is excreted. (Gould, 1983; Larson, 1985).

Milk composition

The composition of milk is complex, containing a large number of constituents. The major components are water, lipids, proteins, lactose and salts. Other important constituents are enzymes, hormones and immunoglobulins (Jensen, 1995). Comparative studies have revealed marked differences in milk composition, but also common features between species (Davies *et al.,* 1983). For instance, primate milk is rich in lactose and poor in protein and fat, while the relationship is the opposite in milk from marine mammals.

The proteins in milk are mainly caseins and whey proteins (Jensen, 1995). Caseins are milk-specific phosphoproteins, which aggregate to micelles in the presence of calcium and phosphate (Jensen, 1995). The caseins can be separated by centrifugation and the resulting whey contains the soluble proteins. In human

milk, α-lactalbumin is the major whey protein, synthesised in the alveolar cells, and specific for milk (Jenness, 1985). Aside from being one component of the enzyme lactose synthase, α-lactalbumin is considered a metalloprotein, having several binding sites for *e.g.* Zn^{2+} (Permyakov *et al.,* 2000). In ruminants, β-lactoglobulin is the most abundant whey protein (Jenness, 1985).

The milk fat supplies energy, essential fatty acids, vitamins and other fat-soluble factors to the suckling (Neville, 2001). Most of the lipid consists of triglycerides, contained in fat droplets called milk fat globules (MFG). The fatty acid components are derived either from maternal diet or maternal storage or are synthesised de novo in the alveolar cells. An enzyme unique to the mammary alveolar cells, thioesterase II, terminates the elongation of the fatty acid after the addition of 8 -14 carbon atoms (Smith *et al.,* 1983).

Milk secretion

Compounds are transported from plasma across the basolateral and mammary epithelial cells, out in the alveolar lumen by different pathways. The secretory mechanisms have been reviewed by (Linzell & Peaker, 1971; Larson, 1985; Shennan & Peaker, 2000; Neville, 2001). Water moves freely across the membranes in response to the osmotic gradient set up by lactose, which is synthesised in the cells. Sodium, potassium and chloride move across the membranes partly by active transport, and partly by moving freely following their electrochemical gradients. Secretory compounds like the major milk proteins and lactose, but also phosphorous and calcium are transported to, or sequestered by, the Golgi apparatus, packed into secretory vesicles and excreted into the lumen by exocytosis. The milk fat globules, containing milk lipids, increase in size as they move towards the apical membrane. There they bud off, sometimes together with small amounts of cytoplasm, encapsuled by fragments of the apical membrane (Kurosumi *et al.,* 1968). This membrane is termed the milk fat globule membrane (MFGM). Blood immunoglobulins are transported through the secretory cells by specific intracellular transport mechanisms. These involve binding to receptors at the basolateral membrane, pinocytos, transport in vesicles, and finally exocytosis at the apical membrane. Tight junctions normally prevent passage of substances between the alveolar cells. During pregnancy, and during milk accumulation or mastitis in ruminants, the tight junctions become leaky and compounds in plasma can pass into the alveolar lumen (Stelwagen *et al.,* 1997; Neville, 2001).

Recently, the identification of transport proteins has improved the understanding of the secretory mechanisms in the mammary epithelial cells. For instance, the lactating mammary gland has a high demand for glucose which is the precursor for lactose and triacylglycerols (Shennan & Peaker, 2000). The GLUT1 glucose transporter is one identified mechanism for uptake of glucose across the basolateral membrane (Shennan & Peaker, 2000). Similarly there are transporter systems supplying the alveolar cells with amino acids and peptides (Groneberg *et al.,* 2002). Increased mRNA levels for several transporter genes, *e.g.* organic cation transporter, multidrug resistance transporter, have been identified in lactating human mammary epithelial cells compared to non-lactating cells (Alcorn *et al.,* 2002). A sodium dependent transporter of iodine on the basolateral

membrane, and the presence of another transporter on the apical membrane has been identified (Spitzweg *et al.,* 1998; Cho *et al.,* 2000; Remilla *et al.,* 2003). Iodine in the maternal diet is readily transferred across the mammary epithelial cells into milk, and may therefore be a matter of concern in cases of radioactive fallouts (Jensen, 1995). Another example is ZnT-4, which is highly expressed in the mammary epithelial cells, the protein being homologous to other known zinc transporters (Huang & Gitschier, 1997; Kelleher & Lönnerdal, 2002). In the lethal milk mutant mouse strain this gene is mutated, resulting in markedly less zinc secretion in milk and mortality of the suckling offspring (Piletz & Ganschow, 1978; Lee *et al.,* 1992). Mortality is prevented if the pups are nursed by dams of other strains, or given zinc orally (Ackland & Mercer, 1992).

The developing central nervous system

A period of rapid synthesis of brain tissue, commonly called the brain growth spurt, occurs during the last trimester and continues for about 18 months after birth in humans. In the rat, the brain growth spurt begins in the immediate postnatal period, with a peak at about 10 days of age. This period is characterised by *e.g.* formation of synaptic contacts and myelin synthesis (Innis, 1991). Brain development is a dynamic process varying in time and speed in different regions of the brain (Clandinin, 1999). During synaptogenesis, when interaction between neurons within and between brain regions is crucial, the neurons are sensitive to disturbances in their synaptic environment (Olney, 2002). Thus, each step in the development becomes a critical basis for development of the next. In addition, the blood-brain barrier limiting the uptake of compounds in the brain in adults is more permeable during development (Saunders *et al.,* 1999). During development some of the neurotransmitters besides transmitting neuronal signals also regulate the development of neurones (Rice & Barone, Jr., 2000; Herlenius & Lagercrantz, 2001; Nguyen *et al.,* 2001). Thus, an insult having a transient effect on neurotransmitters in the adult brain may have permanent effects on the developing central nervous system. For example DDT, pyrethroids, organophosphates, nicotine, paraquat and PCBs have all been shown to cause irreversible behavioural and cholinergic changes in the central system in mice exposed during the brain growth spurt (Eriksson, 1997).

Neurotransmitters and modulators

The neurotransmitters in the central nervous system have important roles in normal functioning and behaviour of the adult individual. They interact with each other in complex networks, for instance in the processes of learning and memory, in which acetylcholine is proposed to have a central role (Decker & McGaugh, 1991) and in locomotion for which *e.g.* dopamine and serotonin interact (Beninger, 1983; Oberlander *et al.,* 1986). The serotonergic system is also involved in anxiety response as shown in animal studies (File *et al.,* 2000). In animal models severe depletion of serotonin in cerebral cortex and hippocampus increases spontaneous activity, impairs learning and influences habituation (Mohammed *et al.,* 1990).

Neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-5 (NT-5), and are present in peripheral and central neurons (Thoenen, 1995). Their function is not fully understood, but involves regulation of survival and differentiation of selective subsets of neurons, and maintaining normal function of neurons during adulthood (Thoenen, 1995). Also, BDNF seems to play a role in learning and memory processes (Tyler *et al.,* 2002; Yamada *et* al, 2002). The neurotrophins bind and activate one or more of the tropomyosin-related kinase (Trk) family of receptor tyrosine kinases, thereby activating intracellular signalling pathways (Patapoutian & Reichardt, 2001). Alterations in BDNF and trkB mRNA have been reported after acute methylmercury and trimethyltin exposure (Andersson *et al.,* 1997a, Andersson *et al.,* 1997b). BDNF and to some extent also NT-3 binds to trkB. Glial fibrillary acidic protein (GFAP) is considered a sensitive biomarker of neurotoxicity, being increased when astrocytes respond to tissue damage or exposure to chemicals like methylmercury or triethyltin (Eng *et al.,* 2000).

Fatty acids

The brain lipid content is about 50-60% of the dry weight, most of the lipids being phospholipids (Sastry, 1985). During neonatal development a rapid accrual of long-chain polyunsaturated fatty acids (LCPUFA), especially arachidonic acid (AA) and docosahexaenoic acid (DHA), occurs in the brain (Innis, 1991; Clandinin, 1999).

Fatty acids are aliphatic chains of carbons with a methyl group at one end of the chain and a carboxyl group at the other. The length of the carbon chain varies as does the number and localisation of double bonds between the carbon atoms. A few words about the nomenclature: fatty acids are commonly described by giving the number of carbons, followed by the number of double bonds, and finally the position from the methyl end (the n- or **ω**-end) where the first double bond occurs, *e.g.* 20:4 (n-6). Fatty acids with 20 carbons are precursors for eicosanoid production in mammals, the most important being AA (Whelan, 1996). Except for the essential fatty acids, see below, fatty acids can be synthesised de novo, catalysed by a multienzyme complex, fatty acid synthase, present in several tissues. The predominant end product of de novo synthesis is palmitic acid, 16:0 (Sastry, 1985). Besides de novo synthesis, fatty acids are also dietary derived.
(Innis, 1991). In neonatal rodents it has been shown that EFA are transported to the brain by highly specialised mechanisms, while saturated and monounsaturated fatty acids are synthesised in the brain (Edmond *et al.,* 1998). In brain, LA and LNA are metabolised to LCPUFA in astrocytes but not in neurons, as shown in primary cultures of rat neurons and astrocytes (Moore *et al.,* 1991).

Unlike plants, animals are unable to insert double bonds at n-6 and n-3 positions, and can therefore not synthesise linoleic (LA), 18:2 (n-6) and linolenic acid (LNA), 18:3 (n-3). These are therefore essential fatty acids (EFA) in the human diet (Innis, 1991). EFA are precursors for LCPUFA, containing 20 or more carbons synthesised via several steps of desaturation and elongation, mainly in the liver (Innis, 1991; Voss *et al.,* 1991; Sprecher *et al.,* 1995) as shown in *Figure 1*. The enzymes active in the process of desaturation and elongation are common for

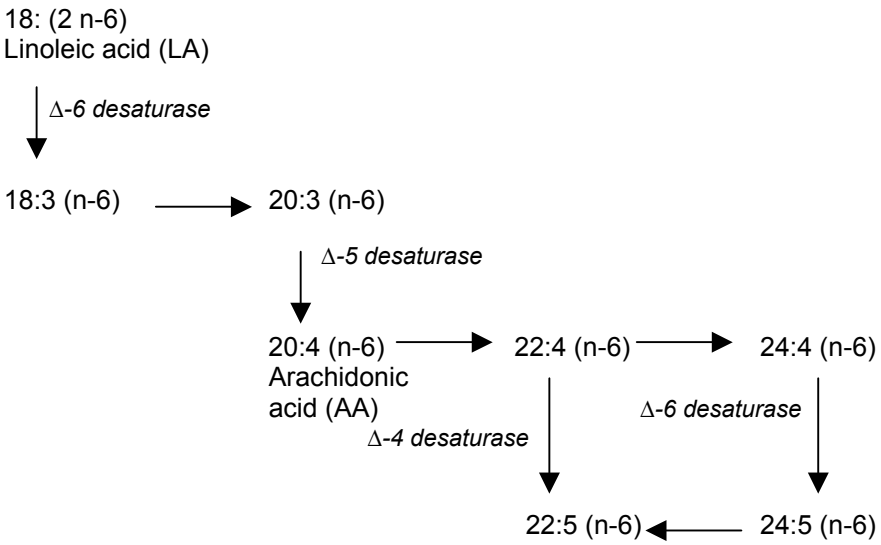

Figure 1a. Schematic presentation of the desaturation and elongation steps involved in the conversion of 18:2 (n-6) to 22:5 (n-6), two alternative pathways. Vertical and horizontal lines represent desaturation and elongation steps, respectively.

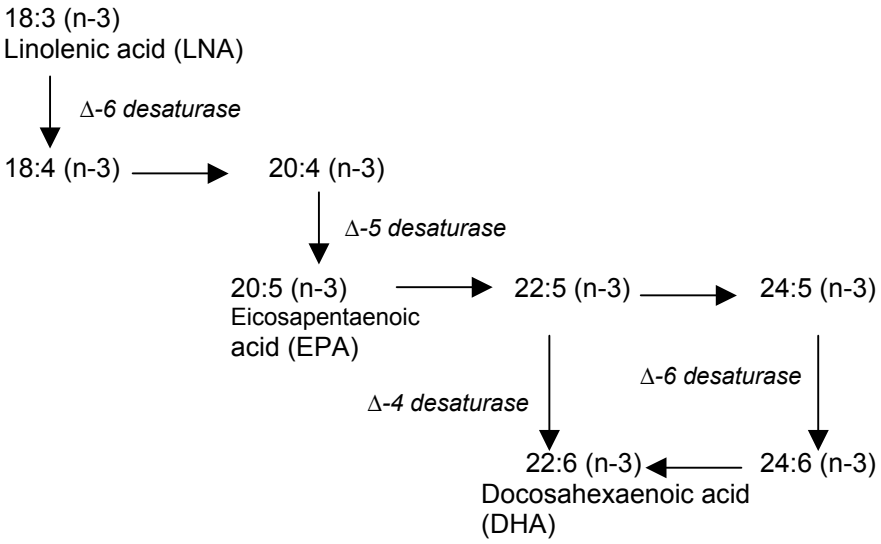

Figure 1b. Schematic presentation of the desaturation and elongation steps involved in the conversion of 18:3 (n-3) to 22:6 (n-3), two alternative pathways. Vertical and horizontal lines represent desaturation and elongation steps, respectively.

the n-6 and n-3 families of fatty acids, resulting in competition between LA and LNA for desaturation. A balance of the ratio n-6/n-3 in the diet is therefore important The fatty acids form larger molecules, of which the two main classes will be shortly described here. A triacylglycerol (also named triglyceride) consists of three fatty acids held in ester linkage to glycerol. Triacylglycerols are stored in droplets in the cytoplasm of many cells, and the function is mainly to store energy within the cell. Phospoholipids are the main constituents of cell- and intracellular membranes. They consist of one or two fatty acids, bound to phosphorylated alcohols, the phosphate group attached to a variety of small polar groups.

