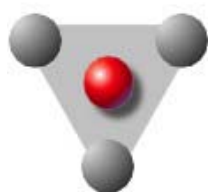


**Comparison of Exposure-Effect  
Pathways to Improve the  
Assessment of Human Health Risks  
of Complex Environmental Mixtures  
of Organohalogenes  
COMPARE**



The main objective of the project is to improve our understanding of comparative pathways for early life-stage exposure and long-term effects of several classes of organohalogenes

## Summary

Contract N°:QKL4-CT2000-00261

European Commission

Quality of Life and Management of Living Resources Programme (QoL)

Key Action 4 - Environment and Health



## *Project Progress Summary*

<b>Title of the project:</b> Comparison of exposure-effect pathways to improve the assessment of human health risks of complex environmental mixtures of organohalogenes		
<b>Acronym of the project:</b> COMPARE		
<b>Type of contract:</b> Shared cost project		<b>Total project cost</b> € 1,874,905
<b>Contract number</b> QLK4-CT2000-00261	<b>Duration</b> 45 Months	<b>EU contribution</b> € 1,874,905
<b>Commencement date</b> 1 January 2001		<b>Period covered by the Final Report</b> 1 January 2001 - 30 September 2004
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<b>Key words</b>  OH-PCBs, Brominated Flame Retardants, Human, Risk Assessment, Exposure		
<b>World wide web address:</b> <a href="http://www.compare-project.info">http://www.compare-project.info</a>		

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## OBJECTIVES

The major objective of this project was to: "provide a mechanism-based approach for the assessment of human health risks from exposure to complex environmental mixtures of halogenated phenolic compounds (HPCs). The Compare project involved studies on synthesis, identification and analysis of HPCs; on maternal-to-fetal transfer kinetics (role of TTR binding protein) in rodents and birds, on in vitro endocrine potency of HPCs; on developmental reproductive and neurobehavioural toxicity in rats, and on clinical epidemiological studies involving a Child Development cohort and a Fishermen and Fishermen's Wives Adults cohort. Finally, all the results from the Compare project were used to perform an integrated and comparative risk assessment for exposure to complex mixtures of environmental chemicals". For this purpose consolidated information on human individual exposure to HPCs, and on adverse health outcome of HPC exposure in laboratory animals, and in human individuals is included as this forms the basis for the integrated and comparative risk assessment.

## RESULTS AND MILESTONES:

### Human exposure assessment to HPCs

An assessment of exposure of human individuals to halogenated phenolic compounds (HPCs) has been performed within the Compare project. This required the development of proper analytical chemical methods for determination of a variety of HPCs representative for several classes of contaminants and/or their metabolites, such as polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs); p,p'-DDE, pentachlorophenol (PCP), several representatives of brominated flame retardants, like 2,4,6-tribromophenol (TBP), brominated diphenylethers, BDE 47, and its hydroxy metabolite (6-OH-BDE 47), and hexabromocyclododecane (HBCDD). These methods were successfully developed by the Group of Prof. Bergman from Stockholm University, including an interlaboratory calibration study, involving three laboratories from participants, showing a coefficient of variation between the laboratories below 10%. Furthermore, these compounds and some of their phenolic metabolites were successfully synthesized by the Stockholm group for the purpose of e.g., reference standards. Finally a stability study was performed and it was concluded that prolonged storage of serum and plasma at - 20°C warranted proper analysis of HPCs with a slight preference towards serum as a matrix.

Analyses of HPCs in human serum have been performed in several study cohorts within and outside of the Compare project. These included: a) the Groningen Child Development Cohort, involving analysis of HPCs in maternal and in fetal cord blood samples from 90 pregnant mothers and their children; b) Swedish Fishermen and their Wives Cohort involving about 400 volunteers; c) two relatively high exposure cohorts, one from Slovakia (close to former PCB factory) and one from Faroe Islands (high dietary intake due to e.g., whale blubber consumption).

