# Risk Assessment of Endocrine Active Compounds

Joseph G. Vos[1,2]

[1]*National Institute of Public Health and the Environment, Bilthoven*
[2]*Faculty of Veterinary Medicine, Utrecht University, Utrecht*

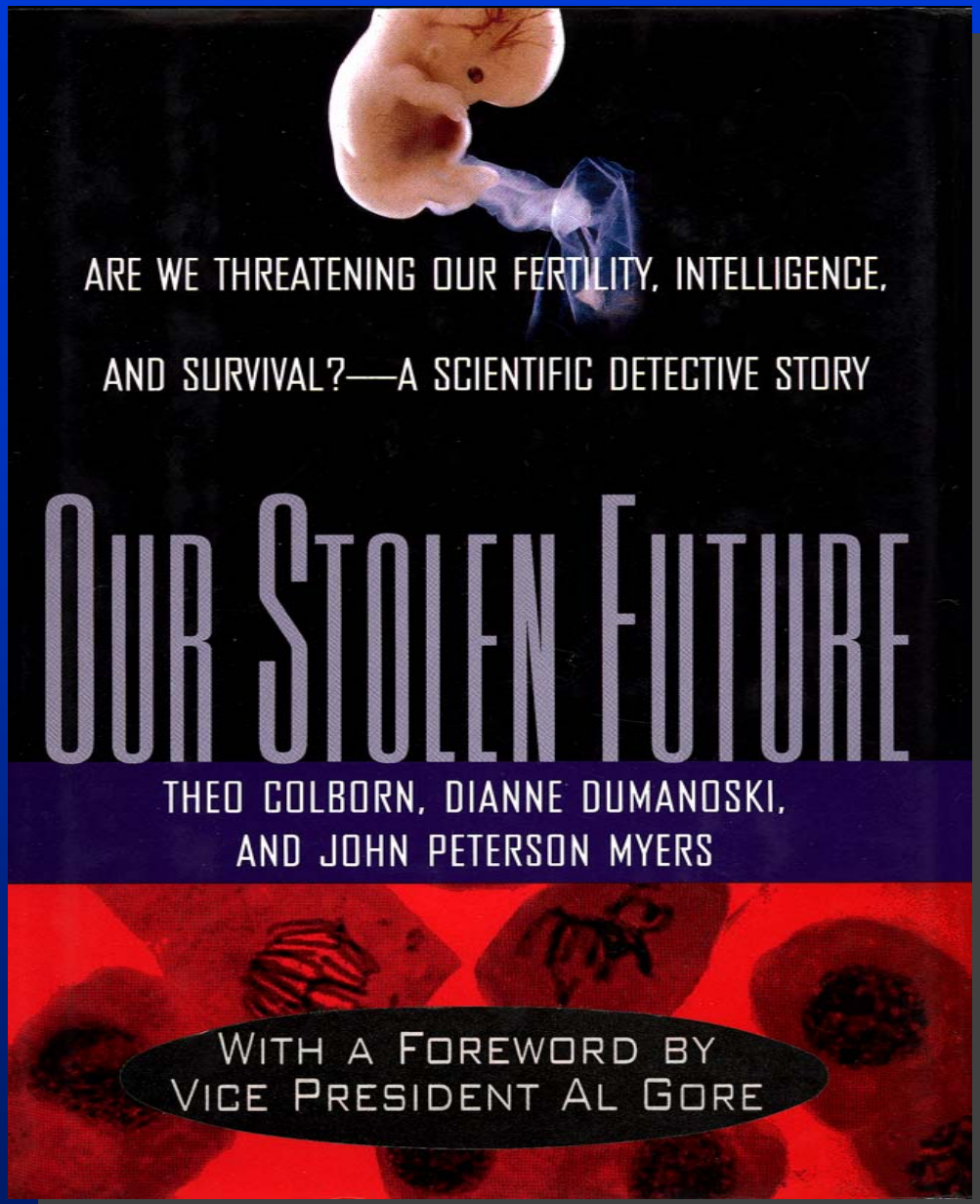
# Definition of Endocrine Disruption

An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

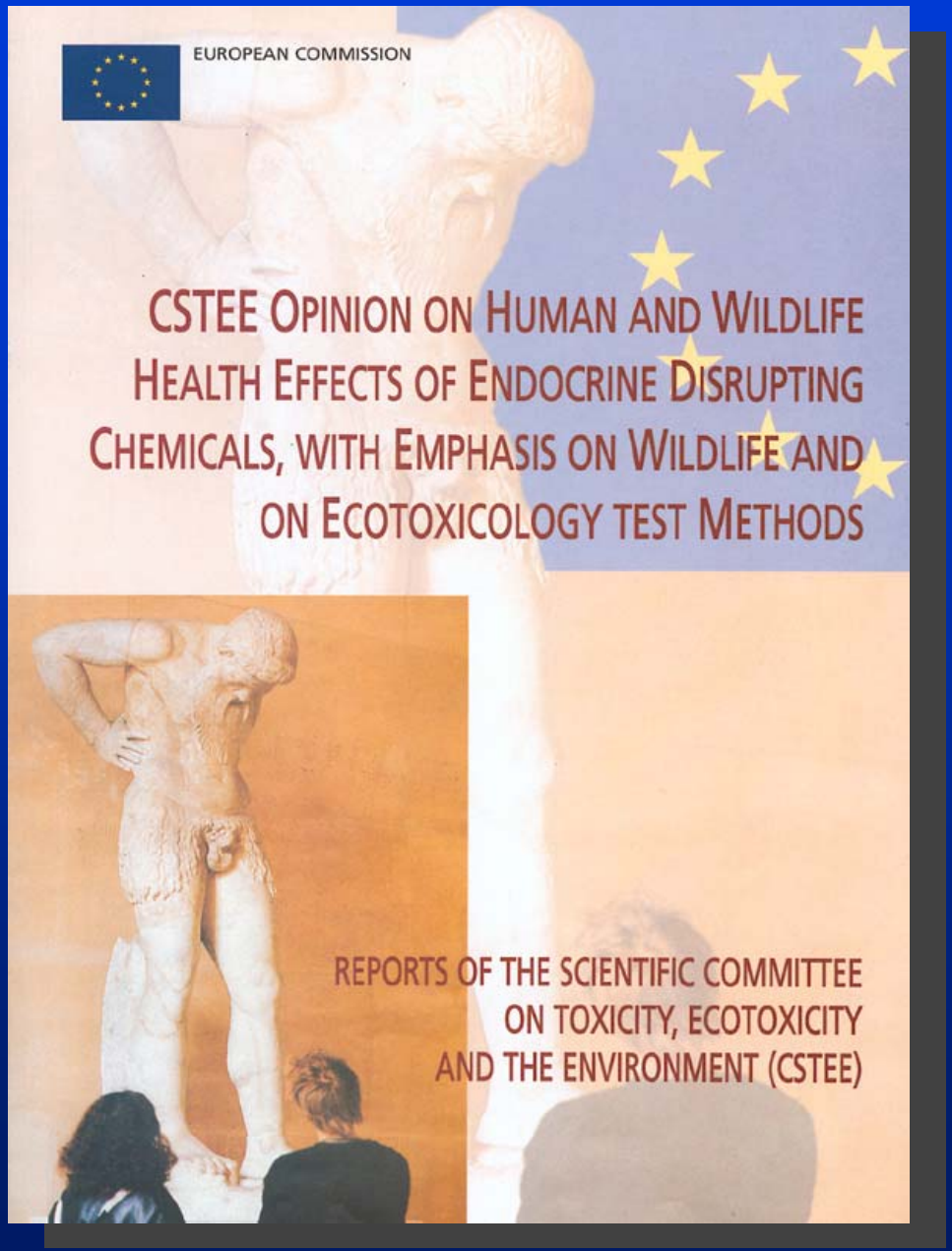
(definition of the IPCS Steering Group, March 1998)

# Introduction

There is general concern of exposure to xenobiotic compounds that are capable of modulating or disrupting the endocrine system. This concern for EDCs is directed at both humans and wildlife.