The high levels of AA, 20:4 (n-6) and DHA, 22:6 (n-3) in the CNS and retina are important for normal function (Innis, 1991). In the cerebral cortex and retina the levels of DHA reaches about 50% of the total phospholipids. Animal studies have shown that diets low in EFA reduce brain AA and DHA levels, and there are indications of a correlation between learning impairment and reduced levels of AA and DHA (Yamamoto *et al.,* 1987; Bourre *et al.,* 1989; Enslen *et al.,* 1991; Frances *et al.,* 1996). Further, AA and its metabolites have been considered as possible candidates for messenger function in the formation of learning and memory in hippocampus (Katsuki & Okuda, 1995). Recent studies indicate that AA and DHA may play a role in dopamine and serotonin metabolism in EFA-deficient piglets and rats (Delion *et al.,* 1996; De la Presa Owens & Innis, 1999; De la Presa Owens & Innis, 2000). In piglets deficient in AA and DHA resulting in altered phospholipid composition in brain, levels of dopamine, serotonin and its metabolite hydroxyindoleacetic acid were decreased, a condition which was not seen after AA and DHA supplementation (De la Presa Owens & Innis, 1999). Breast milk supplies the neonate with EFA, AA and DHA. In human milk AA and DHA are present at concentrations <1% (Jensen *et al.,* 1995).

Cadmium - an environmental contaminant

The adverse health effect considered as the critical after long term exposure to cadmium is renal dysfunction (WHO, 2001). Recently, bone effects, *i.e.* decreased bone density and increased risk for fractures, have been raised as another possible adverse effect at environmental exposure levels (Staessen *et al.,* 1999; Alfvén *et al.,* 2000). Cadmium is also classified as a human carcinogen (IARC, 1993). Food, especially of vegetable origin, is the main source of cadmium exposure in the non-smoking population. Exposure to cadmium via food is inevitable, since cadmium in the soil is readily taken up by plants (Hedlund *et al.,* 1997). Uptake in plants depends on several factors besides levels of Cd in soil, like organic matter content in soil, pH in soil, species and variety (Öborn *et al.,* 1995; Eriksson *et al,* 2000). Cadmium has a natural occurrence in soil, but there is also input from anthropogenic sources like atmospheric deposition and application of fertilisers of varying origin to agricultural soils (Hedlund *et al.,* 1997; Lindén *et al.,* 2003). Measures have been taken in an attempt to keep cadmium exposure on a safe level in food. Such measures are for instance reductions in cadmium emissions and regulations on maximum permitted levels in food and fertilisers (Hedlund *et al.,* 1997; EEC, 2001). The maximum levels for cadmium in food are based on a "Provisional Tolerable Weekly Intake" (PTWI) established by a Joint FAO/WHO Expert Committee (JECFA). The latest update resulted in a PTWI of 7 µg/kg body weight (WHO, 2001).

Kinetics and metabolism

In man, cadmium exposure occurs through ingestion or inhalation, ingestion being the dominating route in the non-smoking and non-occupationally exposed population (WHO, 2001). Data on gastrointestinal absorption in humans are limited, average absorption is reported to be 5% (WHO, 1992), but variations pending *e.g* type of diet is also observed (Sharma *et al.,* 1983; Vahter *et al.,* 1996). Female volunteers exposed to a single dose of stable cadmium isotope absorbed on average 11% of given cadmium dose, with a range of 2-18% (n = 14) (Vanderpool & Reeves, 2001). Higher cadmium levels in urine and blood in environmentally exposed females compared to males have been reported, indicating a higher gastrointestinal absorption in females (Buchet *et al.,* 1990; Baecklund *et al.,* 1999; Olsson *et al.,* 2002). The gastrointestinal absorption of cadmium in animals is dependent on several factors like *e.g* species, cadmium dose and type of diet (Kello & Kostial, 1977; Engström & Nordberg, 1979a; Sasser & Jarboe, 1980; Lehman & Klaassen, 1986). Gastrointestinal absorption is also affected by age and neonatal and young mice have a higher absorption than adult rodents (Kello & Kostial, 1977; Engström & Nordberg, 1979b). Also calcium content in diets alter gastrointestinal absorption, calcium deprivation resulting in increased cadmium absorption and vice versa (Nordberg *et al.,* 1985; Saric *et al.,* 2002). Low iron status increases cadmium absorption in the gastrointestinal tract in rodents and in humans (Flanagan e*t al.,* 1978; Berglund *et al.,* 1994; Schümann *et al.,* 1996).

Once absorbed, cadmium is transported in the blood mainly in the erythrocytes. Cadmium in plasma is initially bound to proteins with high molecular weight generally believed to be albumin, later a fraction is bound to a low molecular

protein of the same size as metallothionein (MT) (Nordberg, 1978). MT is a group of small metalloproteins (6-7 kDa), rich in sulfhydryl groups, readily inducible, with a high affinity for essential and non-essential metals, like zinc, cadmium, mercury and copper. MT can bind up to 7 metals. Interaction between cadmium and zinc in the induction of MT is well established (Nordberg & Nordberg, 2000; Cherian, 1994). Of special importance is the MT induction in the liver, the Cd-MT complex being slowly released into plasma. Cd-MT in plasma is quickly cleared by the renal glomeruli and finally reabsorbed in the renal tubular cells, or excreted in the urine (Nordberg & Nordberg, 1988). Cadmium is selectively accumulated in the renal cortex and the liver, and it is estimated that up to 75% of the total cadmium body burden is present in these tissues. The biological half time for cadmium is extremely long, as it is estimated to be 10-30 years in human kidney cortex (WHO, 1992). In the kidneys Cd-MT is filtered through the glomeruli and reabsorbed in the proximal tubular cells. After degradation in lysosomes, the released cadmium ions induce synthesis of MT. When the synthesising capacity of MT is exceeded, cadmium will induce tubular dysfunction (Nordberg & Nordberg 2000).

The importance of MT in the kinetics and toxicity of cadmium is also proven by the fact that MT knockout mice, with no capacity to synthesise MT, show higher sensitivity to cadmium renal toxicity despite lower cadmium uptake in the kidney (Liu *et al.,* 1998, 2000). Thus, MT protects from cadmium toxicity (Klaassen & Liu, 1998). The precise role of MT under physiological conditions has not been resolved yet (Palmiter, 1998). Besides protecting from cadmium toxicity, this highly conserved metalloprotein is suggested to be involved in the transport and storage of the essential metals zinc and copper, and it also acts as a free radical scavenger (Thornalley & Vasak, 1985; Min *et al.,* 1991; Bauman *et al.,* 1992; Dalton *et al.,* 1994; Klaassen *et al.,* 1999).

Cadmium in brain – uptake and toxicity

Cadmium uptake in brain is age dependent. In adult rats, there is a high cadmium uptake in choroid plexus, while only small amounts pass over to the cerebral compartment via choroid plexus (*i.e.* blood-cerebrospinal fluid barrier) or the blood brain barrier. This results in a low cadmium uptake in the brain parenchyma (Nordberg & Nishiyama, 1972; Arvidson & Tjälve, 1986; Takeda *et al,* 1999). In neonatal rodents there is also an uptake in choroid plexus, but cadmium uptake in brain is higher than in adults (Wong *et al.,* 1980; Choudhuri *et al.,* 1996), and appears to be related to the ontogeny of the brain vascular system (Valois & Webster, 1987). A long half life (i.e. >100 days) for cadmium in brain of neonates was also noted.

Acute cadmium exposure to adult rats and mice causes hemorrhagic lesions in the sensory ganglia, but has no effect on the brain (Gabbiani *et al.,* 1967a). In mice, considerable strain differences in sensitivity to cadmium toxicity in sensory ganglia are reported (Habeebu *et al.,* 2001). Lesions in several regions of the brain and altered spontaneous activity have been reported after administration of a single, sublethal dose of cadmium to young rats (Wong & Klaassen, 1982; Ruppert *et al.,* 1985). After chronic oral cadmium exposure the choroid plexus

was degenerated in adult mice, resulting in increased protein content in the cerebrospinal fluid (Valois & Webster, 1989). Acute exposure to cadmium during the neonatal period on the other hand, results in hemorrhage, edema and necrosis within the brain (Gabbiani *et al.,* 1967b; Webster & Valois, 1981; Wong & Klaassen, 1982). The sensitivity to these profound effects seems to abruptly end at about 3 weeks of age in mice (Webster & Valois, 1981; Valois & Webster, 1987). Further, an increased expression of metallothionein in brain with age, and an inverse correlation between expression of metallothionein and cadmium accumulation in brain was shown in mice (Choudhuri *et al.,* 1996). Thus, an undeveloped blood-brain barrier and a low expression of scavenging metallothionein may facilitate uptake and damage of cadmium to the developing brain.

Changes in neurotransmitter metabolism in the central nervous system after cadmium exposure have been reported. Thus, high doses of cadmium to adult rats resulted in decreased brain levels of serotonin (5-HT), its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and acetylcholine (ACh) (Das *et al.,* 1993). Changes in pup brain levels of 5-HT, dopamine (DA), noradrenaline (NA) or gamma-aminobutyric acid (GABA) have been reported after pre- and postnatal cadmium exposure (Rastogi *et al.,* 1977; Gupta *et al.,* 1993; Antonio *et al.,* 1998; Gutiérrez-Reyes *et al.,* 1998; Lafuente *et al.,* 2000; Esquifino *et al.,* 2001). However, the changes are divergent, possibly explained by differences in experimental design like *e.g.* cadmium dose, timing in age at exposure and measurement of outcome, and brain regions investigated.

Impaired learning performance in rodents has been reported after repeated cadmium exposure (Gupta *et al.,* 1993). Lower repeated cadmium exposure resulted in increased locomotor activity in neonatal rats (Rastogi *et al.,* 1977; Smith *et al.,* 1982). Also prenatal exposure to cadmium has been reported to change normal behaviour in rodents. Alterations in activity, delayed development of motor coordination and decreased learning have been demonstrated in offspring of dams orally exposed to low levels of cadmium during gestation (Ali *et al.,* 1986; Lehotzky *et al.,* 1990). Pre- and postnatal cadmium exposure decreased exploratory behaviour and altered electrophysiological functions in pups (Nagymajtényi *et al.,* 1997; Dési *et al.,* 1998).

To conclude, there are reports of cadmium induced neurobehavioral alterations during development, but the cadmium doses in several of these studies have been relatively high. In addition to behavioural effects, reduction in body weight gain are reported in some of the studies (Rastogi *et al.,* 1977; Wong & Klaassen, 1982; Ruppert *et al.,* 1985; Ali *et al.,* 1986; Gupta *et al.,* 1993; Nagymajtényi *et al.,* 1997).

Aims of the thesis

The overall aim of the thesis was to increase the knowledge of cadmium distribution and adverse effects in the central nervous system, during lactation, and during early life in the offspring, using rodents as a model. The aim has also been to conduct low–dose exposure studies relevant for risk assessment of cadmium.

More specifically, the aims were to study

- distribution of cadmium in the mammary gland and milk during lactation
- the milk transfer of cadmium and the uptake in the suckling pup
- neurochemical effects of cadmium after postnatal exposure via milk
- neurobehavioural effects of cadmium after postnatal exposure via milk
- possible mediators of CNS effects

Materials and methods

The methods used are described in detail in each paper. In this section a short overview of the methods is given. The animal experiments have been performed in line with the Swedish Animal Protection Act and approved by local ethical committees in Uppsala (paper I and II) or South Stockholm (paper III and IV).

Animals and treatment

All dams with litters were housed in separate cages. The animals were fed commercial pelleted diet and given tap water ad libitum. They were kept behind strict hygienic barriers at controlled temperature and humidity, with a 12:12 h light /dark cycle. The litter sizes were adjusted to eight pups per litter on the day of parturition (paper I and II), or day 4 after parturition (paper III and IV). In paper I, radioactive cadmium, ^{109}Cd was administered to lactating dams via intravenous infusion from osmotic minipumps to maintain constant and continuous cadmium exposure for 2 weeks. In paper II lactating dams with litters were exposed to cadmium as $CdCl_2$ in drinking water at a dose of 5 mg Cd/l or control water. The pups were exposed to cadmium either during the suckling period via milk, during three weeks postweaning via drinking water, or during both periods. In paper III

and IV, lactating dams were exposed to cadmium as $CdCl_2$ in drinking water at doses of 0, 5 or 25 mg Cd/l. During the suckling period the pups were exposed to cadmium exclusively via milk. Body weight, feed and water consumption was recorded in paper II, III and IV. In paper I body weights were recorded.

Milking procedure

In paper I and IV, milk was sampled from lactating dams. The pups were separated from the dams 2 h before milking to allow milk to accumulate in the mammary glands, and were returned to the dams when the milking was finished. The dams were anaesthetised with a mixture of Hypnorm (Janssen, Belgium) and Dormicum (Roche, Switzerland), 8.2 mg/kg body weight/ 4.1 mg/kg body weight respectively, administered intraperitoneally. To facilitate milk letdown a subcutaneous injection of 6.25 IU oxytocin/kg body weight (Syntocinon, 5 IU/ml, Sandoz, Basle, Switzerland) was given subcutaneously a few minutes prior to milking. From each dam approximately 2-3 ml milk was obtained by a milking device operated under vacuum as previously described (Oskarsson, 1987).