Conclusions from the human HPC exposure studies are: Human individuals are exposed to HPCs. The levels of HPCs found in background exposure populations analysed range from low PPBs to the PPM level for phenolic metabolites of PCBs and for pentachlorophenol. In contaminated areas the concentrations of HPCs found are around 4 to 10 times higher than in the background population of pregnant mothers in the Netherlands.

The brominated HPCs are present at low PPB levels in human serum and in cord serum. The concentrations normalized for lipid content are in general 1- 4 times higher in the fetus as compared to the mother, indicating that the placenta is no barrier for these compounds. Apparently, the HPC-binding protein, TTR in the serum is not a major factor for the transfer of HPCs from mother to fetus and from blood to brain

### Hazard assessment of HPCs in experimental animals

The hazard assessment of HPCs was based on a) two-generation experimental animal studies, using Wistar rats as model species, focussing on developmental reproductive and neurobehavioural effects, following exposure of dams to HPCs from day 10-16 of gestation; b) kinetic studies in rats and birds and c) in vitro studies on endocrine effects of HPCs. The developmental reproductive studies in rats were performed by the group of Brouwer, at the Institute for Environmental Studies, Vrije Universiteit, Amsterdam, the neurobehavioural studies in rats were performed by the group of Regan.

Dublin University, and at the Amsterdam group, with the help of Lillienthal from University Duesseldorf, Germany.. In Uppsala University, the group of Brandt has performed comparative kinetic and effects studies with HPCs in birds, rats and knockout mice. Finally, the Amsterdam group also performed the in vitro endocrine activity experiments of HPCs.

The preliminary conclusions on developmental toxicity of HPCs are the following: Overall the in vitro as well as the in vivo data generated during the Compare study indicate that PCBs, their hydroxy metabolites (OH-PCBs), and the brominated HPCs can induce long lasting developmental reproductive and neurobehavioural changes in offspring at low dose levels of exposure during pregnancy, i.e., at doses well below the levels that would induce sub-clinical or overt toxicity in dams, or offspring. The HPCs also show endocrine activity in in vitro studies, in particular on the thyroid and the estrogenic pathways. Furthermore, there are changes in thyroid hormone and estrogen levels observed in offspring following exposure of dams to several HPCs. Some of the in vivo changes are already observed at the lowest doses used in this study, therefore it is not possible to give an accurate indication of No Observed Effect Levels (NOELs), but we have Lowest Observed Effect Levels at 0,1 mg/kg per day (at exposure during pregnancy for 7 consecutive days). There are some similarities and also some differences observable between the various classes of HPCs. Overall the PCBs and their hydroxy metabolites are more active on the thyroid system and on developmental neurobehavioural changes on prepulse inhibition and on open field locomotion and neuroplasticity. Effects on the estrous system (hormones and cycle) and on spatial learning are observed in a more comparative way between the chlorinated and brominated HPCs studied.

In birds, the HPCs studied were injected Japanese quail eggs to investigate possible long-term adverse (reproductive and behavioural) effects. Hatchability by a phenolic PCB metabolite (4-OH-CB-107) was reduced in the two highest doses, but no effects on reproductive behaviour, or gonadal weight were observed. Egg laying was not affected. This indicates that the metabolite 4-OH-CB 107 has a low developmental impact on birds, like Japanese quail. 4-OH-CB 187 also showed a high lethality early during embryonic development in the Japanese quail. At a dose not causing embryomortality there were no effects on reproductive behaviour, egg production or plasma concentrations of thyroid hormones in the adult birds. 6-OH-BDE 47 was highly toxic to early embryos