Published by Dutton, NY (1996)




The report of the working group is published in 1999 *(http://europa.eu.int/comm/dg24/health/sc/sct/out37_en.html)*

The wildlife section has been published in Crit. Rev. Toxicol 30 (2000) 71-133

# EDCs and human health effects

**Evidence for decreasing quality of semen during past 50 years**

Elisabeth Carlsen, Aleksander Giwercman, Niels Keiding, Niels Skakkebæk

***British Medical Journal*** 305 (1992) 609-13

# Conclusions CSTEE report on human health effects

- Regarding decrease in sperm concentration and semen volume several reanalyses of the same data have indicated possible bias and confounding in the meta-analysis, and have reached different conclusions with respect to sperm quality, depending on the methodology used. Recent, well designed studies have shown that there are large regional differences in overall sperm quality and time trends, both within and between countries.

- Although there are associations between EDCs, so far investigated, and human health disturbances, a causative role of these chemicals in diseases and abnormalities possibly related to an endocrine disturbance has not been verified. This concerns the reported increased prevalence or incidence of:
  - cryptorchidism or hypospadias in several investigations
  - testicular cancer during the last 30 years
  - prostate cancer in the last decades
  - breast cancer incidence; the available data do not support a causal role for organochlorine compounds
  - declining proportion of male new-borns.

- High accidental exposure to polychlorinated biphenyls and dibenzofurans (PCBs/PCDFs) of pregnant women have led to delays in physical and mental development of the offspring resembling hypothyroidism. There are indications that organochlorine compounds may affect neonatal neurological development, possibly by affecting thyroid hormone status.

# Recommendations on human health

- Further evaluate the human health effects which have been associated with endocrine disrupters and to identify the underlying causes.

- In this, special attention should be given to exceptional high chemical exposures and to the health consequences of phytoestrogens in human food.

# Toxicological test guidelines

- Present regulatory toxicology test guidelines, in particular the guidelines for ecotoxicity testing, cannot detect all endocrine disrupting effects. Therefore, current test guidelines have to be enhanced or new guidelines developed.

- Reliance on *in vitro* assays for predicting *in vivo* endocrine disrupter effects may generate false-negative as well as false-positive results. Therefore, major emphasis should be put on *in vivo* assays.

# Criteria according to Hill (1965): Association versus Causation

- Strength of evidence
- Consistency of evidence
- Specificity of effect
- Temporality of effect
- Dose response
- Plausibility of effect
- Coherence with existing knowledge
- Experimental support (rodents simulation)
- Analogy (structure activity)

- The most prominent and persistent organic pollutants that are associated or even causally linked with endocrine disruption in wildlife and in human individuals are the organohalogens DDT and metabolites, PCBs, PCDDs and PCDFs.

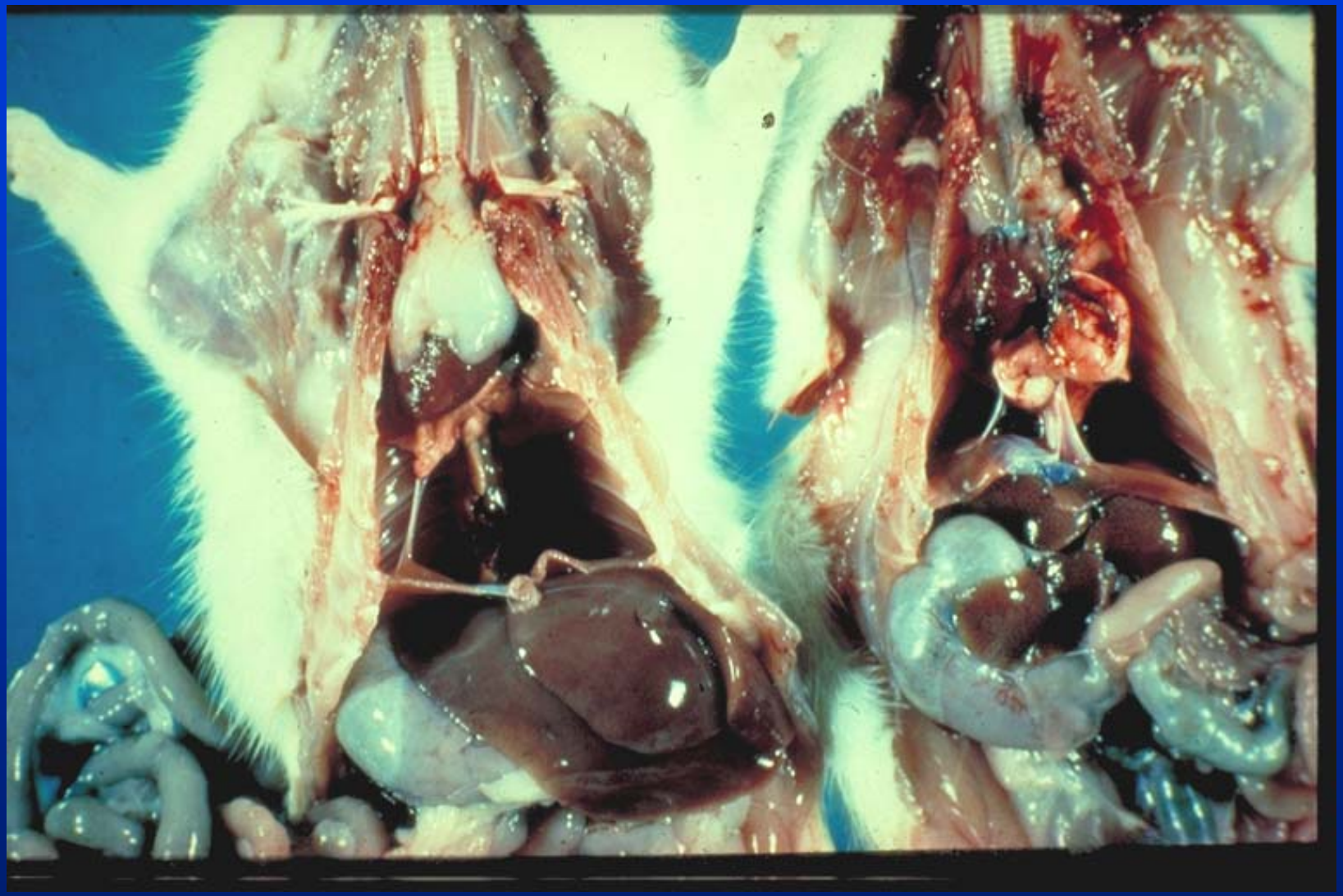
- From an environmental point of view an increasing important group are the brominated flame retardants (BFRs).

