CHAPTER 5: SUMMARY

The production and use of industrial chemicals have increased at an almost exponential rate for the past 50 years, with roughly 10 new chemicals currently being introduced each day. During the same time, a significant rise in the prevalence of developmental human disorders was observed, suggesting that environmental exposure to man-made chemicals is playing an important role in such a trend. Several epidemiological studies support this hypothesis as they found significant correlations between some human disabilities and diseases (*e.g.* reduced male reproductive health, increases in neurodevelopmental disorders) and environmental contaminants (*e.g.* DDT, lead, mercury, PCBs). Among them, the risks posed by the flame retardant polybrominated diphenyl ether (PBDE) is of concern as monitoring studies have detected increasing levels of this persistent

compound in biota samples over the past decades. PBDEs are largely used in industrialized materials due to their potent flame retardant properties, therefore, they are ubiquitous in the environment. Although little is known about their toxicity, experimental studies in rodents indicate that this class of compounds possesses a wide range of toxicity. Effects on thyroid hormone homeostasis, hepatic metabolizing enzymes and neurobehavior have been demonstrated in laboratory animals. However, there is still scarce information on developmental and reproductive effects of PBDEs claiming for more experimental investigations. The exposure during critical periods of organogenesis and the sensitivity of developmental processes to relatively small changes in endogenous steroid levels suggest that endocrine disruption during development may have long-lasting deleterious effects (organizational effects) which are often permanent

In this thesis, several aspects of PBDE 99 toxicity were studied experimentally in rats using doses close to human exposure. Briefly, two experiments were conducted in which pregnant Wistar rats were treated orally by gavage with 60µg or 300µg PBDE 99 / kg body weight on gestation day six. In experiment I, tissue distribution of PBDE 99, effects on thyroid hormonal status and hepatic enzyme activities were evaluated in dams and offspring during lactation. The second experiment was designed to investigate the possible long term effects of PBDE 99 that can become apparent during puberty and adulthood. In order to assess thyroid hormone mediated-effects, an additional reference group for thyroid hormone effects was treated with the goitrogen 6-n-propyl-2-thiouracil (PTU) by placing 5mg of PTU / liter drinking water from GD 7 – 21. Consistent data are presented here showing that, at least in rats, developmental exposure to low doses PBDE 99 adversely affects offspring health.

In experiment I, thyroid hormone (TH) concentrations were measured in dams and offspring along the lactational period. Briefly, thyroid hormone effects were translated into decreases in thyroxin (T4) concentration induced by PBDE 99 affecting dams at the beginning of lactation and offspring at the end of lactation. Additionally, slight changes in hepatic metabolizing enzymes were also observed. Ethoxyresorufin-*O*-deethylase (EROD) activity was significantly reduced in dams whereas male offspring had increased EROD levels by the end of lactation. Moreover, analyzing the phase II metabolic enzyme uridine diphosphoglucuronosyl transferase (UDPGT), only offspring have been affected by PBDE 99 displaying a slight increase in UDPGT activity on PND 1. Since the thyroid hormone signal is essential for several physiological processes, alteration of TH during critical periods of development may lead to organizational effects compromising offspring health. Furthermore,

the fluctuation of hepatic enzyme activities may have biological relevance as the clearance rate and formation of toxic metabolites are unbalanced in exposed animals. Small changes in the metabolic clearance can expose the developing organism to high levels of reactive metabolites and /or the xenobiotic itself.

In this thesis, consistent data are presented demonstrating that PBDE 99 is very persistent as significant amounts of the substance were found in rat tissues (liver and adipose tissue) approximately 37 days after the administration. Furthermore, placental transfer of PBDE 99 seems to be very effective as high concentrations of the parent compound were detected in offspring tissue at the first day of life. Due to high levels of PBDE 99 in dams adipose tissue, it is reasonable to assume that milk transfer is an important way of exposure.

In experiment II, developmental exposure to low doses of PBDE 99 caused long-lasting behavioral changes in offspring. Using a robust method to assess basal locomotor activity (Mobilitron ®), rat offspring were hyperactive on PND 36 after pre- and postnatal exposure to 300µg PBDE 99 / kg BW. Later at puberty (PND 71), the effect persisted but at this time animals exposed to 60µg PBDE 99 were also hyperactive. The neurobehavioral effects observed in rats in this study support the prerogative that environmental pollutants are playing an important role in the incidence of neurobehavioral disorders in children.

Persistent effects on the male reproductive system were also seen in offspring after pre- and postnatal exposure to low doses of PBDE 99. At adulthood, subtle changes in reproductive organ weights were observed followed by significant decreases in sperm and spermatid numbers, as well as daily sperm production. A flow cytometry analysis of germ cells, on the basis of their DNA ploidy/stainability level, confirmed that PBDE 99 affects spermatogenesis in adult male rats. These changes were revealed by an increased number of diploid cells with a concomitant reduction of haploid cells. The effect became clear when the ratio of haploid / diploid cell was plotted. In order to investigate possible mechanisms involved, morphometric analysis of the seminiferous tubules was performed. It seems that decreases in daily sperm production are independent from Sertoli cell number as we did not find any difference in the morphometric analysis. Moreover, at adulthood testosterone and LH levels were within control range, suggesting a minor role of steroid hormones in such a decline. However, we can not rule out the possibility of an early depression of FSH and testosterone in those rats during a critical phase of development which might have affected the spermatogenesis at adulthood.

In summary, the main results presented in this study are:

- Tissue distribution data of PBDE 99 indicates that offspring were exposed *in utero* and during lactation;

- Thyroid hormone homeostasis was altered in dams and offspring exposed to PBDE 99;

- Offspring exposed to PBDE 99 during critical periods of development were hyperactive. This effect persisted at least until puberty;

- Male reproductive health was affected in offspring at adulthood, translated into reduced spermatogenesis.

Low dose effects have been demonstrated in several experimental studies. Concerning the risk assessment of environmentally persistent compounds, the question if studies carried out with high doses are sufficient for comprehensive evaluation of risks is controversially discussed. Therefore, there is a need for studies at low dose levels in order to get a complete toxicity profile of a substance. In this context, this thesis presents consistent data demonstrating that low dose exposure to PBDE 99 may lead to a broad range of functional effects in offspring. Moreover, low dose exposure to PBDE 99 during critical periods of development may lead to organizational effects in rats which were translated into impairment of male reproductive health at adulthood. In the point of view of human risk assessment, this is the lowest dose of PBDE 99 reported to date to have an *in vivo* effect.

Another relevant aspect investigated in this study regards the tissue distribution of PBDE 99. The issue of toxicokinetic is extremely important for human risk assessment, but often is not included in developmental and reproductive toxicity studies. In this study, the quantification of PBDE 99 concentrations in rat tissues provides a useful tool for risk assessors due to the possibility of comparing the experimental data with the human exposure situation. In this study, adipose tissue concentration in dams exposed to the lowest dose of PBDE 99 were only 4.2- and 28.6-fold higher than the highest and mean PBDE 99 levels reported in human breast adipose tissue, respectively. This work can contribute to the evaluation of PBDE 99 for human risk assessment. Consistent evidences have shown that at least in rats, environmental levels of PBDE 99 pose risks for the developing organism. Therefore, the results indicate that the subpopulation at risk for adverse effects of environmentally relevant (low dose) exposures to PBDEs is the developing organism.