Determination of cadmium and zinc

Levels of radioactive cadmium, ^{109}Cd, in samples were determined using a gamma counter at the characteristic line of 88 keV photoemmision (paper I). When analysing non-radioactive cadmium and zinc, vessels and utensils were acid washed in order not to contaminate the samples. The tissues were dry ashed or digested in a microwave oven. Cadmium was determined by graphite furnace atomic absorption spectrometry (GFAAS), with Zeeman background and zinc by flame atomic absorption spectrometry (FAAS). In parallel with the samples in paper II, reference material of bovine liver was analysed. The results from cadmium analyses deviated from the certified concentrations, 0.78 ± 0.05 mg/kg w.w and 0.50 ± 0.03 mg/kg w.w, respectively. In parallel with the milk samples reference material, A11 milk powder from International Atomic Energy Agency (IAEA) was analysed. In paper III, reference materials, BCR 186 (pig kidney) and BCR 184 (bovine muscle), (Community Bureau of Reference, BCR, Brussels, Belgium) were run in each round of microwave digestion. The results were well within the 95% confidence interval of the certified value.

Metallothionein

In paper I, the relative binding of ^{109}Cd to the metallothionein (MT) in the cytosolic fraction was studied. Liver and mammary tissue were homogenised and centrifuged at 12 000 × *g* for 4 min. The supernatants were stored at –70 ºC before ultra-centrifugation at 100 000 × *g* for 2 h. The pellets and the ultra-centrifugation supernatants were analysed for radioactivity in the gamma counter. Proteins were separated by gel filtration on a Sephadex G-75 column. Fractions were analysed for radioactivity and the absorbance was measured at 280 nm.

Autoradiography

In paper I, four lactating BSVS mice were injected intravenously with $^{109}CdCl_2$ in physiological saline (5 µCi/dam; specific activity 1.87 µCi/µg Cd) on day 13 of lactation. At 4, 24, and 72 h and 7 days after administration one dam and one pup from each litter were killed by exposure to CO_2, embedded in carboxymethyl cellulose and frozen in hexane cooled with dry ice. Sagittal whole-body sections (20 µm) attached onto tape were taken through the whole animals in a cryostat at –20 ºC as originally described by Ullberg (1977). The sections were freeze dried at –20 ºC and apposed against X-ray films at 4 ºC. The sections and films were separated and developed after 23 days for sections of dams and 9 months for sections of pups.

Neurochemical tests

In paper II, effects on monoaminergic and cholinergic transmitter systems as well as neurotrophins, and other markers of toxicity were characterised. Glial fibrillary acidic protein (GFAP), acetylcholinesterase (AChE), and tyrosine hydroxylase (TH) were studied by immunohistochemistry. Sections of hippocampus and cerebral cortex were stained with cresyl violet and examined using epifluorescence microscopy. In situ hybridization was used for identification of mRNAs for BDNF, NT-3 and trkB. Concentrations of neurotransmitters were determined after sonication and centrifugation of brain tissue. Acetylcholine (ACh) levels were measured using HPLC and electrochemical detection. Noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were separated on a reverse phase column and detected electrochemically.

Behavioural tests

In paper III behavioural tests were performed on rat pups exposed to cadmium via milk. Spontaneous locomotor behaviour was measured by placing the rats individually in plastic cages equipped with photobeams. The activity was measured by recording breakage of the beams during 6 consecutive periods of 10 minutes. Small movement activity (repetitive breaking of the same beam), ambulation (breaking of consecutive beams), rearing (breaking of high level beams) and total activity (the sum of all counts) were recorded. A rotarod/treadmill was used for testing motor coordination. The animals were individually placed on a rotating rod, located 1 m above the floor. The time until the rat fell off the rod was registered. Learning and memory were tested in a water E-shaped maze. A hidden ladder was placed at the left arm and the rat was placed in the water in the central arm of the maze in a standardised manner. The time for the rat to locate the ladder and numbers of correct first choices were recorded. A week later the trial testing memory was conducted. The elevated plus-maze was used for testing of anxiety. The apparatus consists of 4 crossed arms, two being open and two being enclosed, placed about 0.65 m above the floor. The rat was placed in the centre of the maze, and the number of entries in open and closed

arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 minutes were recorded. The Morris water maze was applied as another way of testing learning. The rats were individually placed in a circular pool filled with tap water. A small platform was located 1 cm below the water surface, and the time for the rat to find the platform was recorded. Five consecutive trials were performed, each from a new starting point and the test was repeated for 3 consecutive days.

Fatty acid analysis

In paper IV lipids in milk, liver and brain were analysed for fatty acid composition. First, the lipids were extracted according to Hara & Radin (1978) using hexan:isopropanole. In the case of liver, the lipids were separated into membrane lipids, *i.e.*, phospholipids, and triglycerides using preparative thin-layer-chromatography. The solvent system used was hexane:diethylether:acetic acid 85:15:1. The fatty acids were converted to methyl esters (FAME) for further analysis by gas chromatography according to the methods of Dutta & Appelqvist (1989) and Appelqvist (1968). A fused silica capillary column was used for the separation of fatty acid methyl esters using helium as a carrier gas, using a flame ionization detector and split/splitless injector. The peak areas were identified by comparing the retention times with those of standard FAME samples.

Statistics

In paper I, II, and III, analysis of variance (ANOVA) was used for comparing the groups. If there were significant differences, Scheffé's, Fisher's or Tukey's post hoc tests were applied. In the case of two groups only (paper II), Student's t-test was applied. The data from the spontaneous locomotor activity test, Morris water maze and the E-maze (paper III) were tested by repeated measures ANOVA. Normality distribution was tested before analysis, and in papers III and IV also equality of variances. If the criteria for parametric methods were not fulfilled, the Kruskal Wallis test was used. In all papers, differences were considered statistically significant at $p < 0.05$. The statistical analyses have been conducted using software Minitab (Minitab Inc.) and StatView (SAS Institute, N.C., U.SA).

Results and discussion

Kinetics of cadmium in lactating rodents and their offspring

In paper I, intravenous infusion was used in order to maintain a low and continuous cadmium exposure. In dams infused with cadmium from day 3 to day 16 after parturition, cadmium levels increased in plasma and milk in a dose-dependent manner, and were approximately twice as high on day 16 of lactation compared to day 10. The milk/plasma ratio varied between 2 and 6, indicating an active transport of cadmium from plasma to milk. Whole blood levels of cadmium

were low. The proportion of cadmium in plasma in relation to whole blood ranged from 7% in the lowest dose group to 19% in the highest. The small amounts of cadmium available in plasma may partly explain the low levels of cadmium transferred to milk. For comparison, milk/plasma ratios of methylmercury, inorganic mercury and lead in rodents, after a single i.v. dose, were approximately 0.10, 0.25 and 100, respectively (Palminger Hallén & Oskarsson, 1993; Sundberg *et al.,* 1998).

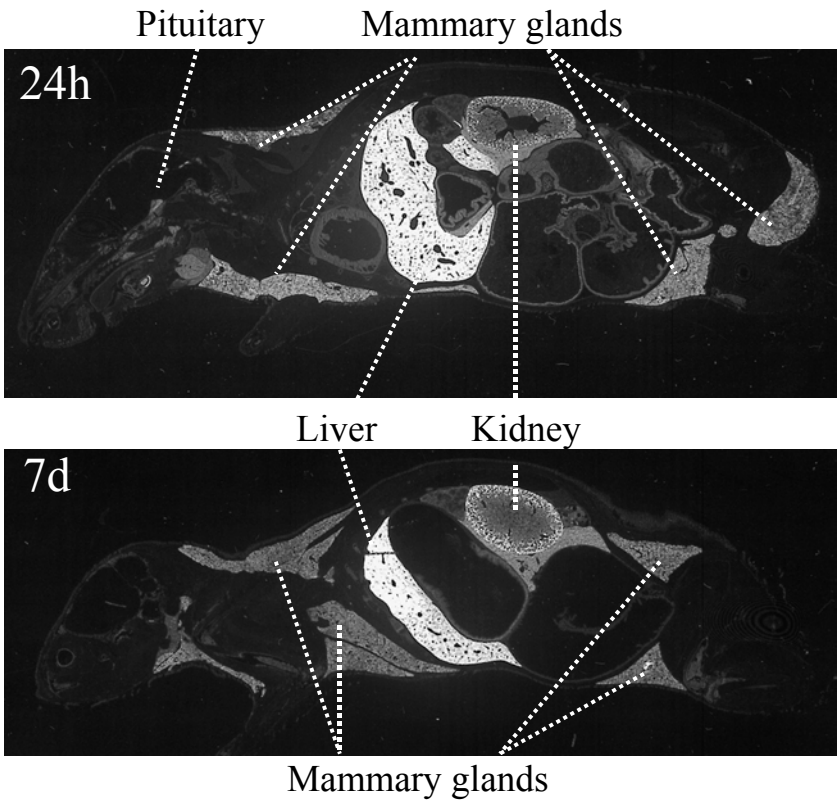

Figure 2. Autoradiograms of lactating mice on day 13 of lactation, given an intravenous injection of $^{109}CdCl_2$ (5µCi) in saline and killed 24 h and 7 days later. At 24 h there is a high level of radioactivity (white areas) in liver, kidney cortex and mammary gland. A high uptake in the intestinal mucosa is also visible.The distribution pattern is similar after 7 days.

Cadmium exposure during lactation resulted in a considerable uptake and long retention of cadmium in the mammary tissue of rats and mice, in consistency with previous reports (Bhattacharyya *et al.,* 1981, 1982). The uptake was rapid, as shown in lactating mice. Whole-body autoradiography after a single intravenous injection of tracer dose of $^{109}CdCl_2$ (*Figure 2a*) showed a high uptake of radioactivity in liver, kidney, pancreas and mammary tissue. This pattern persisted 7 days after exposure (*Figure 2b*).

In the mammary gland the radioactivity was distributed in a non-uniform pattern with a high uptake in the epithelium of the lactiferous ducts and a low uptake in the lumen. Cadmium kidney concentrations of the suckling pups increased with dose, although the levels were 3 to 4 orders of magnitude lower than in the kidneys of the dams. A high correlation between cadmium concentrations in milk and kidney of pups was found, indicating that cadmium concentrations in pup kidneys could be used as a biomarker for cadmium exposure, despite the immature renal function. At 24 h and at 7 days after exposure of the dams, ^{109}Cd was present in the stomach and intestinal content and mucosa of the suckling pup (*Figure 3a*).

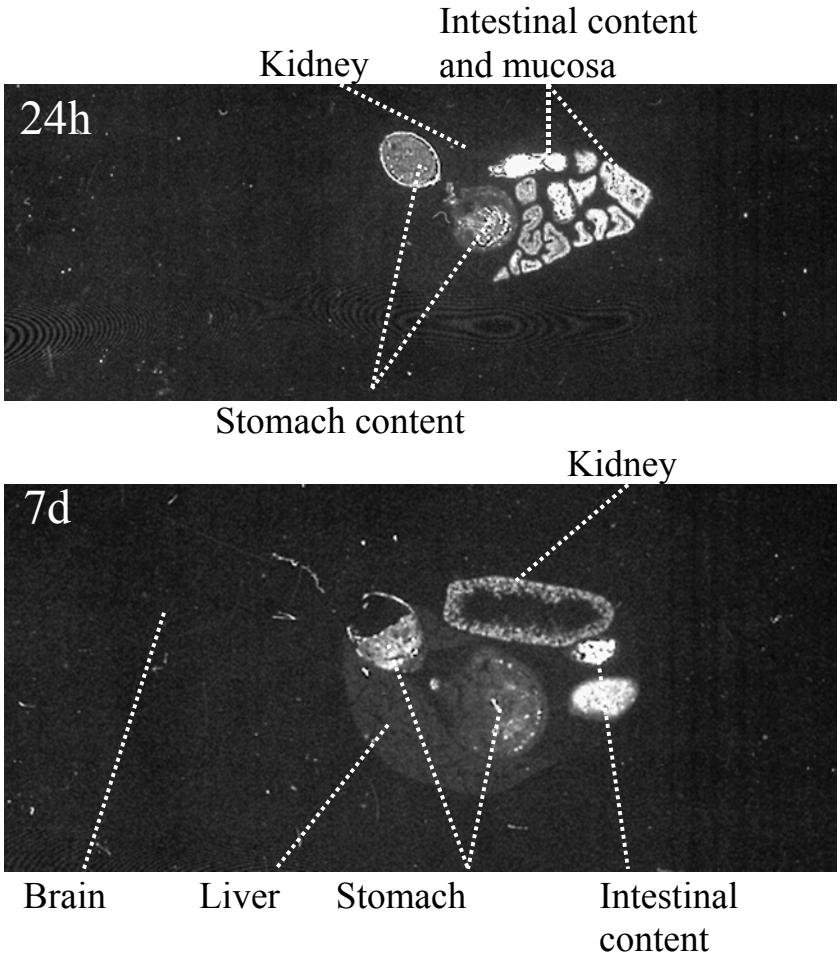

Figure 3. Details of autoradiograms of a suckling pup 24 h and 7 days after an intravenous injection of $^{109}CdCl_2$ (5 μCi) in saline given to the dam on day 13 of lactation. At 24 h a high level of radioactivity was observed in the stomach and intestinal content as well as in mucosa, but there was no radioactivity in the kidney or any other tissue. At 7 days uptake of radioactivity was observed in the kidney cortex and liver