# Chemicals of environmental concern discussed in this presentation

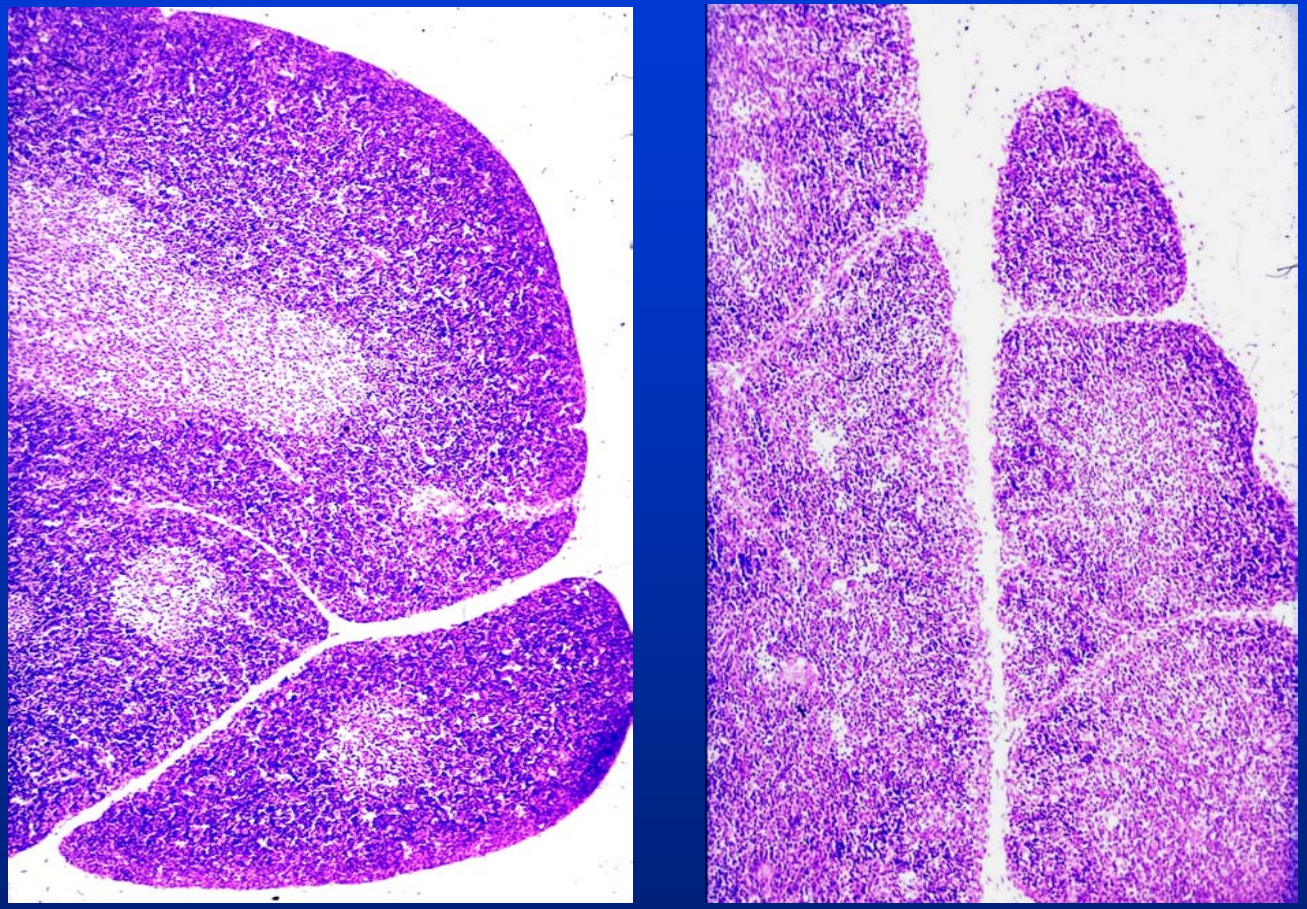
- Polychlorinated biphenyls (PCBs): immune effects in children following perinatal exposure and the role of immunotoxicity in the mass mortality in seals in Europe in 1988 (and 2002).

- Polybrominated diphenyl ethers (PBDEs): exposure, toxic effects, risk assessment (EU FIRE project)

Studies in laboratory animals implicate many organochlorines (including PCBs, PCDFs and PCDDs) in immunosuppression and increased susceptibility to infectious diseases in several species



TCDD-induced thymus atrophy in the rat



Thymus cortex atrophy in rat as result of reduced cell divisions following exposure to TCDD

# Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children

Weisglas-Kuperus et al., **Environ. Health Perspect.** 108 (2000) 1203

**Table 2.** Prevalence of infectious and allergic diseases and effects of prenatal and current PCB body burden.

| | Prevalence *n* (%) (*n* = 175) | Prenatal PCB exposure ΣPCB Maternal OR (95% CI)[a] | *p*-Value | Current PCB body burden ΣPCB at 42 months OR (95% CI)[a] | *p*-Value |
|---|---|---|---|---|---|
| Infectious diseases | | | | | |
| Middle-ear infections (1 or more episodes) | 103 (58.9) | 0.89 (0.65–1.23) | 0.49 | 1.27 (0.61–2.64) | 0.52 |
| Recurrent middle-ear infections (6 or more episodes) | 21 (12.0) | 1.37 (0.87–2.17) | 0.17 | 3.06 (1.17–7.98) | 0.02* |
| Pneumonia | 5 (2.9) | 0.41 (0.10–1.63) | 0.21 | 0.01 (0.01–29.68) | 0.13 |
| Scarlatina | 13 (7.4) | 1.00 (0.56–1.80) | 0.98 | 0.59 (0.08–4.03) | 0.59 |
| Chicken pox | 130 (74.3) | 1.43 (0.92–2.24) | 0.11 | 7.63 (1.21–48.54) | 0.03* |
| Other infectious diseases | 15 (8.6) | 1.04 (0.60–1.82) | 0.87 | 0.85 (0.27–2.67) | 0.79 |
| Hospital admissions for infectious diseases | 7 (4.0) | 1.04 (0.44–2.46) | 0.93 | 0.68 (0.01–25.05) | 0.37 |
| Allergic diseases | | | | | |
| Eczema | 42 (24.0) | 1.18 (0.82–1.71) | 0.37 | 0.92 (0.41–2.08) | 0.84 |
| Allergic reaction | 14 (8.0) | 0.62 (0.29–1.32) | 0.22 | 0.01 (0.01–0.37) | 0.01* |
| Asthma or bronchitis | 30 (17.1) | 0.87 (0.55–1.40) | 0.56 | 0.38 (0.06–2.57) | 0.32 |
| Coughing, chest congestion, or phlegm lasting for 10 days or more[b] | 48 (27.4) | 1.08 (0.75–1.54) | 0.69 | 1.12 (0.58–2.16) | 0.74 |
| Attacks of shortness of breath with wheeze[b] | 17 (9.7) | 0.44 (0.18–0.99) | 0.05* | 0.34 (0.02–4.49) | 0.41 |

