

Project Summary

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Title of the project: Developmental Neurotoxicity of Polybrominated Diphenyl-Ethers: Mechanisms and Effects.		
Acronym of the project PBDE-NTOX		
Type of contract Shared-cost		Total project cost 1258877 €
Contract number QLK4-CT-1999-01562	Duration 36 Months	EU contribution (i) 800000 €
Commencement date 1 February 2000		Period covered by the progress report 1 February 2000 – 31 January 2003
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Key words (5 maximum - Please include specific keywords that best describe the project). PBDE, neurotoxicity, developmental, mechanisms, behaviour		
World wide web address (the project's www address)		

List of participants Provide all partners' details including their legal status in the contract i.e., contractor, assistant contractor (to which contractor?).

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Section 2: Project Progress Report

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Objectives:

The goal of the project is to elucidate the effects of developmental exposure of animals to the flame retardant Polybrominated Diphenylethers (PBDE) on the central nervous system. Epidemiological studies indicate a marked increase of PDBE levels in breast milk. In contrast, effects of PBDEs on the developing CNS are largely unknown. Therefore, aims of the present proposal are: To assess several neurobehavioral endpoints after perinatal treatment of animals for characterisation of possible impairments. To relate neurobehavioral effects to electrophysiological effects in the different brain areas. To compare perinatal with adult exposure in order to examine if the developing CNS is particularly susceptible to PDBEs. To compare different animals species for generalisation to the findings. To characterise mechanisms of PDBE neurotoxicity at cellular and molecular levels including neurotransmitters, receptors and intracellular signal transduction pathways. To examine if exposure alters sexual differentiation of the brain. Different dose levels of PBDE's will be studied. PBDE exposure will be compared with PCB-induced effects to relate findings to a better examined substance group.

Results and Milestones:

No signs of general developmental PBDE99 toxicity could be detected using the main reproduction parameters like offspring number and offspring weight in the range of the PBDE doses perinatally applied during these experiments. The development of bodyweights in the offspring showed minor deviations, mainly due to the treatment with Aroclor1254 and to a lesser extent due to PBDE99 treatment.

The neurobehavioral experiments - performed in mice as well as in rats – revealed a species dependent pattern of changes. Open field habituation of mice was changed due to the perinatal PBDE99 treatment whereas such effects could not be detected in rats. The context conditioned fear in rats showed changed reactivity as did some vocalisations in mice. The haloperidol induced catalepsy in rats showed increased latencies and discrimination performance in the y-maze was elevated.

Synaptic plasticity as a well known model for learning and memory in rats was dose dependently reduced by the perinatal PBDE99 treatment in the main parameters slope and amplitude of excitatory postsynaptic potentials. These changes could be shown to be expressed in different degrees in different central nervous system areas like visual cortex layer III/IV and hippocampal area CA1.

The results of the studies in mice convincingly showed that either gestational or early postnatal exposure of animals to PBDE's significantly interfered with several neurobehavioral endpoints. The main result in this connection may be that the treatment occurred during a time window of central nervous system development which in animals includes the perinatal period as well as the time period of "brain growth spurt", i.e. covering a period of development which in humans begins with the third trimester of pregnancy and continues throughout the first 2 years of life. The results of the project work exactly refer to developmental, perinatal neurotoxicity. After perinatal treatment (gestational day 6 to postnatal day 21) several neurobehavioral endpoints were investigated. Daily assessment of somatic, sensory motor and neuromotor development was performed from birth to postnatal day 21. This investigation also referred to the assessment of ultrasonic vocalisation, an endpoint, which refers to emotional development. Open field behaviour and activity trends like habituation were measured. The results show that there seems

to be an u-shape dose response relationship between exposure dose of PBDE99 and several neurobehavioral endpoints. These data are corroborated by investigations aiming to the spontaneous behaviour in 2 month and 4 month old mice treated once at postnatal day 10. The experimental outcome shows impediment by PBDE99 and PBDE153 of activity during the first test period (60 min) while towards the end of the experiment the animals became hyperactive. Similar inferences on spontaneous behaviour were found in experiments using PCB52 for comparison. If learning and memory performances of these animals were tested in young adulthood (6 month old mice) dose response related defects due to PBDE99 and PBDE153 exposure were found.

These experimental results obtained in the different neurobehavioral paradigms clearly show that PBDE's seem to induce neurobehavioral deficits if the animals were treated perinatally. There is an astonishing similarity to the perinatal developmental neurotoxicity of PCB's which obviously is alerting in view of the well documented perinatal developmental PCB neurotoxicity in humans.

Studies of the mode of PBDE99 action used different types of cell cultures in vitro to analyse general changes of intracellular calcium levels ($[Ca^{++}]_i$) and intracellular signal transduction chains. Oscillations of $[Ca^{++}]_i$ were induced in neurones by PBDE99 which were similar to those described in the literature for ortho-substituted PCB congeners. Analogous to these the PBDE99 induced changes of $[Ca^{++}]_i$ occurred in the low micro-molar concentration range.

Changes of intracellular signal transduction in neurones due to the perinatal in vivo treatment using PBDE99 could be detected as significant interferences with the NO-cGMP pathway; interestingly changes in this pathway analogous in mode of action and degree occurred in neurones in vitro treated with concentrations of PBDE99 in the micro-molar range. This finding is important for the use of in vitro data in order to explain PBDE99 neurotoxicity mechanisms in vivo.