This is in accordance with previously reported high cadmium uptake and retention in the intestinal mucosa of rodent pups during the suckling period (Eklund *et al.,* 2001; Brako *et al.,* 2003). Retention of cadmium in the kidney cortex of suckling pups was observed in the autoradiograms at 7 days after exposure of the dams, indicating that cadmium accumulates in the proximal tubuli in the offspring as in the adult kidney (*Figure 3b*). Also the liver of the suckling pups had some uptake of radioactivity. Hepatic MT concentrations are higher in neonatal rodents compared to adults (Wong *et al.,* 1980; Goering & Klaassen, 1984; Brako *et al.,* 2003), and probably cadmium is transported to the kidney complexed to MT, as shown in adult rodents (Nordberg & Nordberg, 2000). The proportion of radioactive cadmium eluted in the fraction with a similar molecular weight as metallothionein after gel filtration increased in a dose-dependent manner in mammary tissue (57 ± 11%, 73 ± 11%, 85 ± 3%), and in liver (88 ± 1%, 96 ± 1%, 98 ± 1%) in control, low and high dose, respectively. In the liver cytosol the difference was statistically significant between all dose groups, while in the mammary gland the difference was significant between the high dose and control group only. Metallothionein has been found in mammary gland cells in non-pregnant and pregnant guinea pigs and in mice (Nishimura *et al.,* 1989; Lee *et al.,* 1992). It has also been shown that plasma levels of metallothionein increase in rats during gestation (Chan *et al.,* 1993), and that metallothionein is induced in liver, kidney and duodenum in mice during lactation (Solaiman *et al.,* 2001). A suggested reason for the observed MT induction during gestation and lactation is the increased demand for essential trace elements like zinc and copper, which also could result in the high uptake of cadmium in the mammary tissue. On the other hand, Brako and coworkers (2003) showed that cadmium uptake in mammary tissue as well as in the liver was higher in MTI/MTII knock-out mice compared to in wildtype mice. The high cadmium uptake in the liver and mammary gland of MT knockout mice may at least in part be explained by higher amounts of cadmium available in plasma. It is not known what cadmium is bound to in the tissues of the MT-knockout mice. Evidently there are other binding sites in liver and mammary gland, besides MT. By comparing the retention of cadmium in knock-out mice and wildtype mice it has been shown that MT plays a major role in the elimination of cadmium from other tissues than the kidney (Liu *et al.,* 1996). Also, at marginal zinc intakes in lactating rats, zinc concentrations in plasma, mammary gland, and milk were maintained, while mammary gland MT levels were decreased (Kelleher & Lönnerdal, 2002). A possible explanation could be that there is no need for zinc storage in the mammary gland under conditions of zinc deficiency. Zinc bound to MT may undergo exchange reactions transferring zinc to other proteins (Klaassen *et al.,* 1999). Cadmium has a higher affinity for MT than zinc and the Cd-MT complex is more stable in the tissues. This could explain the cadmium-induced increase of zinc in milk in our study, *i.e.* MT binding sites for zinc in the mammary gland were depleted due to cadmium uptake, and thus more zinc was available for excretion. Further, the data may indicate that MT induction was saturated in the mammary tissue, since there was no increase in the fraction of cadmium eluted in the MT fraction in the high compared to the low dose group. This was in contrast to the liver, where the fraction of cadmium eluted in the MT fraction increased during the whole dose range.

To conclude, the results in our study demonstrate a low, but consistent, transfer of cadmium via milk to the suckling pup. The uptake and retention of cadmium in the mammary tissue was prominent, and a dose-related increase in proportion of cadmium in the cytosolic MT–fraction was observed. The role of MT in uptake and retention of zinc and cadmium in the mammary epithelial cells remains unclear, but it seems probable that MT functions as a storage protein in the tissues.

Cadmium in milk and effects on milk secretion

^{109}Cd was distributed in fat (46-52%), casein fraction (40-46%), and whey fraction (6-8%) in milk (paper I). In human milk significant amounts (15-30%) of copper, zinc, calcium and iron are associated with the fat fraction, mainly bound to the outer fat globule membrane (Fransson & Lönnerdal 1982, 1984). It has been suggested that iron and zinc bind to xanthine oxidase and alkaline phosphatase present in the outer fat globule membrane in human milk (Fransson & Lönnerdal 1982, 1984). Both enzymes are metal-carrying; alkaline phosphatase with four specific binding sites for zinc and xanthine oxidase with eight specific binding sites for iron per molecule (Rumball & Baker, 1985). Since cadmium is known to interact with zinc, it may be speculated that cadmium in the milk fat fraction also binds to these enzymes. There were no alterations of protein or lactose concentrations in milk following cadmium exposure. Neither were there were any effects on DNA or RNA levels or the RNA/DNA ratio, or any abnormalities in the histological evaluation of the mammary tissue at any dose level.

The binding of cadmium to the casein fraction might also be explained by the similarities between cadmium and zinc. Zinc is bound to the phosphoserine groups in casein in the human and bovine milk (Singh *et al.,* 1989). Cadmium also interacts with calcium (Goyer, 1997), and since casein is rich in calcium, this could also explain the presence of cadmium in the casein fraction. There are large interspecies differences in casein concentration in milk. For instance, in human milk the casein levels are 2-6 mg casein/ml compared to 60-90 mg casein/ml in rat milk (Jenness, 1973; Lönnerdal & Fossum, 1985). Lead is known to be excreted via milk in relation to casein concentrations, and consequently, lead excretion varies between species pending casein content (Palminger Hallén & Oskarsson*.,* 1995). Cadmium levels in milk are low in all species investigated; rodents (Lucis *et al.,* 1972; Tanaka *et al.,* 1972), sheep (Houpert *et al.,* 1997), cattle (Smith *et al.,* 1991) and humans (Vuori et al., 1983; Dabeka *et al.,* 1986; Schramel *et al.,* 1988; Palminger Hallén *et al.,* 1995; Biego *et al.,* 1998; Rodríguez Rodríguez *et al.,* 1999), which indicate that the mechanism for cadmium excretion in milk differs from lead excretion, despite the high fraction present in the casein fraction in both cases.

Cadmium in the whey fraction was low and may partly consist of Cd-MT complex. In human breast milk, free from lipid and lipid associated proteins, thus corresponding to the casein and whey fractions in our study, cadmium was found in the same fractions as the metallothionein standard (Michalke & Schrammel, 1990). The discrepancy may depend on species differences.

Zinc levels in milk were positively correlated to cadmium levels in milk ($y = 0.61x + 9.0$; $r^2 = 0.26$; $p = 0.03$). Zinc concentrations in milk are highly conserved over a wide range of dietary zinc intake in both humans and rodents, indicating that zinc transport from the mammary gland to milk is tightly regulated (Kelleher & Lönnerdal, 2002). Thus, the small increase in milk zinc concentrations found here indicates that cadmium treatment imposed a pathway, although minor, for zinc excretion in milk. Excretion of zinc and cadmium in milk in rats may not depend on identical excretion mechanisms. After single doses of radioactive isotopes of zinc or cadmium, since zinc levels in milk showed a rapid peak and decline, compared to cadmium, which was excreted at continuously low levels and with a slow decline (Lucis *et al.,* 1972).

The increased zinc excretion in our study may be explained by cadmium-induced depletion of intermediate intracellular binding sites for zinc, as discussed above, preceding the excretion via zinc transporter proteins, as proposed by Kelleher & Lönnerdal (2002). Another possibility is that cadmium in the mammary gland modulates the zinc transporter proteins. In liver cells it has been shown that cadmium and zinc induce mRNAs for ZnT-1, a zinc export protein (Langmade *et al,* 2000), which is also identified in mammary gland (Kelleher & Lönnerdal, 2002). However, at present there are no reports on interactions between cadmium and ZnT-4, the highly expressed zinc transporter in mammary epithelial cells (Huang & Gitschier, 1997).

A significant decrease ($p = 0.03$) in medium-chain fatty acids (8:0-14:0) and an increase in 16:0 ($p = 0.007$) were observed in milk of rats in the high dose group compared to the control group (paper IV). An explanation to this finding could be reduced activity of an enzyme specific for the mammary tissue, thioesterase II, which contains three cystein residues (Randhawa & Smith, 1987). The medium-chain fatty acids are synthesised de novo in the mammary epithelial cells through the activation of thioesterase II. Thioesterase II terminates the fatty acid chain at 8-12 carbons. In the absence of thioesterase II, the elongation proceeds to 16 carbons (Barber *et al.,* 1997). The medium chain fatty acids are considered beneficial for the neonate since they are rapidly absorbed in the gastrointestinal tract (Williamson, 1985; Hamosh, 1987). Milk from lactating mothers with preterm newborns have higher levels of medium-chain and long-chain fatty acids. A decrease in medium-chain fatty acid intake of the pups in the present study could possibly result in decreased body weight, but no such alterations were seen.

Neurochemical effects in sucklings after postnatal cadmium exposure via milk

The serotonergic system was found to be the most susceptible transmitter system in developing brain after cadmium exposure (paper III). Serotonin (5-HT)levels in cerebral cortex were reduced to approximately 70% of the levels in the control group in animals exposed during suckling or during both the suckling and postweaning periods. Further, levels of the metabolite of serotonin, 5-hydroxyindoleacetic acid, were reduced to approximately 60% of the control in cerebral cortex and hippocampus in the group exposed during suckling. In striatum the pattern was similar to that in the cerebral cortex and hippocampus, but the differences between treatment groups did not reach significance. Changes in neurotransmitter metabolism in the developing CNS after cadmium exposure have been reported, as previously described. In contrast to our results, increased 5-HT levels in pup brains were demonstrated after pre- and postnatal cadmium exposure (Rastogi *et al.,* 1977; Antonio *et al.,* 1998). The contradicting results may be due to differences in experimental design *e.g.* cadmium dose, timing in age at exposure or measurement of outcome, and brain regions investigated. Tyrosine hydroxylase, which is the rate limiting enzyme for synthesis of dopamine and noradrenaline, was decreased in the cerebral cortex of rats exposed to cadmium during both suckling and postweaning. This is in consistency with observations by Gutiérrez-Reyes and coworkers (1998), who reported a decrease in striatal tyrosine hydroxylase activity in newborn rats exposed parenterally to cadmium. There was a tendency to decreased noradrenaline levels in cerebral cortex and hippocampus that could be a result of reduced activity of tyrosine hydroxylase, but the findings could also be due to a reduced number of noradrenergic nerve terminals. Dopamine levels were not significantly altered by treatment. No changes in acetylcholine levels were found, in spite of a decrease of acetylcholine esterase in the cerebral cortex of rats exposed to cadmium during both suckling and postweaning. A cadmium-induced reduction in synthesis or transport of acetylcholinesterase could explain the observation. There were no alterations in mRNA levels of BDNF, NT-3, or trkB. No morphological alterations were observed.

It can be concluded that the serotonergic pathway was the most sensitive to cadmium exposure via milk in the neonate during the suckling period. It can be noted that compared to previous reports on neurochemical effects after postnatal cadmium exposure, the cadmium dose in the present study was very low, *i.e.* 0.8-1.2 mg Cd/kg/day p.o., to the lactating dam. It can also be concluded that the indications of cadmium altering CNS neurotransmitter metabolism during development, need further exploration.

Neurobehavioural effects in rats after cadmium exposure via milk

Cadmium-related increase in locomotor activity was observed during the initial periods of the spontaneous locomotor test (paper III). Ambulation data are illustrated in *Figure 4a*. Repeated measures ANOVA showed that the between-group main effect for ambulation was significant [$F(2,27)= 5.3$; $p=0.011$]. Also, significant treatment by time interactions were found for ambulation [$F(10,135)= 5.4$; $p<0.0001$)], small movements [$F(10,135)= 3.2$; $p<0.0001$], rearing [$F(10,135)= 6.0$; $p<0.0001$], total activity [$F(10,135)= 6.2$; $p<0.0001$)]. Multiple comparisons between groups within each 10 minute period, showed that the high dose group were significantly more active than the control group during the initial 20 minutes of the test, with the exception of rearing, for which the outcome was different from control in the first 10 minutes only. Cadmium concentrations in the kidney were used as biomarker for biological dose, see discussion below. By using linear regression analysis the individual data from all groups were used, and a significant linear relationship was found between outcome in the activity test and cadmium levels in kidneys of pups ($p < 0.01$; R^2 ranging between 0.26-0.32 for ambulation, small movements and total activity). In *Figure 4b*, linear regression analysis of the ambulation data is shown. There was no indication of a threshold level and we conclude that hyperactivity was correlated to the biological cadmium dose. No alterations in learning, memory or anxiety responses were found. In contrast, decreased learning ability as well as hypoactivity was reported in weaned rats perinatally exposed to cadmium for 30 days (Gupta *et al.,* 1993). The cadmium exposure used in the present study is markedly lower, which may explain why no effects on learning were observed. The behavioural response is similar to what Rastogi and coworkers described (1977) after repeated oral exposure in neonatal rats, which could indicate that the observed neurobehavioural effects are induced by cadmium directly, and not mediated by maternal effects resulting in altered milk composition. However, in order to elucidate this, a study exposing neonates orally to cadmium is necessary. No differences were found between groups in the outcome of the rotarod test when the first and second trials were analysed separately.