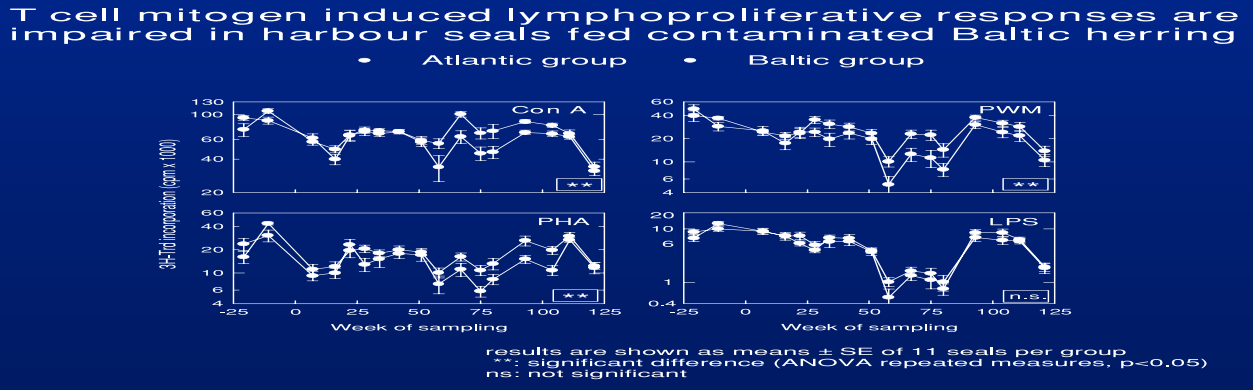
[a]Corrected for sex, early feeding type (breast-fed or formula-fed), duration of breast-feeding during infancy (less or more than 16 weeks), parity (firstborn or second born), maternal education and parental occupation (low), tobacco smoking by one or both parents (yes or no), family history of atopy in one or more parents (yes or no), and day care or nursery school attendance for the child (yes or no). [b]In the previous 12 months. *Significant at the ≤ 0.05 level.

The authors conclude that in Dutch preschool children the effects of perinatal background exposure to PCBs and dioxins persist into childhood and might be associated with a greater susceptibility to infectious diseases. Common infections acquired early in life may prevent the development of allergy, as exposure might be associated with a lower prevalence of allergic diseases.

# The role of PCBs as immune suppressor in the virus-induced mass mortality in seals: link between field, semi-field and laboratory studies

- In 1988, a mass mortality killed 60% of the population of harbor seals (*Phoca vitulina*) in northwestern Europe, or 20,000 animals.

- A new virus was discovered to be responsible: phocid (seal) distemper virus (PDV), closely related to dog distemper virus and human measles virus.

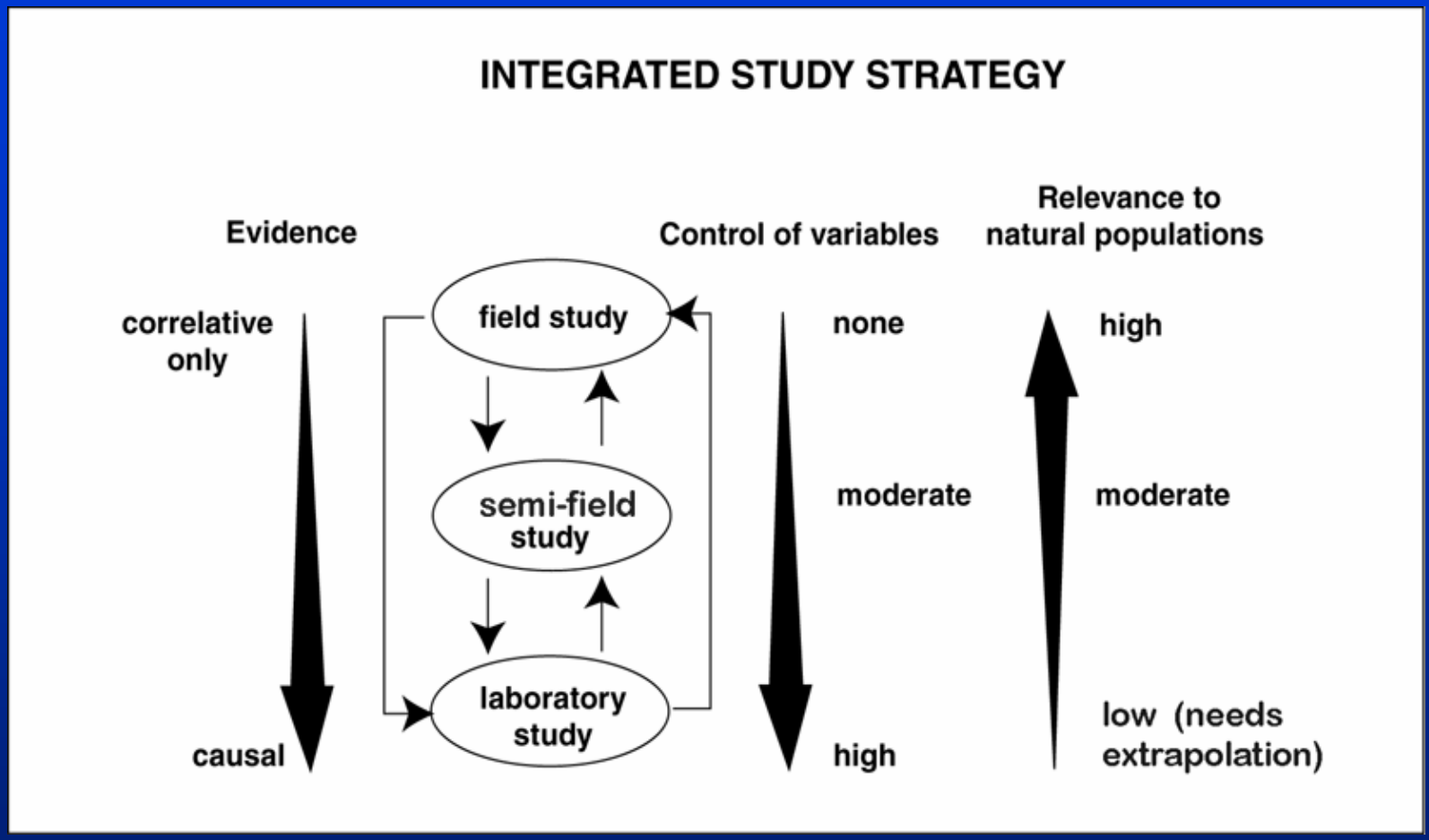


- Following the 1988 epizootic there was a rapid recovery, but the new outbreak in 2002 killed 20,500 animals (that were not immunologically protected).

- The severity of this virus epizootic thus raised concern that organochlorine chemical pollution of the environment had aggravated the severity and extent of the outbreak.

- For certain chemicals, including PCBs, PCDFs and PCDDs, large interspecies differences are found.

- The risk assessment process for these compounds benefits from data obtained by testing the species itself, either in semi-field studies or in studies performed in the laboratory.