Referring to the studies of mechanistic aspects of PBDE neurotoxicity there seems to be also a remarkable similarity between mechanisms of action of PBDE99 as compared to ortho-substituted PCB's: In a neuronal cell line as well as in astrocytes, i.e. the two main cell types of the central nervous system, acute PBDE99 treatment in vitro induced repetitively occurring bursts of intracellular calcium levels, which have been recently described also to occur upon PCB treatment in several central nervous system preparations. A preliminary evaluation of dose response relationship showed that PBDE99 seems to be even more potent in inducing these calcium level changes as compared to the non coplanar PCB congener PCB47. Another important evidence for similarity of neurotoxicity between PBDE's and PCB's comes from long term exposure (9 to 14 days) experiments performed in cerebellar neurone cultures. PBDE's and a PCB-mixture (Aroclor) seem to be neurotoxic in the same range of concentrations between low micromolar and 100 μ M levels of treatment. Good evidences could be obtained for the impediment of the glutaminergic receptor-nitric oxid-cGMP pathway due to the PBDE treatment in these cells, a phenomenon which could also be seen in preliminary experiments in primary cultures of neurones derived from rats prenatally exposed to PBDE's. These experiments show as significant milestones of the project for the first time not only similarity in mechanisms of action between PBDE's and PCB's but also give evidences for mechanistic interferences with signal transduction pathways in neurones treated in vitro as well as in neuronal cultures obtained from exposed animals.

Our data also provide in vivo evidence for an interaction of PBDE with neuroendocrine mechanisms specifically during ontogeny. Treatment with PBDE 99 from gestational days 10 to 18, which because of bioaccumulation resulted in additional exposure during the early postnatal period, was followed by changes in onset of puberty and reproductive organ weights. Changes in

sexual development occurred in form of a delayed puberty onset and a reduced anorganic genital distance. Serum concentrations of estradiol and testosterone were reduced in the adult male offspring. Significant delayed changes in mRNA levels of sex hormone target genes occurred in sexually dimorphic brain regions, ventral and dorsal prostate and uterus of adult offspring. At brain level, developmental exposure to PBDE 99 affected the sexual dimorphism of progesterone receptor mRNA expression of the ventromedial hypothalamic nucleus (VMN) of adult offspring, and also interfered with expression of estrogen receptor alpha and preproenkephalin. In good correspondence to these findings the sweet preference of male offspring was statistically significantly increased.

It is remarkable that all the changes due to the perinatal PBDE99 treatment could be detected as long lasting and persisting into the adulthood after the peri-natal exposure to PBDE99. Impairment of learning and memory could even be detected in high aged animals showing PBDE99 tissue concentrations not different from untreated controls. It is therefore suggested that there is not only strong evidence for developmental PBDE99 neurotoxicity but also for the irreversibility of the PBDE99 effects induced during gestation.

Benefits and Beneficiaries:

Community added value and contribution to EU policies

As given in the introduction there still is a growing exposure to PBDEs in both environment and human beings in European and especially in north-american countries. On the other hand, there is a considerable lack of information about effects of PBDEs. This creates a problem of potential adverse effects to human health and developmental impairments in children. Thus, the project provided results which can be used as a basis for improvement of the quality of live by regulatory actions in the EU. For this purpose, the project used an integrated approach by combining cellular, electrophysiological, biochemical and behavioural techniques for which each of the participating laboratories had a known experience. This combination was ideally suited for the evaluation of PBDE effects on the developing nervous system and indeed provided effects on learning and memory, emotional, social and sexual behavior together with investigations of their underlying processes in nerve cells and tissues. Particular attention will be given to sexual differentiation of brain. In addition, the application of different dose levels and the comparative behavioural studies in two animal species delivered important basic knowledge for the risk management in order to secure environmental health. This broad investigation considerably profited from the participation of several laboratories in different countries specialised in different disciplines of neurotoxicology.

Contribution to Community social objectives

The regulation of environment and health aspects of widely used and distributed chemicals like PBDEs is necessary for maintenance of life quality. Previous experience with related substances, e.g. PCBs - which show similar properties as chemical and thermal stability, lipophilicity, persistence in biota, accumulation in food chains - exemplifies the need to control the distribution, usage and exposure to such compounds. For instance, PCBs which were at the beginning thought to be biologically inert are wide-spread in the environment, found at elevated levels in fat tissue of many animal species and human beings. The occurrence in human milk results in exposure of babies through nursing, so that breast-fed babies face the highest exposure levels in the general population, occupational exposure excluded. This developmental exposure has been shown to result in delays of neurobehavioral maturation in European and American studies. In order to manage environmental health risks and to prevent probably harmful effects, thorough knowledge on effects of maternal exposure and the possible long-term effects on

neurodevelopment is required. As yet, the toxicological effects of PBDEs, in particular, on nervous system and behaviour after developmental exposure are largely unknown. The integrative experimental approach of the present project provides a basis for assessing exposure risks of PBDE99. The achieved results data effectively contributed to a solid data basis which is needed for the decision whether regulations of these increasingly used substances are needed or not.

In this context the press release of “Great Lakes Chemical Corporation” (as of nov. 3rd 2003) is remarkable ‘that it will cease production of penta-PBDE flame retardant by the end of 2004’.

Exploitation and dissemination

The results obtained in this project have been published in qualified peer-reviewed journals and discussed with the scientific community on several relevant conferences. These included the meetings of the Society of Toxicology (USA), Society of Neuroscience (USA), International Neurotoxicology Association, EUTOX, European Teratology Society and meetings of national societies, respectively. All partners of this project have a proven record of publications of their research activities and were regularly present at the relevant conferences. To secure a coordinated progress members of all different groups have been meeting regularly once a year to the exchange of information during the project progress.