In conclusion, this study indicates that neurobehavioral effects may occur in the offspring of lactating dams at lower cadmium exposure levels than previously reported.

Body weights

In paper I and II, there were no significant differences in body weights between the groups of pups or dams. In paper III there were no differences in body weights in dams. In the litters, a significantly lower body weight was recorded at one occasion, postnatal day (PND) 17, in the high dose group compared to low dose and control groups. At PND 1, 4, 7, 10, and from PND 24 and onwards, there were no significant alterations in body weights between the treatment groups. It is known that malnutrition resulting in a persistent and more severe decrease in body weight is accompanied by increased activity in pups (Almeida *et al.,* 1993).

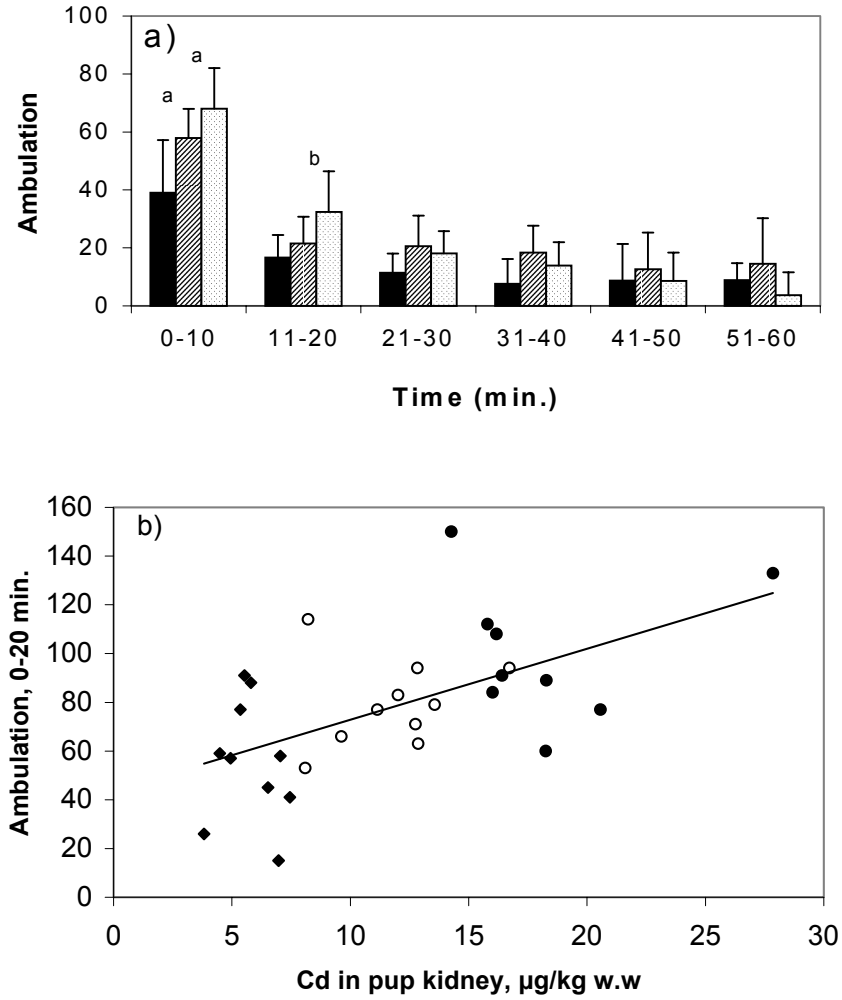

Figure 4a. Ambulation performance in the spontaneous locomotor activity test at PND 39-42 (*n*=10). Rats were exposed to cadmium during suckling via cadmium exposed dams until PND 17. The dams were exposed to cadmium chloride in drinking water at doses of 0, 5 or 25 mg Cd/l. Results are presented as mean values ± SD
[a] Significantly different from control $p< 0.01$, [b] Significantly different from control $p< 0.01$

b) The relationship between ambulation performance in the spontaneous locomotor activity test and individual Cd concentrations in pup kidney (µg/kg w.w.) sampled immediately after completion of the test. Pups were exposed to cadmium during suckling via cadmium exposed dams until PND 17. The dams were exposed to cadmium chloride in drinking water at doses of 0, 5 or 25 mg Cd/l. The relationship between ambulation and Cd in pups kidney is described by the equation: $y = 43.7 + 2.9x$, $p = 0.001$ and $r^2 = 0.32$. Diamonds: control group; open circles: low dose group; filled circles: high dose group.

The observed transient reduction in body weights by 7% on PND 17 in the present study is not a plausible explanation for the increased activity in exposed pups. In paper IV no body weight differences were observed in the female pups at sacrifice on PND 19.

Possible explanations of the CNS effects

Cadmium concentrations in the brain of pups were below the detection limit, i.e. <0.004 µg/g w.w. (paper II). Still, there were pronounced reductions in serotonin and its metabolite 5-hydroxyindoleacetic acid in cerebral cortex and hippocampus of rats exposed to cadmium during suckling. There may be two reasons for these findings: either the developing central nervous system is very sensitive to cadmium, or cadmium exerts some effect on the milk composition affecting the newborn. The non-detectable cadmium concentrations in brain are in contrast to other studies, where increased levels of cadmium are reported in brain after perinatal cadmium exposure (Gulati *et al.,* 1986; Gupta et al., 1993; Gupta et al., 1995; Antonio *et al.,* 1998; Gutiérrez-Reyes *et al.,* 1998; Antonio *et al.,* 1999; Méndez-Armenta *et al.,* 2001). However, there are differences in study design, *e.g.* cadmium dose, route and duration of exposure, which can explain this. Also, we chose to withdraw the source of exposure, namely cadmium in the drinking water, from postnatal day 17 until weaning. This was done in order to avoid the suckling pups to be directly exposed to cadmium by drinking water. Quality assurance of the cadmium analyses is also an important issue when interpreting the data, and information of this is often lacking. In paper II, analyses of reference material resulted in higher cadmium levels than the certified value, indicating contamination problems in these samples or too high signals. Anyway, the discrepancy does not imply that the cadmium concentrations in the brains were underestimated in this experiment (paper II). Altogether, from the present work (paper I), and previously reported studies it can be concluded that cadmium is distributed to the developing brain in very small amounts.

Zinc levels in brain of pups were in line with previously reported levels (Kishi *et al.,* 1982; Barański,1986; Gupta & Shukla, 1996; Erickson *et al.,* 1997), and not altered by cadmium exposure (paper IV). This is in contrast to observations made by Gupta & Shukla (1996) and Barański (1986) after cadmium exposure. However, they exposed the animals prenatally which may explain the difference. Also, the distribution of zinc in brain seems to be age dependent. In mice, zinc levels are higher in hippocampus than in other brain regions (Shin-ichi *et al.,* 1997). On the other hand, in brains of 22 day old rats zinc was evenly distributed, while zinc levels were higher in hippocampus than in other brain regions in adult rats (Kishi *et al.,* 1982). In the present study, zinc was analysed in whole brain at postnatal day 20 and at 6 weeks of age, and the zinc concentrations were remarkably constant in exposed and control groups as well as in both age groups. This indicates that cadmium treatment did not cause any change in whole brain or regional zinc levels.

The fatty acid composition in the brains of pups was characterised by a low inter-individual variation. There were no major changes in the fatty acid composition due to cadmium treatment. Levels of DHA were not altered. The (n-

6)/(n-3) ratio was not altered by treatment, indicating that the activity of the rate limiting Δ-6 desaturase was not affected. However, a small but significant increase in the proportion of 20:3(n-6) was observed, 0.84% ± 0.04 and 0.78% ± 0.01, in the high compared to the control group, respectively ($p = 0.037$). Despite 20:3(n-6) being a precursor to AA, there were no alterations in AA levels in the brain. It may be speculated that increased amounts of AA are actually synthesised, the turnover of AA in brain phospholipids is indeed very rapid (Washizaki et al (1994), and further metabolised to its eicosanoid metabolites with the formation of fatty acid radicals and oxygen radicals. It is known that AA is involved both in processes leading to tissue damage and in beneficial biological actions in the brain (Katsuki and Okuda, 1995). Thus, highly controlled levels of AA are essential for the physiological functions of the nervous system. However, it can not be excluded that the significant effect on 20:3(n-6) is spurious and further investigations are needed to confirm the results and elucidate the mechanism.

Further studies are needed in order to clarify this issue. For instance, analyses of eicosanoid metabolites, or studies of the activity of enzymes in the AA metabolic pathway should be investigated in hippocampus and cerebral cortex in the developing brain after cadmium exposure.

Cadmium exposure and biological dose in lactating rats and their offspring

Cadmium doses used in these studies have generally been low. Kidney cadmium levels have been used as a marker for biological dose in adults. However, renal function is not fully developed at birth in humans or rodents. Glomerular function develops to adult capacity during the first 6 months of life in humans, and adult renal tubuli function is reached at approximately 1 year of age (Alcorn & McNamara, 2002). Likewise, adult glomerular filtration rate is not reached in young rats until at 7 weeks of age (Provoost *et al.,* 1983; Fleck, 1992).

The exposure regimens and the resulting cadmium kidney levels in dams and pups are shown in Table 1. Since one of the endpoints of using animal models in toxicology is extrapolation to humans, a comparison to human exposure and cadmium kidney levels is also made in Table 1. Cadmium intake via food is approximately 10-20 µg/day in most Western diets, which corresponds to approximately 0.1-0.4 µg Cd/kg body wt. per day (WHO, 2001). One might argue that the doses given in the present work are high compared to human cadmium intake via food. On the other hand, in most previous studies on developmental neurotoxicity of cadmium, even higher doses and often parenteral administration have been used.

The cadmium exposure in the present study could also be compared by means of the kidney cadmium concentrations. Histopathological and biochemical changes in rat renal tubules are reported at Cd levels in rat kidney ranging from 40-200 mg Cd/kg wet weight (WHO, 1992). This should be compared with the observed CNS effects in offspring reported in the present study, at kidney Cd levels of 4-13 mg Cd/kg wet weight in lactating dams after 3 weeks of oral Cd exposure. It should also be emphasised that the pups have been exposed to, and absorbed, much lower

levels of cadmium. Thus, the results indicate that neurochemical and neurobehavioural effects during development may be a more sensitive target for cadmium toxicity in animal models than renal dysfunction.

Cadmium levels in rat milk obtained in the present study were 0.10 - 5.4 ng/ml (paper I). In human milk similar cadmium levels have been reported: < 0.002-4 ng/ml (Vuori *et al.,* 1983; Dabeka *et al.,* 1986; Biego *et al.,* 1988; Schramel *et al.,* 1988; Palminger Hallén *et al.,* 1995; Rodríguez Rodríguez *et al.,* 1999) giving support for the view that the cadmium exposure in the present study is a low dose regimen.

Table 1. *Summary of the exposure regimens used and the resulting biological doses, using kidney as a biomarker. For comparison, human exposure data are also given*

Species, and source of exposure	Duration of exposure, (days after parturition, or postnatal day)	Daily cadmium exposure, µg/kg body wt./day	Average kidney cadmium concentration*	Reference
Rat				
Drinking water				Paper II
Dams	0 - 17	700 - 1060	2.2	
Suckling pups	0 - 17		0.02	
Weaned pups	19 - 42	800 - 1200	1.21	
Suckling and postweaning	0 -17 + 19 - 42	800 – 1200**	1.26	
Drinking water				Paper III and IV
Dams	0 – 17	1100 - 4800	4 - 13	
Suckling pups	0 -17		0.02 - 0.03	
Human				
Food	Whole life	0.1 - 0.3		WHO, 2001
			6-11***	****
PTWI*****	Whole life	1	< 40***	WHO, 2001

* µg/g wet weight; whole kidney
** excluding exposure via milk
*** whole kidney concentrations estimated by dividing kidney cortex concentration by 1.25 in accordance to (Svartengren *et al.,* 1986)
**** data on non-smokers only (Elinder *et al.,* 1976; Nilsson *et al.,* 1995; Friis *et al.,* 1998; Lyon *et al.,* 1999; Nilsson *et al.,* 2000)
***** Provisional Tolerable Weekly Intake, 7 µg/kg body wt./week (renal dysfunction being the critical effect)

Concluding remarks

Using radioactive ^{109}Cd, it was shown that cadmium is transferred via milk to the pup. A strong correlation between cadmium concentration in pup kidney and cadmium concentration in the kidney and milk of the lactating dam was found, showing that cadmium is transferred via milk to the suckling pup. In addition, this indicates that cadmium kidney concentrations can be used as a biomarker for biological dose in pups, despite immature renal function.

Autoradiography showed a high uptake and retention of cadmium in the mammary epithelial cells during lactation. In lactating rat mammary tissue cadmium was found in a peak eluted at a similar molecular size as metallothionein after gel filtration. The data thus indicate that cadmium to a large extent is bound to metallothionein in the cytosol of the mammary epithelial cells. This could explain the high cadmium concentration in the mammary gland and the low cadmium concentration in the milk. In rat milk, cadmium was mainly bound to the fat and casein fractions.

In pups with brain cadmium levels below the detection limit, a marked decrease in serotonin and its metabolite 5-hydroxyindoleacetic acid was observed in cerebral cortex and hippocampus. The pups were exposed to cadmium via the milk from exposed dams. Further, there was a tendency to decreased levels of noradrenaline but the differences did not reach significance.

Pups exposed to Cd via milk from lactating rats showed increased spontaneous locomotor activity during the first period of the test compared to control. A positive linear correlation was found between initial spontaneous locomotor activity and kidney cadmium concentrations in the pups. Outcomes in tests measuring learning, memory, or anxiety were not altered by treatment.