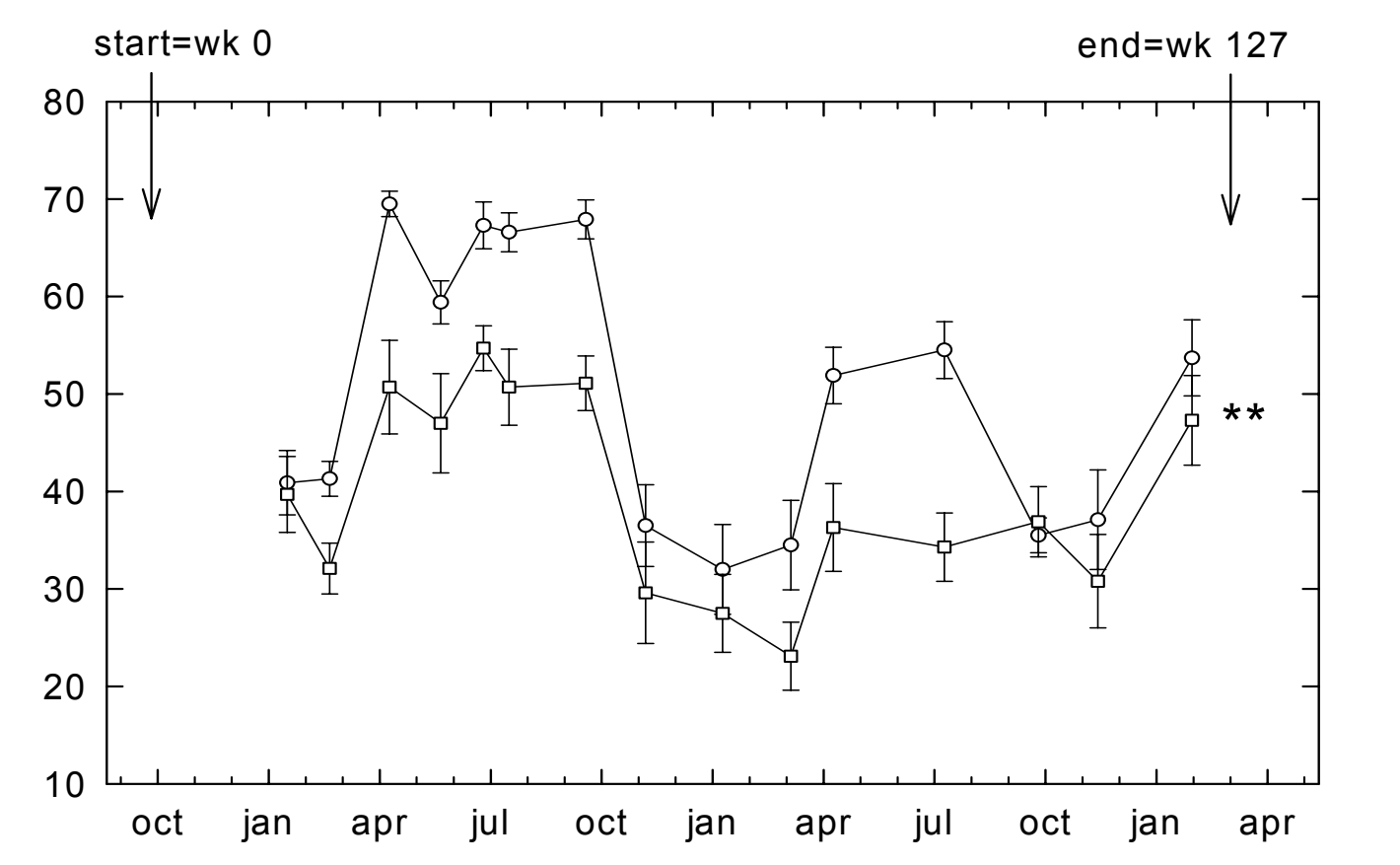

Integrated approach to study the causes of wildlife diseases

(after: Vethaak, 1993)

# Semi-field study in harbor seals

- In order to investigate whether the immune system of seals inhabiting polluted areas is impaired, a study with harbor seals (*Phoca vitulina*) was carried out under semi-field conditions.

- In this study two groups of 11 seals (7 females, 4 males) were fed herring for a period of 2.5 years from either the polluted Baltic Sea or the relatively uncontaminated Atlantic Ocean.




NK cell activity in blood mononuclear cells from seals fed herring from either the Atlantic Ocean (round symbols) or the Baltic Sea (square symbols); **P<0.01

(from: Ross et al., Aquat. Toxicol. 34 (1996) 71)

Summary of Immunotoxicological Effects in a Semi-field Study of Harbour Seals Fed Herring from the Baltic Sea*

| Immune parameter | Effect |
|---|---|
| NK cell activity | ↓ |
| T-cell mitogen response (PHA, Con A, PWM) | ↓ |
| Mixed lymphocyte response | ↓ |
| Antigen specific proliferation (rabies, tetanus) | ↓ |
| Specific antibody production | -/↓ |
| Delayed-type hypersensitivity to ovalbumin | ↓ |
| Neutrophils in circulation | ↑ |

* As compared to results of the group of seals fed herring from the relatively uncontaminated Atlantic Ocean