#### Human clinical-epidemiological studies on HPCs and health outcome

In the Compare project human clinical-epidemiological studies on possible associations between health outcome and exposure to HPCs were performed as well. Two study cohorts were included in the Compare project, namely: a human adult cohort: the Swedish Fishermen and fishermen's wives studied by the group of Prof. Hagmar, Lund University and a human infant cohort: the Groningen Child Development Cohort, studied by the group of Prof. Sauer, Groningen University. The Swedish Fishermen and fishermen's wives cohort study was aimed at exposure to the HPCs: CB 153, p,p'DDE, the hydroxyPCBs and BDE 47. East and West coast fishermen and their wives were compared, due to their different dietary and environmental exposure level to HPCs. The health outcome was primarily focused on fracture incidence, bone mineral density and endometriosis i.e., markers sensitive to e.g. estrogenic effects. The Groningen Child Development Cohort was aimed at exposure of mother's and their children (cord blood) to the following HPCs: 4-OH-CB-107, 4-OH-CB-187 and 4-OH-CB-146, PCB 153, TBBPA, 2,4,6-TBP and the BDEs 99, 100, 153, 154 and HBCDD. The developmental health outcome parameters in the children studied were aimed at neurological, immunological and reproductive development. A total of 90 mothers and their children were included in the study. Blood withdrawal was at w 20 and w 35 of pregnancy as well as cord blood and child blood at 3 months and at 18 months of age. Investigation of development of the children was performed at 14 days of age, at 3, 10 and 18 months of age.

### Conclusions from the human data

The conclusion from the Swedish Fishermen and Fishermen's Wives cohort study are, that the results observed do not support an association between fish born-HPC exposure and bone fracture incidence, or bone mineral density changes. The conclusion from the Groningen Child Development study is preliminary, because the data are still not fully evaluated.

From the data obtained so far, there are at best some weak associations observable between HPC exposure in mothers and some neurological development parameters in the children. In addition, there are some weak associations between maternal HPC exposure and thyroid hormone and sex hormone parameters in boys at 3 months of age. It should be noted however, that the possible influence of confounding factors on these weak associations is not fully evaluated yet.

### Integrated and comparative risk assessment

The LOAEL for effects on estrous cycle length in offspring, changes in open field locomotion and decreases in serum TT4 levels were observed at the lowest dose applied of 0,1 mg/kg body weight of 4-OH-CB-107 per day for 7 consecutive days during pregnancy in rats. The serum level of 4-OH-CB-107 in rat offspring from dams from the 0,1 mg/kg exposure group on postnatal day 4 was on average 0,6 µg/gram plasma, or 85,7 µg/g lipid in plasma. Here it is assumed that the HPC plasma concentration on PND 4 is a fair representation of the prenatal plasma level of the offspring, i.e., during the critical window of development of reproductive and neurobehavioural capacity in rats. It is also assumed that the no observable adverse effect level (NOAEL) is about 1/5<sup>th</sup> of the LOAEL, or at a dose level of 0,02 mg/kg and that the corresponding plasma levels in rat offspring on PND would also be at or around 1/5<sup>th</sup> of the level observed at the LOAEL dose level, or 0,12 µg /g plasma, or about 17 µg /g lipid in plasma.

When the rat offspring plasma level of 17 µg /g lipid estimated on the basis of a NOAEL exposure level of 0,02 mg/kg is compared to the cord serum levels from the human child development cohort, of on average 7,7 ng/g lipid and a maximal observed level of 22,5 ng/g lipid for 4-OH-CB-107 there is a difference of about a factor 1000. This margin of safety would normally be sufficient to cover the uncertainty in extrapolating from laboratory animals to human individuals

However, if the individual HPCs (OH-PCBs and PCP) are summed-up, based on similar modes of action on e.g., the thyroid axis, the average concentration in cord serum of total T4-EQ was 2,5 µgT4-EQ/g lipid with a maximal observed concentration of 5,7 µgT4-EQ/g lipid in cord serum. The concentration of T4-EQ estimated to be present in serum from rat offspring at PND 4 from dams exposed to an estimated NOAEL dose of 0,02 mg/kg would be around 220 µgT4-EQ/g lipid, assuming that a linear relationship exists between external exposure and internal serum levels for HPCs in rats. Assuming further that PCP also would produce effects in vivo similar to the hydroxy-PCBs, this would suggest that the margin of safety between experimental animal studies and human individual exposure levels may only be in the order of a factor of 50 when expressing internal exposure levels of HPCs as µgT4-EQ/g lipid. This margin of safety may not be sufficient enough to cover all uncertainty in extrapolation from experimental animals to human infants. This situation is even worse when considering the contaminated sites in Slovakia or the high background exposure area in the Faroe Islands. Plasma concentrations of hydroxylated PCBs were respectively 3-40 times higher and 5-10 times higher than the concentrations in mothers from the Groningen child development cohort. Moreover, PCP was not even determined yet in the Faroe Island, or the Slovakia plasma samples. This would suggest that there is very little margin of safety left in these contaminated areas with respect to possible consequences for reproductive and neurobehavioural development.