Cadmium exposure altered the fatty acid composition in the liver and milk of the dams, and a minor modification was seen in the brain of the pups. The long-chain polyunsaturated fatty acids, which are important for normal development of the CNS, were unaltered, as were zinc levels in the brain. The results showed that cadmium treatment decreased the levels of medium chain fatty acids in milk while the levels of 16:0 were increased. This indicates that cadmium may inhibit the activity of thioesterase II present in the cytosol of the rat mammary epithelial cells. The Cd-induced CNS effects in offspring can probably not be due to changes in fatty acid composition or zinc levels in brain. It seems likely that the CNS effects are due to a direct effect of Cd in the brain.

Overall, the results indicate that neurochemical and neurobehavioral effects during development may be a more sensitive endpoint for cadmium toxicity in animal models than renal dysfunction. In most studies on cadmium neurotoxicity, high doses and parenteral exposure routes have been applied. Further research in developmental neurotoxicity of cadmium at relevant biological doses, as well as elucidation of the mechanisms for the observed effects would be of importance for risk assessment of cadmium in infants and children.

References

Ackland, M.L. & Mercer, J.F.B. 1992. The murine mutation, lethal milk, results in production of zinc deficient milk. *Journal of Nutrition 122,* 1214-1218.

Alcorn, J. & McNamara, P.J. 2002. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clinical Pharmacokinetics 41,* 959-998.

Alcorn, J., Lu, X., Moscow, J.A., McNAmamra, P.J. 2002.Transporter gene expression in lactating and nonlactating human mammary epithelial cells during real-time reverse transcription-polymerase chain reaction. *The Journal of Pharmacology and Experimental Therapeutics 303,* 487-496.

Alfvén, T., Elinder, C.G., Carlsson, M., Grubb, A., Hellström, L., Persson, B., Pettersson, C., Spång, G., Schütz, A., Järup, L. 2000. Low level cadmium exposure and osteoporosis. *Journal of Bone Mineral Research 15,* 1579-1586.

Ali, M.M., Murthy, R.C., Chandra, S.V. 1986. Developmental and longterm neurobehavioral toxicity of low-level in utero Cd exposure in rats. *Neurobehavioral Toxicology and Teratology 8,* 463-468.

Almeida, S.S., Garcia, R.A., de Oliveira L.M. 1993. Effects of early protein malnutrition and repeated testing upon locomotor and exploratory behaviors in the elevated plus-maze. *Physiology & Behavior 54,* 749-752.

Andersson, H., Lindqvist, E., Olson, L. 1997a. Downregulation of brain-derived neurotrophic factor mRNA in adult rat brain after acute administration of methylmercury. *Molecular and Chemical Neuropathology 31,* 225-233.

Andersson, H., Wetmore, C., Lindqvist, E., Luthman, J., Olson, L. 1997b. Trimethyltin exposure in the rat induces delayed changes in brain-derived neurotrophic factor, fos and heat shock protein 70. *Neurotoxicology 18,* 147-159.

Antonio, M.T., Benito, M.J., Leret, M.L., Corpas, I. 1998. Gestational administration of cadmium alters the neurotransmitter levels in newborn rat brains. *Journal of Applied Toxicology 18,* 83-88.

Antonio, M.T., Corpas, I., Leret, M.L. 1999. Neurochemical changes in newborn rat's brain after gestational cadmium and lead exposure. *Toxicology Letters 104,* 1-9

Appelqvist, L.Å. 1968. Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissues with special remarks on preventing the accumulation of lipid contaminants. *Arkiv för Kemi 28,* 550-570.

Arvidson, B. & Tjälve, H. 1986. Distribution of ^{109}Cd in the nervous system in rats after intravenous injection. *Acta neuropathologica (Berlin) 69,* 111-116.

Baecklund, M., Pedersen, N.L., Björkman, L., Vahter, M. 1999. Variation in blood concentrations of cadmium and lead in the elderly. *Environmental Research 80,* 222-230.

Barański, B. Effect of maternal cadmium exposure on postnatal development and tissue cadmium, copper and zinc concentrations. 1986. *Archives of Toxicology 58,* 225-260.

Barber, M.C., Clegg, R.A., Travers, M.T., Vernon, R.G. 1997. Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta 1347,* 101-126.

Bauman, J.W., Madhu, C., McKim, J.M., Liu, Y., Klaassen, C.D. 1992. Induction of hepatic metallothionein by paraquat. *Toxicology and Applied Pharmacology 117,* 233-241.

Beninger, R.J. 1983. The role of dopamine in locomotor control. *Brain Research Reviews 6,* 173-196.

Berglund, M., Åkesson, A., Nermell, B., Vahter, M. 1994. Intestinal absorption of dietary cadmium in women depends on body iron stores and fibre intake. *Environmental Health Perspectives 12,* 1058-1066.

Bhattacharyya, M.H., Whelton, B.D., Peterson, D.P. 1981. Gastrointestinal absorption of cadmium in mice during gestation and lactation. I. Short-term exposure studies. *Toxicology and Applied Pharmacology 61,* 335-342.

Bhattacharyya, M.H., Whelton, B.D., Peterson, D.P. 1982. Gastrointestinal absorption of cadmium in mice during gestation and lactation. II. Continuous exposure studies. *Toxicology and Applied Pharmacology 66,* 368-375.

Biego, G.E., Joyeux, M., Hartemann, P. and Debry, G. 1998. Determination of mineral contents in different kinds of milk and estimation of dietary intake in infants. *Food Additives and Contaminants 15,* 775-781.

Bourre, J.M., Francois, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G., Durand, G. 1989. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *Journal of Nutrition 119,* 1880-1892.

Brako, E.E., Wilson, A.K., Jonah, M.M., Blum, C.A., Cerny, E.A., Williams, K.L., Bhattacharyya, M.H. 2003. Cadmium pathways during gestation and lactation in control versus metallothionein 1,2-knockout mice. *Toxicological Sciences 71,* 154-163.

Buchet, J.P., Lauwerys, R., Roels, H., Bernard, A., Bruaux, P., Claeys, F., Ducoffre, G., de Plaen, P., Staessen, J., Amery, A., Lijnen, P., Thijs, L., Rondia, D., Sartor, F., Saint Remy, A., Nick, L. 1990. Renal effects of cadmium body burden of the general population. *Lancet 336,* 699-702; 1991 337, 1554.

Chan, H.M., Tamura, Y., Cherian, G.M., Goyer, R.A. 1993. Pregnancy-associated changes in plasma metallothionein concentration and renal cadmium accumulation in rats. *Proceedings of the Society for Experimental Biology and Medicine 202,* 420-427.

Cherian, M.G. 1994. Metallothionein and its interaction with metals. In: *Toxicology of metals. Biochemical aspects. Vol. 115, Handbook of Experimental Pharmacology* Eds. Goyer, R.A. and Cherian, M.G. Springer Verlag, Berlin. pp 121-137.

Cho, J.Y., Leveille, R., Kao, R., Rousset, B., Parlow, A.F., Burak, W.E. Jr., Mazzaferri, E.L., Jhiang, S.M. 2000. Hormonal regulation of radioiodide uptake activity and Na^+-I^- symporter expression in mammary glands. *The Journal of Clinical Endocrinology & Metabolism 85,* 2936-2943.

Choudhuri, S., Liu, W.L., Berman, N.E.J., Klaassen, C.D. 1996. Cadmium accumulation and metallothionein expression in brain of mice at different stages of development. *Toxicology Letters 84,* 127-133.

Clandinin, M.T. 1999. Brain development and assessing the supply of polyunsaturated fatty acid. *Lipids 34,* 131-137.

Dabeka, R.W., Karpinski, K.F., McKenzie, A.D., Bajdik, C.D. 1986. Survey of lead, cadmium and fluoride in human milk and correlation of levels with environmental and food factors. *Food and Chemical Toxicology 24,* 913-921.

Dalton, T., Palmiter, R.D., Andrews, G.K. 1994. Transcriptional induction of the mouse metallothionein-I gene in hydrogen peroxide-treated Hepa cells involves a composite major late transcription factor/antioxidant response element and metal response promoter elements. *Nucleic Acids Research 22,* 5016-5023.

Das, K.P., Das, P.C., Dasgupta, S., Dey, C.D. 1993. Serotonergic-cholinergic neurotransmitters' function in brain during cadmium exposure in protein restricted rat. *Biological Trace Element Research 36,* 119-127.

Davies, D.T., Holt, C., Christie, W.W. 1983. The composition of milk. In: *Biochemistry of lactation.* Ed. T.B. Mepham, Elsevier Science Publishers B.V., pp. 72-108.

De la Presa Owens, S. & Innis, S.M. 1999. Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotonergic neurotransmitters in frontal cortex caused by a linoleic and α-linolenic acid deficient diet in formula-fed piglets. *Journal of Nutrition 129,* 2088-2093.

De la Presa Owens, S., Innis, S.M. 2000. Diverse, region-specific effects of addition of arachidonic and docosahexaenoic acids to formula with low or adequate linoleic and α-linolenic acids on piglet brain monoaminergic neurotransmitters. *Pediatric Research 48,* 125-130.

Delion, S., Chalon, S., Guilleoteau, D., Besnard, J.C., Dwand, G. 1996. Alpha-linoleic acid dietary deficiency alters age-related changes of dopaminergic and serotonergic neurotransmission in the rat frontal cortex. *Journal of Neurochemistry 66,* 1582-1591.

Dési, I., Nagymajtényi, L., Schultz, H. 1998. Behavioural and neurotoxicological changes caused by cadmium treatment of rats during development. *Journal of Applied Toxicology 18,* 63-70.
Decker, M.W. & McGaugh, J.L. 1991. The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse 7,* 151-168.
Dutta, C.D. & Appelqvist, L.Å. 1989. The effect of different cultural conditions on the accumulation of depot lipids notably petroselinic acid during somatic embryogenesis in *Daucus carota L. Plant Science 64,* 167-177.
EEC. 2001. European Council Regulation. Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal L 077,* 0001-0013.
Edmond, J., Higa, T.A., Korsak, R.A., Bergner, E.A., Lee, W.N. 1998. Fatty acid transport, and utilization for the developing brain. *Journal of Neurochemistry 70,* 1227-1234.
Eklund, G., Grawé, K.P., Oskarsson, A. 2001. Bioavailability of cadmium from infant diets in newborn rats. *Archives of Toxicology 75,* 522-530.
Elinder, C-G., Kjellström, T., Friberg, L., Lind, M., Linnman, L. 1976. Cadmium in kidney cortex, liver and pancreas from Swedish autopsies. *Archives of Environmental Health 31,* 292-302.
Eng, L.F., Ghirnikar, R.S., Lee, Y.L. 2000. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochemical Research 25,* 1439-1451.
Engström, B., Nordberg, G.F. 1979a. Dose dependence of gastrointestinal absorption and biological half-time of cadmium in mice. *Toxicology 13,* 215-222.
Engström, B. & Nordberg, G.F. 1979b. Factors influencing absorption and retention of oral ^{109}Cd in mice: age pretreatment, and subsequent pretreatment with non-radioactive cadmium. *Acta pharmacologica et toxicologica 45,* 315-324.
Enslen, M., Nilan, H., Malnoe, A. 1991. Effects of low intake of n-3 fatty acids during development on brain phospholipid fatty acid composition and exploratory behaviour in rats. *Lipids 26,* 203-208.
Erickson, J.C., Hollopeter, G., Thomas, S.A., Froelick, G.J., Palmiter, R.D. 1997. Disruption of the metallothionein-III gene in mice: analysis of brain zinc, behavior, and neuron vulnerability to metals, aging, and seizures. *The Journal of Neuroscience 17,* 1271-1281.
Eriksson, J. Stenberg, B., Andersson, A., Andersson, R. 2000. Tillståndet i svensk åkermark och spannmålsgröda. *Swedish Environmental Protection Agency, Report No. 5062.* ISSN 0282-7298.
Eriksson, P. 1997. Developmental neurotoxicity of environmental agents in the neonate. *Neurotoxicology 18,* 719-726.
Esquifino, A.I., Seara, R., Fernández-Rey, E., Lafuente, A. 2001. Alternate cadmium exposure differentially affects the content of gamma-aminobutyric acid (GABA) and taurine within the hypothalamus, median eminence, striatum and prefrontal cortex of male rats. *Archives of Toxicology 75,* 127-133.
File, S.R., Kenny, P.J., Cheeta, S. 2000. The role of dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. *Pharmacology Biochemistry and Behavior 66,* 65-72.
Flanagan, P.R., McLellan, J.S., Haist, J., Cherian, M.G., Chamberlain, M.J., Valberg, L.S. 1978. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterology 74,* 841-846.
Fleck C. 1992. Renal transport of endogenous amino acids. I. Comparison between immature and adult rats. *Renal Physiology and Biochemistry 15,* 257-265.
Frances, H., Monier, C., Clement, M., Lecorsier, A., Debray, M., Bourre, J.M. 1996. Effect of dietary α-linolenic acid deficiency on habituation. *Life Sciences 58,* 1805-1816.
Fransson, G.B. & Lönnerdal, B. 1982. Zinc, copper calcium and magnesium in human milk. *Journal of Pediatrics 101,* 504-508.
Fransson, G.B. & Lönnerdal, B. 1984. Iron, copper, zinc, calcium, and magnesium in human milk fat. *American Journal of Clinical Nutrition 39,* 185-189.
Friis, L., Petersson, L, Edling, C. 1998. Reduced cadmium levels in human kidney cortex in Sweden. *Environmental Health Perspectives 106,* 175-178.