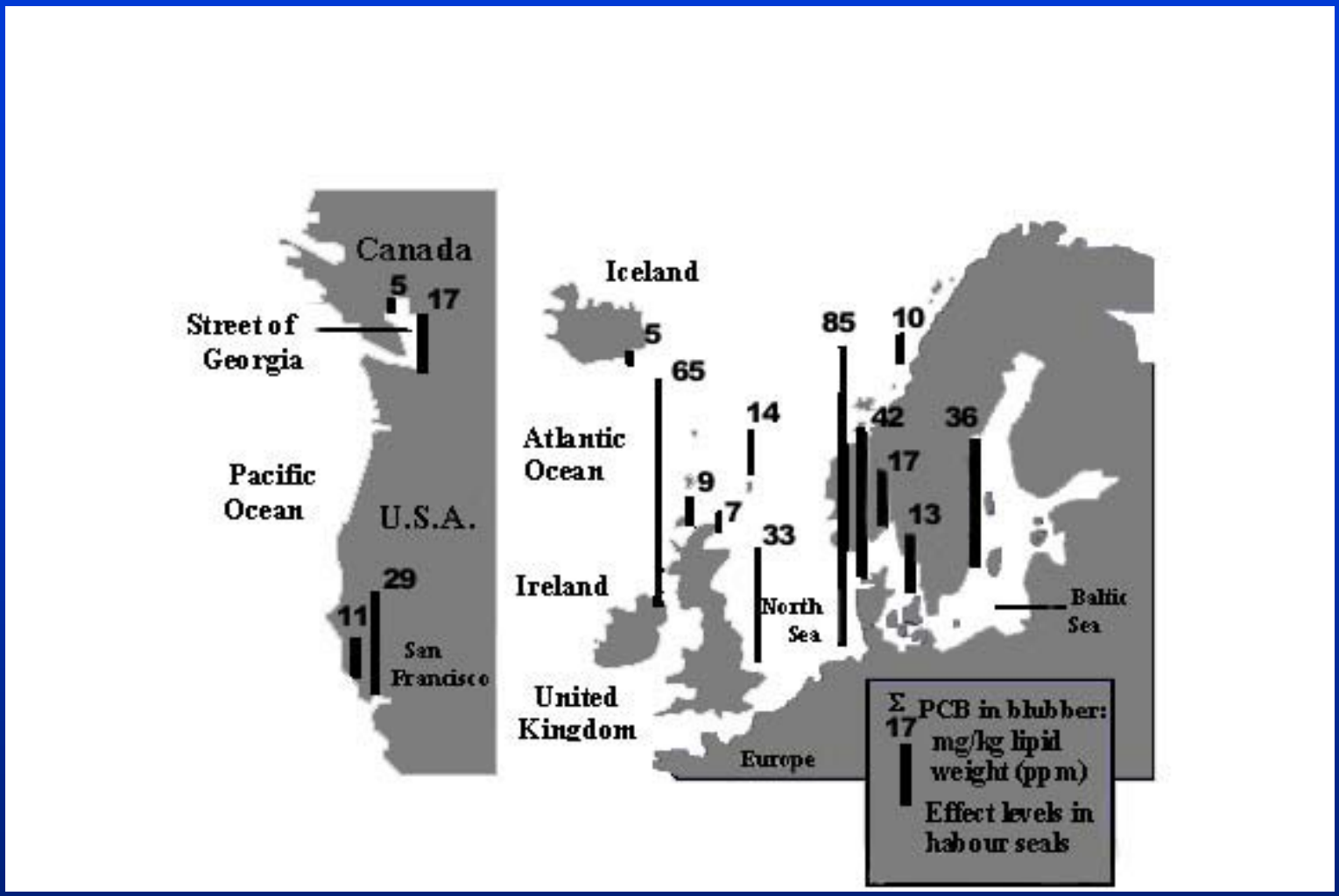
# Conclusions

- Results of the semi-field study showed that seals fed fish from the Baltic Sea had suppressed immune function as measured by NK cell activity and T-cell function; both being crucial to anti-virus defences, including the clearance of morbillivirus infections

- Results of studies in laboratory animals using the same herring batches, and TCDD as a positive control group, suggested that perinatal exposure to environmental contaminants represents a greater immunotoxic threat than exposure as a juvenile or adult.

- Additional studies showed that serum vitamin A levels and thyroid hormone levels were decreased in the Baltic group as compared to the Atlantic group, also implicating PCBs.

- These data indicate that current concentrations of PCBs in the aquatic food chain in northwestern Europe are immunotoxic to marine mammals.




Mean blubber concentrations of PCBs in harbour seals (*Phoca vitulina*)

(after: Ross et al., Toxicol. 112 (1996) 157)

- PCB levels in free-ranging harbour seals inhabiting many coastal areas of northwerstern Europe may be at risk to immunotoxicity.

- This may predispose these populations to an increased severity of infectious disease outbreaks, such as the 1988 and 2002 seal distemper virus mass mortalities.

# Risk Assessment of Brominated Flame Retardants as Suspected Endocrine Disrupters for Human and Wildlife Health

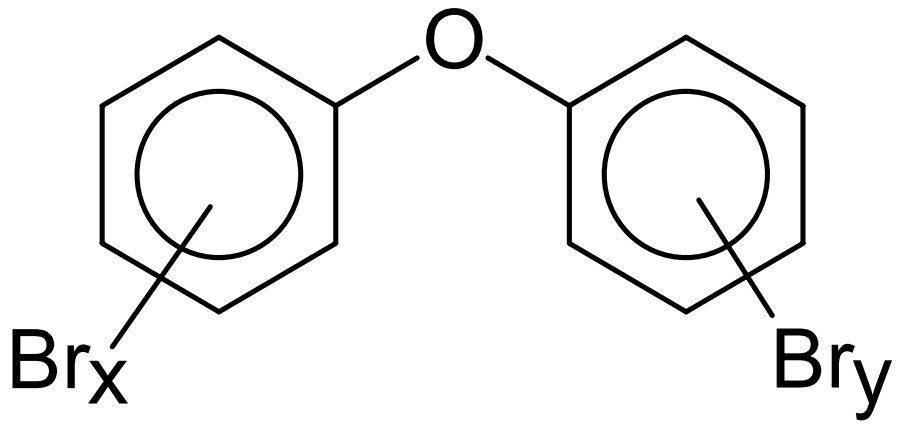
BFRs are made up of structurally very different chemicals with a wide variety in physicochemical and reactivity characteristics and include:

- polybrominated diphenyl ethers (PBDEs),
- tetrabromobisphenol A (TBBP-A) and
- hexabromocyclododecane (HBCD).

They are widely used in polymers and textiles, and applied in construction materials, in carpets and furniture and in electric and electronic equipment.

The annual market demand in 1999 has been estimated to 67,000 tons for PBDEs and 121,000 tons for TBBPA.

# Chemical structure of polybrominated diphenyl ethers




x + y = 1-10

PBDE

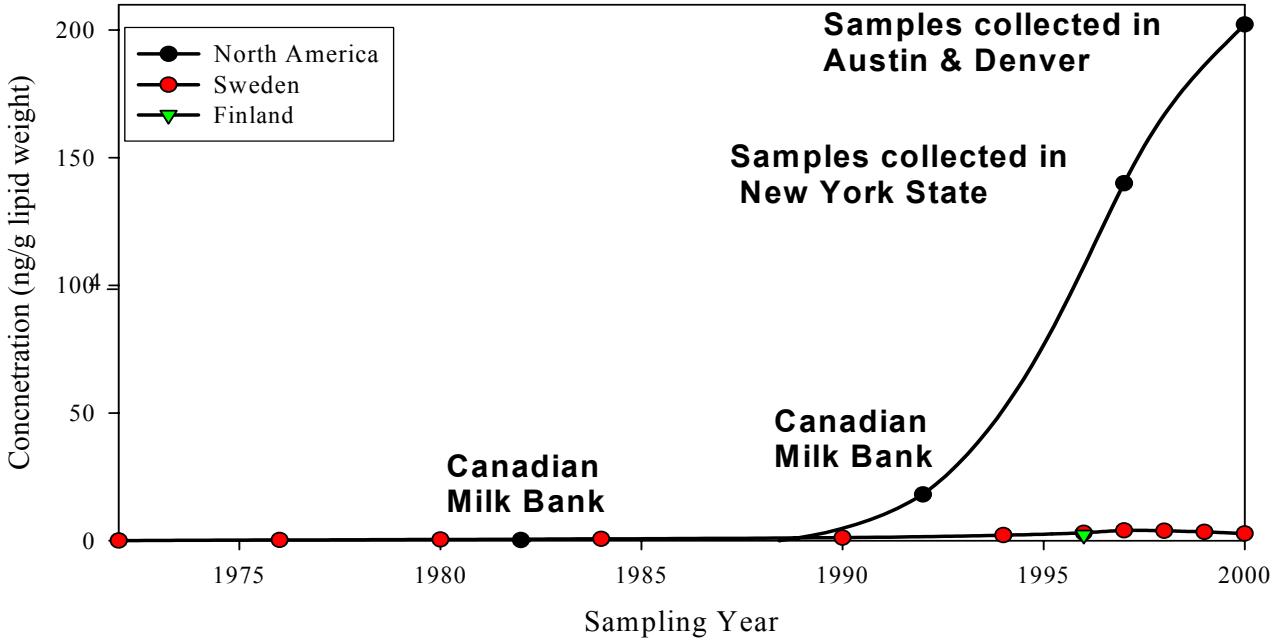
# Exposure data

- Due to the high lipophilicity and persistency PBDEs bioaccumulate with BDE-47, BDE-99, BDE-100 and BDE-153 being the dominant congeners.

- In human milk, mean PBDE levels reported for northern Europe ranged between 4 and 16 ng/g lipid. Levels are increasing in human milk: a study in Sweden showed a doubling in concentration every five year over the period 1972 to 1997, BDE-47 being the predominant congener (from 1998 to 2000 a decrease in PBDE levels was noticed, which can be a consequence of the phase out of the commercial pentaBDE in Sweden).