The levels of brominated HPCs observed in human adult and infant serum are generally 3-10 times lower than the levels chlorinated HPCs. On the other hand the observed effects of brominated HPCs in experimental animal studies were much less pronounced and required exposure levels of 50 to 250 times higher than of the chlorinated HPCs investigated. Therefore, it is concluded that there is presently sufficient margin of safety for brominated HPCs and that current background exposure levels of human individuals to brominated HPC does not pose any risk for adverse health effects.

In terms of sources of exposure to hydroxylated PCBs it is estimated that the main source of exposure would be the internal production by metabolism of the parent PCB congeners. The possible sources of external exposure are limited to eggs, liver, or liver products, blood, or blood products, due to the specific distribution pattern of hydroxyl-PCBs. However, these external sources are most likely outnumbered by far by the internal production due to metabolism of PCBs. This is certainly not the case for pentachlorophenol (PCP), which is still used as wood preservative in some areas of the world. Exposure to PCP via food and environment will continue to exist for a long time to come. PCP is the dominating contributor to the total T4-EQ as well as the predominant chlorinated HPC present in human serum, i.e., 10 times higher concentration than any of the other chlorinated HPCs present. This pinpoints to a urgency to further investigate exposure and effect levels for pentachlorophenol, as well as options to replace and reduce environmental contamination.

Based on the above risk assessment possible effects in human individuals, in particular in children are not very likely to occur in background exposure situations, like the Groningen Child Development cohort. In fact, the results from this child development study indicate that there are no obvious effects from HPC exposure observable in the children involved in the cohort. There are some findings observed in the Child development study which suggest some weak but significant correlations between HPC exposure and some aspects of endocrine and neurobehavioural development. It should be noted however, that a proper evaluation of the data and of the possible impact of confounding factors is not completed yet.

Final conclusions: Human individuals are exposed to a mixture of different HPCs, derived from internal (metabolism) as well as external sources. Background exposure levels in human infants to HPCs, when considering individual congeners, suggests that there still is a sufficient margin of safety left to avoid occurrence of adverse developmental health outcome due to these compounds. However, when considering a summing-up of chlorinated HPCs as  $\mu\text{T4-EQ/g}$  lipid in cord serum, there is only a small margin of safety left of about a factor 45 for children born to mothers exposed to background levels of HPCs. This margin of safety does probably not exist when considering human individuals, in particular children, living in high exposure areas, like Faroe islands and at contamination sites in Slovakia. In other words, adverse developmental health effects from chlorinated HPCs in human children originating from contaminated, or otherwise high background exposure area cannot be excluded. The results from the Compare study with respect to the brominated HPCs studied do not support that an additional risk for adverse health effects can be expected at current background exposure levels of the brominated HPCs investigated in human individuals.

## **BENEFITS AND BENEFICIARIES:**

The main benefit from the results of the Compare study is the integrated and comparative risk assessment of the chlorinated and brominated halogenated phenolic compounds (HPCs) investigated. It is stressed that this risk assessment provides a preliminary state of the art situation, since evaluation, and interpretation of the Compare datasets is still ongoing. This risk assessment is timely and there is a great need for this information regarding questions about the safety of e.g., brominated chemicals that are presently widely used globally in a wide variety of applications. The main beneficiaries of the Compare study results with respect to the risk assessment performed are national and international regulatory bodies, chemical industry and the general public.

## **FUTURE ACTIONS:**

Continuation of the data evaluation and interpretation process of the Compare study results and dissemination of results through e.g., scientific publications outside of the Compare project term.