Gabbiani, G., Gregory, M., Bais, D. 1967a. Cadmium-induced selective lesions of sensory ganglia. *Journal of Neuropathology and Experimental Neurology 26,* 498-506.
Gabbiani, G., Baic, D., Diesel, C. 1967b. Toxicity of cadmium in the central nervous system. *Experimental Neurology 18,* 154-160.
Goering, P.L. & Klaassen, C.D. 1984. Resistance to cadmium-induced hepatotoxicity in immature rats. *Toxicology and Applied Pharmacology 74,* 321-329.
Gould, S.F. 1983. Anatomy of the breast. In*: Lactation. Physiology, nutrition, and breast-feeding.* Eds. Neville, M.C. and Neifert, M.R. Plenum Press, New York and London, pp. 23-47.
Goyer, R.A. 1997. Toxic and essential metal interactions. *Annual Reviews Nutrition 17,* 37-50.
Groneberg, D.A., Döring, F., Theis, S., Nickolaus, M., Fischer, A., Daniel, H. 2002. Peptide transport in the mammary gland: expression and distribution of PEPT2 mRNA and protein. *American Journal of Physiology Endocrinology and Metabolism 282,* E1172-E1179.
Gulati, S., Gill, K.D., Nath, R. 1986. Effect of cadmium on lipid composition of the weanling rat brain. *Acta pharmacoogica et toxicologica 59,* 89-93.
Gupta, A., Gupta, A., Murthy, R.C., Ali, M.M., Chandra, S.V. 1993. Neurobehavioral and neurochemical changes in growing rats after cadmium exposure. *Toxicological and Environmental Chemistry 39,* 153-160.
Gupta, A., Gupta, A., Shukla, G.J. 1995. Development of brain free radical scavenging system and lipid peroxidation under the influence of gestational and lactational cadmium exposure. *Human & Experimental Toxicology 14,* 428-433.
Gupta, A. and Shukla, G.S. 1996. Ontogenic profile of brain lipids following perinatal exposure to Cd. *Journal of Applied Toxicology 16,* 227-233.
Gutiérrez-Reyes, E.Y., Albores, A., Ríos, C. 1998. Increase of striatal dopamine release by cadmium in nursing rats and its prevention by dexamethasone-induced metallothionein. *Toxicology 131,* 145-154.
Habeebu, S.S., Liu, Y., Park, J.D., Klaassen, C.D. 2001. Strain differences in the toxicity of cadmium to trigeminal ganglia in mice*. Toxicology and Applied Pharmacology 177,* 200-207.
Hamosh, M. 1987. Lipid metabolism in premature infants*. Biology of the Neonate 52 Suppl 1,* 50-64.
Hara, A. & Radin, N.S. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry 90,* 420-426.
Hedlund, B., Eriksson, J., Petersson Grawé, K., Öborn, I. 1997. Kadmium – tillstånd och trender. *Swedish Environmental Protection Agency, Report No. 4759.* ISSN 0282-7298. In Swedish.
Herlenius, E. & Lagercrantz, H. 2001. Neurotransmitters and neuromodulators during early human development. *Early Human Development 65,* 21-37.
Houpert, P., Federspiel, B., Milhaud, G. 1997. Toxicokinetics of cadmium in lactating and nonlactating ewes after oral and intravenous administration. *Environmental Research 72,* 140-150.
Huang, L & Gitschier, J. 1997. A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nature Genetics 17,* 292-297.
IARC. 1993. International Agency for Research on Cancer. Monographs: *Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Vol. 58*. IARC, Lyon, France.
Innis, S. 1991. Essential fatty acids in growth and development*. Progress in Lipid Research 30,* 39-103.
Jenness, R. 1973. Caseins and caseinates micelles of various species*. Netherlands Milk and Dairy Journal 27,* 251-257.
Jenness, R.R. 1985. Biochemical and nutritional aspects of milk and colostrum. In: *Lactation*. Ed. Larson, B. The Iowa University Press, Ames, Iowa, USA, pp 164-197.
Jensen, R.G. 1995. *Handbook of milk composition.* Ed. Jensen, R.G., Academic Press, San Diego, CA., USA. 919 pp.

Katsuki, H. & Okuda, S. 1995. Arachidonic acid as a neurotroxic and neurotrophic substance. *Progress in Neurobiology 46,* 607-636.
Kelleher, S.H. & Lönnerdal, B. 2002. Zinc transporters in rat mammary gland respond to marginal zinc and vitamin A intakes during lactation. *Journal of Nutrition 132,* 3280-3285.
Kello, D. & Kostial, K. 1977. Influence of age and milk diet on cadmium absorption from the gut. *Toxicology and Applied Pharmacology 40,* 277-282.
Kishi, R., Ikeda, T., Miyake, H., Uchino, E., Tsuzuki, T., Inoue, K. 1982. Regional distribution of lead, zinc, iron and copper in suckling and adult rat brains. *Brain Research 251,* 180-182.
Klaassen, C.D. & Liu, J. 1998. Induction of metallothionein as an adaptive mechanism affecting the magnitude and progression of toxicological injury. *Environmental Health Perspectives 106 Suppl 1,* 297-300.
Klaassen, C.D., Liu, J., Choudhuri, S. 1999. Metallothionein: Amn intracellular protein to protect against cadmium toxicity. *Annual Reviews in Pharmacology and Toxicology 39,* 267-294.
Kurosumi, K., Kobayashi, Y., Baba, N. 1968. The fine structure of mammary glands of lactating rats, with special reference to the apocrine secretion. *Experimental Cell Research 50,* 177-192.
Lafuente, A., Márquez, N., Pérez-Lorenzo, M., Pazo, D., Esquifino, A.I. 2000. Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary-testicular axis function in the rat. *Food and Chemical Toxicology 38,* 913-923.
Langmade, S.J., Ravindra, R., Daniels, P.J., Andrews, G.K. 2000. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. *The Journal of Biological Chemistry 275,* 34803-34809.
Larson, B. 1985. Biosynthesis and cellular secretion of milk. In: *Lactation*. Ed. Larson, B. The Iowa University Press, Ames, Iowa, USA, pp 129-163.
Lee, D.Y. Shay, N.F., Cousins, R.J. 1992. Altered zinc metabolism occurs in murine lethal milk syndrome. *Journal of Nutrition 122,* 2233-2238.
Lehman, L.D. & Klaassen, C.D. 1986. Dosage-dependent disposition of cadmium administered orally to rats. *Toxicology and Applied Pharmacology 84,* 159-167.
Lehotzky, K., Ungváry, G., Polinák, D., Kiss A. 1990. Behavioural deficits due to prenatal exposure to cadmium chloride in CFY rats. *Neurotoxicology and Teratology 12,* 169-172.
Lindén, A., Olsson, I.-M., Bensryd, I., Lundh, T., Skerfving, S., Oskarsson, A. Monitoring of cadmium in the chain from soil via crops and feed to pig blood and kidney. *In press*
Linzell, J.L. and Peaker, M. 1971. Mechanism of milk secretion. *Physiological Review 51,* 564-597.
Liu, J., Liu, Y., Michalska, A.E., Choo, K.H.A., Klaassen, C.D. 1996. Distribution and retention of cadmium in metallothionein I and II null mice. *Toxicology and Applied Pharmacology 136,* 260-268.
Liu, J., Liu, Y., Habebuu, S.S., Klaassen, C.D. 1998. Susceptibility of MT-null mice to chronic $CdCl_2$-induced nephrotoxicity indicates that renal injury is not mediated by the CdMT complex. *Toxicological Sciences 46,* 197-203.
Liu, Y., Liu, J., Habebuu, S.S., Waalkes, M.P., Klaassen, C.D. 2000. Metallothionein-I/II null mice are sensitive to chronic oral cadmium induced nephrotoxicity. *Toxicological Sciences 57,* 167-176.
Lucis, O.J., Lucis, R., Shaikh, Z.A. 1972. Cadmium and zinc in pregnancy and lactation. *Archives of Environmental Health 25,* 14-22.
Lyon. T.D.B., Aughey, E., Scott, R., Fell, G.S. 1999. Cadmium concentrations in human kidney in the UK: 1978-1993. *Journal of Environmental Monitoring 1,* 227-231.
Lönnerdal, B. & Fossum, E. 1985. Casein content of human milk. *American Journal of Clinical Nutrition 41,* 113-120.
Méndez-Armenta, M., Barroso-Moguel, R., Villeda-Hernández. J., Nava-Ruíz, C., Ríos, C. 2001. Histopathological alterations in the brain regions of rats after combined treatment with cadmium and dexamethasone. *Toxicology 161,* 189-199.

Michalke, B. & Schrammel, P. 1990. Protein fractionation and Cd-speciation in human breast milk by HPLC and voltammetry. *Journal of Trace Element and Electrolytes in Health and Disease 4,* 163-167.

Min, K.D., Terano, Y., Onosaka, S., Tanaka, K. 1991. Induction of hepatic metallothionein by nonmetallic compounds associated with acute-phase response in inflammation. *Toxicology and Applied Pharmacology 111,* 152-162.

Mohammed, A.K., Magnusson, O., Maehlen, J., Fonnum, F., Norrby, E., Schultzberg, M., Kristensson, K. 1990. Behavioural deficits and serotonin depletion in adult rats after transient infant nasal viral infection. *Neuroscience 35,* 355-363.

Moore, S.A., Yoder, E., Murphy, S., Dutton, G.R., Spector, A.A. 1991. Astrocytes, not neurons, produce docosahexaenoic acid (22:6 ω-3) and arachidonic acid (20:4 ω-6). *Journal of Neurochemistry 56,* 518-524.

Nagymajtényi, L., Schultz, H., Dési, I. 1997. Behavioural and functional neurotoxicological changes caused by cadmium in a three-generational study in rats. *Human & Expermiental Toxicology 16,* 691-699.

Neville, M.C. 2001. Anatomy and physiology of lactation. In: Breastfeeding 2001, Part I: The evidence for breastfeeding. *Pediatric Clinics of North America 48,* 13-34.

Nguyen, L., Rigo, J.M., Rocher, V., Belachew, S., Malgrange, B., Rogister, B., Leprince, P., Moonen, G. 2001. Neurotransmitters as early signals for central nervous system development. *Cell and Tissue Research 305,* 187-202.

Nilsson, U., Schütz, A., Skerfving, S., Mattsson, S. 1995. Cadmium in kidneys in Swedes measured in vivo using X-ray fluorescence analysis. *International archives of occupational and environmental health 67,* 405-411.

Nilsson U, Schütz A, Bensryd I, Nilsson A, Skerfving S, Mattsson S. 2000. Cadmium levels in kidney cortex in Swedish farmers. *Environmental Research 82,* 53-59.

Nishimura, N., Nishimura, H., Tohyama, C. 1989. Localization of metallothionein in female reproductive organs of rat and guinea pig. *The Journal of Histochemistry and Cytochemistry 37,* 1601-1607.

Nordberg, G.F. & Nishiyama, K. 1972. Whole-body and hair retention of cadmium in mice. *Archives of Environmental Health 24,* 209-214.

Nordberg, M. 1978. Studies on metallothionein and cadmium. *Environmental Research 15,* 381-404.

Nordberg, M. & Nordberg, G.F. 2000. Toxicological aspects of metallothionein. *Cellular and Molecular Biology 46,* 451-463.

Oberlander, C., Blaquière, B., Pujol, J-F. 1986. Distinct functions for dopamine and serotonin in locomotor behaviour: evidence using the 5-HT_1 agonist RU 24969 in globus pallidus-lesioned rats. *Neuroscience Letters 67,* 113-118.

Olney, J.W. 2002. New insights and new issues in developmental neurotoxicology. *Neurotoxicology 23,* 659-668.

Olsson, IM., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S., Oskarsson, A. 2002. Cadmium in blood and urine-impact of sex, age, dietary intake, iron status, and former smoking-association of renal effects. *Environmental Health Perspectives 110,* 1185-1190.

Oskarsson, A. 1987. Effects of disulfiram on milk transfer and tissue distribution of lead in the neonatal rat. *Toxicology Letters 36,* 73-79.

Palminger Hallén, I.and Oskarsson, A. 1993. Dose dependent transfer of ^{203}lead to milk and tissue uptake in suckling offspring studied in rats and mice. *Pharmacology and Toxicology 73,* 174-179.

Palminger Hallén, I. & Oskarsson, A. 1995. Bioavailability of lead from various milk diets studied in a suckling rat model. *Biometals 8,* 231-236.

Palminger Hallén, I., Jorhem, L., J:son Lagerkvist, B., Oskarsson, A. 1995. Lead and cadmium levels in human milk and blood. *Science of the Total Environment 166,* 149-158.

Palmiter, R.D. 1998. The elusive function of metallothioneins. *Proceedings of the National Academy of Sciences USA 95,* 8428-8430.

Patapoutian, A. & Reichardt, L.F. 2001. Trk receptors: mediators of neurotrophin action. *Current Opinion in Neurobiology 11,* 272-280.