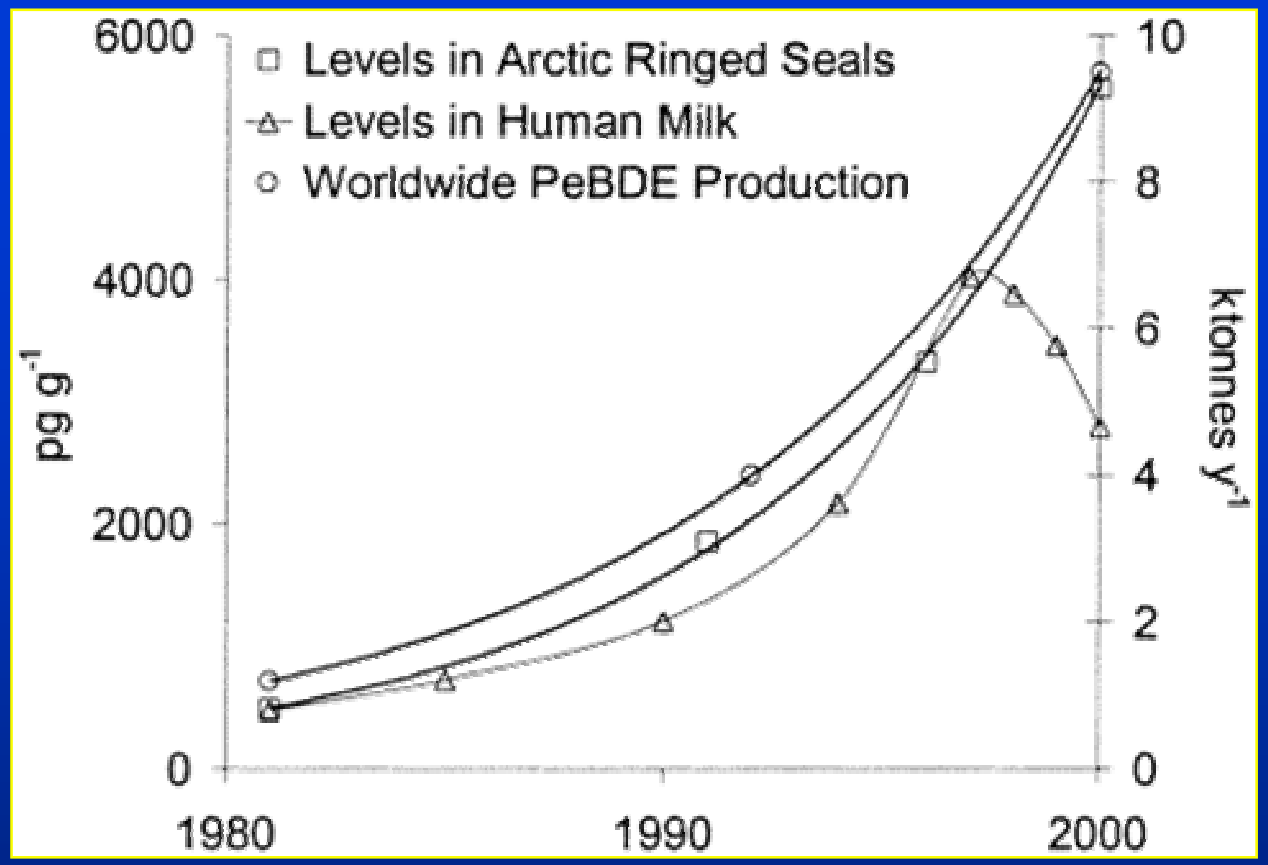
- Recently levels of approximately 200 ng/g lipid were reported in a pooled sample of mothers milk from the U.S.

# Comparison Between Concentrations of PBDEs in Breast Milk from North America and Europe




Canadian Milk Bank and New York State from Ryan and Patry 2000, Denver and Austin results from Papke et al 2001; Swedish data from Meironyte Guvernius and Noren 2001, Finnish data from Strandman et al. 2000

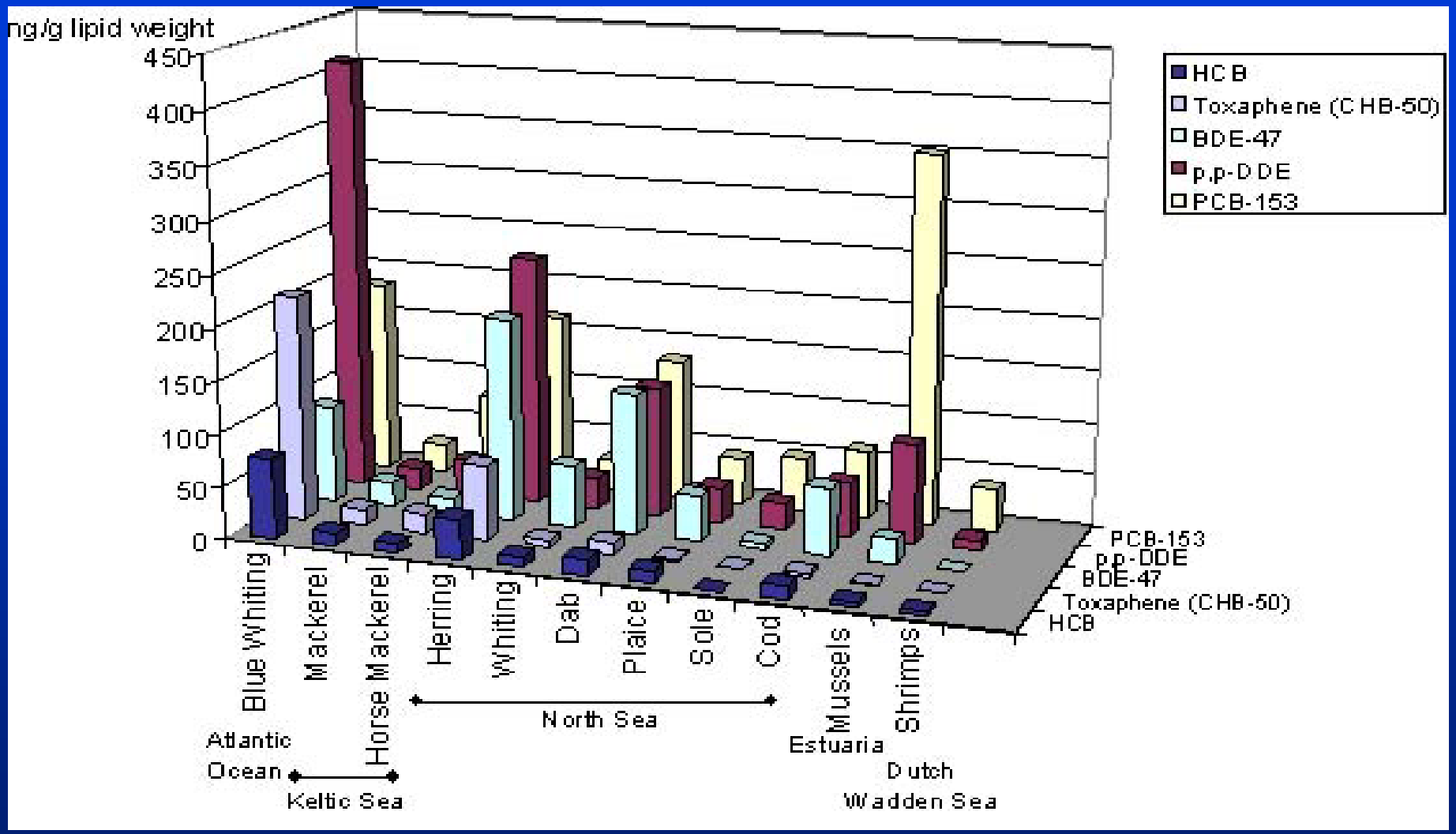
Note that the levels in North America appear much higher as compared to data from northern Europe