Permyakov, E.A. & Berliner, L.J. 2000. α-Lactalbumin: structure and function. *FEBS Letters 473,* 269-274.
Piletz, J.E. & Ganschow, R.E. 1978. Zinc deficiency in murine milk underlies expression of the lethal milk (lm) mutation. *Science 199,* 181-183.
Provoost, A.P., de Keijzer, M.H., Wolff, E.D., Molenaar, J.C. 1983. Development of renal function in the rat. The measurement of GFR and ERPF and correlation to body and kidney weight. *Renal Physiology 6,* 1-9.
Randhawa, Z.I. & Smith, S. 1987. Complete amino acid sequence of the medium-chain S-acyl fatty acid synthetase thio ester hydrolase from rat mammary gland. *Biochemistry 26,* 1365-1373.
Rastogi, R.B., Merali, Z., Singhal, R.L. 1977. Cadmium alters behaviour and the biosynthetic capacity for catecholamines and serotonin in neonatal brain**.** *Journal of Neurochemistry 28,* 789-794.
Remilla, J.A. and Hill, M.A. 2003. Prolactin regulation of the pendrin-iodide transporter in the mammary gland. *American Journal of Physiological Metabolism 284,* E25-E28.
Rice, D. and Barone Jr, S. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives 108 (suppl. 3),* 511-533.
Rodríguez Rodríguez, E.M., Delgado Uretra, E., Díaz Romero, C. 1999. Concentrations of cadmium and lead in different types of milk. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A 208,* 162-168.
Rumball, S.V. & Baker, E.N. 1985. Trace elements in human milk: Structural aspects. In: *Human Lactation: Milk Components and Methodologies* Eds. Jensen, R.J., Neville, M.C., Plenum Press, New York, pp 237-242.
Ruppert, P.H., Dean, K.F., Reiter, L.W. 1985. Development of locomotor activity of rat pups exposed to heavy metals. *Toxicology and Applied Pharmacology 78,* 69-77.
Saric, M.M., Blanusa, M., Piasek, M., Varnai, V.M., Juresa, D., Kostial, K. 2002. Effect of dietary calcium on cadmium absorption and retention in suckling rats. *Biometals 15,* 175-182.
Sasser, L.B. & Jarboe, G.E. 1980. Intestinal absorption and retention of cadmium in neonatal pigs compared to rats and guinea pigs. *Journal of Nutrition 110,* 1641-1647.
Sastry, P.S. 1985. Lipids of the nervous tissue: Composition and metabolism. *Progress in Lipid Research 24,* 69-176.
Saunders, N.R., Habgood, M.D.,Dziegielewska, K.M. 1999. Barrier mechansims in the brain, II. Immature brain. *Clinical and Experimental Pharmacology and Physiology 26,* 85-91.
Schramel, P., Hasse, S., Ovcar-Pavlu, J. 1988. Selenium, cadmium, lead and mercury concentrations in human breast milk, in placenta, maternal blood, and the blood of the newborn. *Biological Trace Element Research 15,* 111-124.
Schümann, K., Friebel, P., Schmolke, G., Elsenhans, B. 1996. State of iron repletion and cadmium tissue accumulation as a function of growth in young rats after oral cadmium exposure. *Archives of Environmental Contamination and Toxicology 31,* 483-487.
Sharma, R.P., Kjellström, JT., McKenzie, J.M. 1983. Cadmium in blood and urine among smokers and non-smokers with high cadmium intake via food. *Toxicology 29,* 163-171.
Shennan, D.B. & Peaker, M. 2000. Transport of milk constituents by the mammary gland. *Physiological Reviews 80,* 925-951.
Shin-ichi, O., Koroptnick, J., Cherian, G.M. 1997. Regional brain distribution of metallothionein, zinc and copper in toxic milk mutant and transgenic mice. *Toxicology 124,* 1-10.
Singh, H., Flynn, A., Fox, P.F. 1989. Binding of zinc to bovine and human milk proteins. *Journal of Dairy Research 56,* 235-248.
Smith, M.J., Pihl, R.O., Garber, B. 1982. Postnatal cadmium exposure and longterm behavioural changes in the rat. *Neurobehavioral Toxicology and Teratology 4,* 283-287.
Smith, R.M., Leach, R.M., Muller, L.D., Griel, L.C., Baker, D.E. 1991. Effects of long-term dietary cadmium chloride on tissue, milk, and urine mineral concentrations of lactating dairy cows. *Journal of Animal Science 69,* 4088-4096.

Smith, S., Pasco, D., Nandi, S. 1983. Biosynthesis of medium-chain fatty acids by mammary epithelial cells from virgin rats. *Biochemical Journal 212*, 155-159.

Solaiman, D., Jonah, M.M, Miyazaki, W., Ho, G., Bhattacharyya, M.H. 2001. Increased metallothionein in mouse liver, kidneys, and duodenum during lactation. *Toxicological Sciences 60,* 184-192.

Spitzweg, C., Joba, W., Eisenmenger, W., Heufelder, A.E. 1998. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of it complementary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa. *The Journal of Clinical Endocrinology and Metabolism 83,* 1746-1751.

Sprecher, H., Luthria, D.L., Mohammed, B.S., Baykousheva, S.P. 1995. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *Journal of Lipid Research 36,* 2471-2477.

Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R. 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Public Health and Environmental Exposure to Cadmium (PheeCad) Study Group. *Lancet 353,* 1140-1144.

Stelwagen, K., Farr, V.C., McFadden, H.A., Prosser, C.G., Davis, S.R. 1997. Time course of milk accumulation-induced opening of mammary gland tight junctions, and blood clearance of milk components. *American Journal of Physiology 273,* R379-386.

Sundberg, J., Jönsson, S., Karlsson, M.O., Palminger Hallén, I., Oskarsson, A. 1998. Kinetics of methylmecury and inorganic mercury in lactating and nonlactating mice. *Toxicology and Applied Pharmacology 151,* 319-329.

Svartengren, M., Elinder, C-G., Friberg, L., Lind, B. 1986. Distribution and concentration of cadmium in human kidney. *Environmental Research 39,* 1-7.

Takeda, A., Takefuta, S., Ijiro, H., Okada, S., Oku, N. 1999. ^{109}Cd transport in rat brain. *Brain Research Bulletin 49,* 453-457.

Tanaka, M., Matsusaka, N., Yuyama, A., Kobayashi, H. 1972. Transfer of cadmium through placenta and milk in the mouse. *Radioisotopes 21,* 34-46.

Thoenen, H. 1995. Neurotrophins and neuronal plasticity. *Science 270,* 593-598.

Thornalley, P.J. & Vasak, M. 1985. Possible role for metallothionein in protection against radiation–induced oxidative stress: kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochimica et Biophysica Acta 27,* 36-44.

Tyler,W.J., Alonso, M., Bramham, C.R., Pozzo-Miller, L.D. 2002. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signalling in hippocampal-dependent learning. *Learning and Memory 9,* 224-237.

Ullberg, S. 1977. The technique of whole body autoradiography. Cryosectioning large specimen. Science Tools: LKB Instrumental Journal. Special issue on whole body autoradiography, pp 1-29.

Vahter, M., Berglund, M., Nermell, B., Åkesson, A. 1996. Bioavailability of cadmium from shellfish and mixed diet in women. *Toxicology and Applied Pharmacology 136,* 332-341.

Valois, A.A. & Webster, W.S. 1987. The choroid plexus and cerebral vasculature as target sites for cadmium following acute exposure in neonatal and adult mice: an autoradiographic and gamma counting study. *Toxicology 46,* 43-55.

Valois, A.A. & Webster, W.S. 1989. The choroid plexus as a target site for cadmium toxicity following chronic exposure in the adult mouse: an ultrastructural study. *Toxicology 55,* 193-205.

Vanderpool, R.A. & Reeves, P.G. 2001. Cadmium absorption in women fed processed edible sunflower kernels labeled with a stable isotope of cadmium, ^{113}Cd. *Environmental Research A 87,* 69-80.

Voss, A., Reinhart, M., Sankarappa, S., Sprecher, H. 1991. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *Journal of Biological Chemistry 266,* 19995-20000.

Vuori, E., Vetter, M., Kuitunen, P., Salmela, S. 1983. Cadmium in Finnish breast milk, a longitudinal study. *Archives of Toxicology 53,* 207-211.

WHO, 1986. Principles for evaluating health risks from chemicals during infancy and early *childhood: the need for a special approach. Environmental* Health Criteria 59.

International Programme on Chemical Safety. World Health Organization, Geneva, 73 pp.
WHO. 1992. Cadmium. *Environmental Health Criteria 134.* International Programme on Chemical Safety. World Health Organization, Geneva, 280 pp.
WHO. 2001. Cadmium. *In: Safety evaluation of certain food additives and contaminants. Fifty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (*JECFA*). WHO Food Additives Series 46,* pp. 247-305 World Health Organization, Geneva.
Washizaki, K., Smith, Q.R., Rapoport, S.I., Purdon, A.D. 1994. Brain arachidonic acid incorporation and precursor pool specific activity during intravenous infusion of unesterified [3H]arachidonate in the anaestetized rat. *Journal of Neurochemistry* 63, 727-736.
Webster, W.S. & Valois, A. 1981. The toxic effects of cadmium on the neonatal mouse CNS. *Journal of Neuropathology and Experimental Neurology 40,* 247-257.
Whelan, J. 1996. Antagonistic effects of dietary arachidonic acid and n-3 polyunsaturated fatty acids. *Journal of Nutrition 126, Suppl. 4,* 1086S-1091S.
Williamson, D.H. 1985. Ketone body metabolism during development. *Federal Proceedings 44,* 2342-2346.
Wong, K.L., Cachía, R., Klaassen, C.D. 1980. Comparison of the toxicity and tissue distribution of cadmium in newborn and adult rats after repeated administration. *Toxicology and Applied Pharmacology 56,* 317-325.
Wong, K.L. & Klaassen, C.D. 1982. Neurotoxic effects of cadmium in young rats. *Toxicology and Applied Pharmacology 63,* 330-337.
Yamada, K., Mizuno, M., Nabeshima, T. 2002. Role for brain-derived neurotrophic factor in learning and memory. *Life Sciences 70,* 735-744.
Yamamoto, N., Saitoh, M., Moriuchi, A., Nomura, M., Okuyama, H. 1987. Effects of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *Journal of Lipid Research 28,* 144-151.
Öborn, I., Jansson, G., Johnsson, L. 1995. A field study on the influence of soil pH on trace element levels in spring wheat (*Triticum aestivum*), potatoes (*Solanum tuberosum*) and carrots (*Daucus carota*). *Water Air and Soil Pollution 85,* 835-840.

Acknowledgements

This work was carried out at the Department of Pharmacology and Toxicology, SLU and the National Food Administration in Uppsala. Parts of the work were done in collaboration with the Department of Food Sciences and the Department of Food Hygiene, SLU, and AstraZeneca R&D, Safety Assessment, Södertälje.

A thesis is the result of a teamwork, and I therefore wish to express my sincere gratitude to all of those who have supported me in different ways through the years, and especially:

Agneta Oskarsson, my supervisor, for active supervision, support and encouragement, always inspiring me. Agnetas wide experience in the field of toxicology and her strong drive has been a prerequisite for the progress and completion of this work. I have learnt a lot from you during the years, Agneta!

Leif Busk and *Anders Glynn*, former and present head, respectively, at the Division of Toxicology at the National Food Administration, for very much appreciated support and encouragement during the years.

Lars Åke Appelgren for taking me on board at the Department of Pharmacology and Toxicology and for his kind support and interest in my work.

My co-authors *Helena Andersson*, *Elisabeth Jalkesten*, *Elisabet Lindqvist*, *Jan Luthman*, *Lars Olsson* and *Anna Teiling-Gårdlund* for sharing with me their knowledge and experience in neuroscience.

My co-authors *Paresh Dutta* and *Jana Pickova* for introducing me into the field of lipid analysis, for their kindness and patient willingness to guide me through the jungle of peaks in the GC chromatograms.

Bengt Danielsson for the opportunity to conduct the behavioural study in the excellent laboratory facilities at AstraZeneca R&D.

For expert technical laboratory support: *Dagmar Brabencova*, *Lena Carlsson*, *Ella Cari Enquist*, *Inga Lill Gadhasson*, *Joakim Engman*, *Lars Jorhem*, *Elvy Netzel*, *Birgitta Sundström*, and *Birgitta Öhman* at the National Food Administration, *Agneta Boström* at the Department of Pharmacology and Toxicology, *Seved Helmersson* at the Department of Food Hygiene, *Anders Högberg*, *Lars Johnsson* and *Christina Nilsson* at the Department of Food Science, Swedish University of Agricultural Sciences, and the staff taking care of the animals at AstraZeneca R&D. It has been a real pleasure working with all of you!

Gunilla Eklund, *Anna Lindén* and *Ing-Marie Olsson*, my nice cadmium-colleagues, for introducing me into the details of FAAS and GFAAS.

Johanna Sundberg and *Ira Palminger Hallén* for our nice companionship during the early days of the Milk Project.

Hans Tjälve, for kindly and critically reading the manuscript of this thesis.

All former and present colleagues at the Division of Toxicology, National Food Administration, and at the Department of Pharmacology and Toxicology, SLU, for creating a friendly atmosphere to work in.

My strong *Mother* and *Father* (you are still remembered Dad), for their caring and for giving me a stable platform in life. My sister *Brita* and brother *Anders*, for always being around.

All friends and relatives for helping me make things go around despite thesis writing, with child-care, bike-in restaurant meals at Malma Backe, and for being around and encouraging me.

My family: *Janne*, my love and best friend, for his never-ending support and belief in my capabilities. *Matilda* and *Markus* for being my greatest joy. ♥♥♥

I also would like to thank The Swedish Council for Forestry and Agricultural Research (SJFR) and The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) for financial support.