Comparison of PBDE levels in ringed seals from the Canadian Arctic, PBDE levels in human milk from Sweden, and worldwide commercial penta-BDE production

(from: Ikonomou et al, Environ Sci Technol 36 (2002) 1886-1892)

Recent data from the Netherlands Institute for Fisheries Research (RIVO) show that in different fish species collected in 2000 from the North Sea and the Celtic Sea levels of BDE-47 were similar as levels of PCB 153 and p,p-DDE. Levels of hexachlorobenzene (HCB) and Toxaphene (CHB-50) were lower.




Contaminant profile of fish, mussels and shrimp caught in 2000 in different waters (pooled samples of 25 animals each)

# Toxic effects data

There is a lack of information regarding the potential of BFRs to cause endocrine disruption. Alteration of endocrine function by BFRs is realistic as there are resemblances in toxicological effects between PCBs and PBDEs:

- thyroid toxicity
- neurobehavior disturbances
- immunotoxicity
- estrogenic activity *in vitro*.

# Conclusions

- From the present human and environmental monitoring data as well as the toxicological data it is concluded that they are far too incomplete and insufficient to perform an adequate human and ecological risk assessment.
- Levels of PBDEs in wildlife as well as in humans are still increasing.
- Systematic monitoring and generation of toxicity data should answer the question whether margins of safety are sufficient. This is the aim of the FIRE project

# Risk Assessment of Brominated Flame Retardants as Suspected Endocrine Disrupters for Human and Wildlife Health

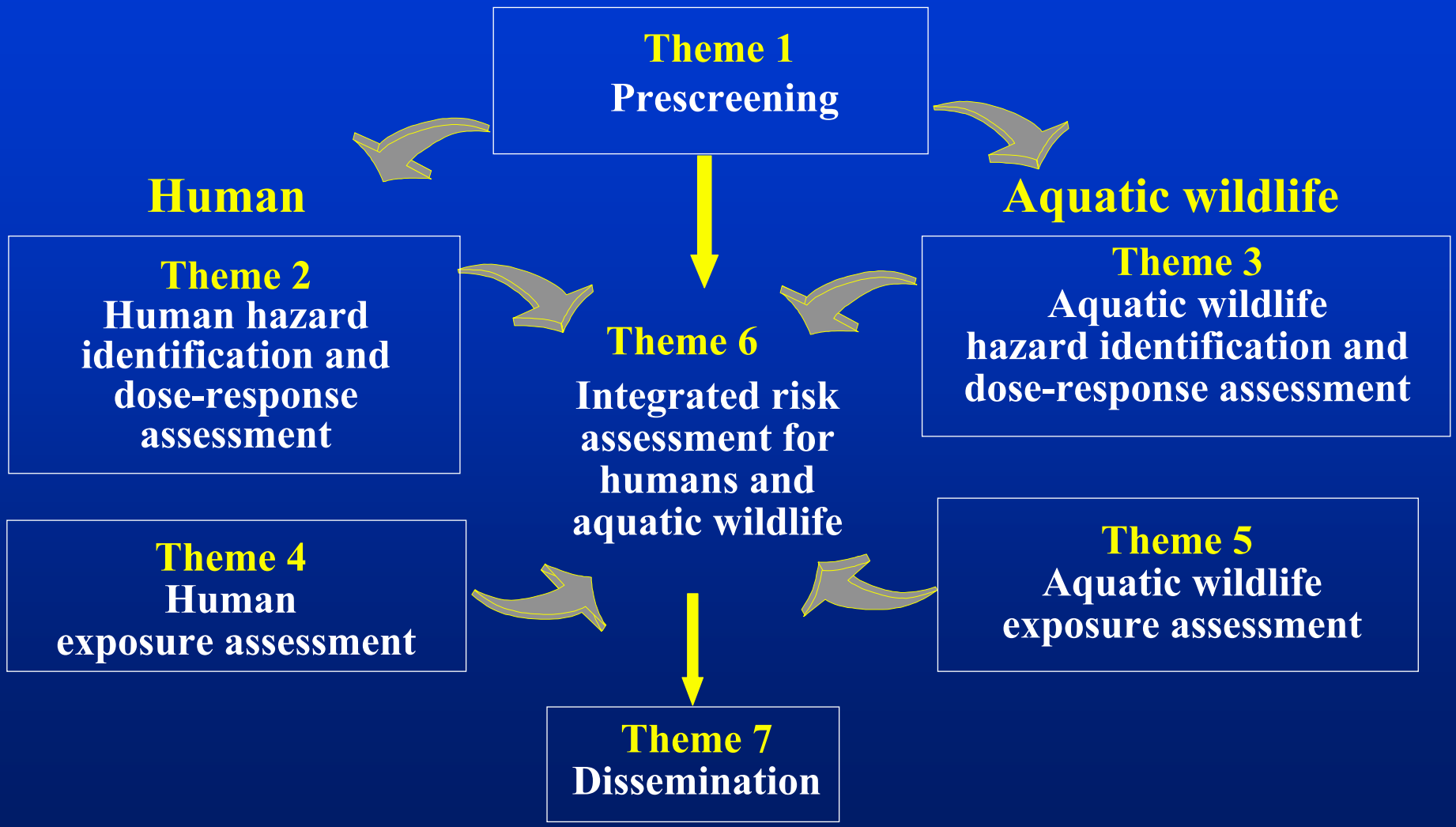
Flame retardants Integrated Risk assessment for Endocrine disruption

FIRE

# FIRE

- 7 countries: The Netherlands, Norway, Czech Republic, Germany, Sweden, United Kingdom, Belgium
- 19 partners
- Duration: 3.5 years (December 2002 - June 2006)
- Total cost: 6.811.999 Euro
- EC contribution: 4.862.885 Euro
- Website: www.rivm.nl/fire
- FIRE is part of the EUs new Cluster of Research on Endocrine Disruption in Europe (CREDO)

# Workplan


Theme 1
Prescreening
Human
Aquatic wildlife
Theme 2
Human hazard identification and dose-response assessment
Theme 3
Aquatic wildlife hazard identification and dose-response assessment
Theme 6
Integrated risk assessment for humans and aquatic wildlife
Theme 4
Human exposure assessment
Theme 5
Aquatic wildlife exposure assessment
Theme 7
Dissemination

# Thank you for your attention

more information: j.vos@rivm.nl

# EU-Flame retardants

Pentabromodiphenyl ether (pentaBDE) and Octabromodiphenyl ether (octaBDE)

Banned for all uses in Directive 2003/11 as of 15 August 2004

# EU-Flame retardants

Polybrominated diphenyl ethers  banned for use in electronics as of 1 July 2006

- national bans already in force will apply
- commision to review ban as soon as possible for decaBDE