ADVERSE DEVELOPMENTAL EFFECTS IN PIGS FOLLOWING *IN UTERO* AND LACTATIONAL EXPOSURE TO ORGANOCHLORINES: EFFECTS ON IMMUNE FUNCTION

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Introduction

Organochlorine compounds (OCs) can be found in virtually all ecosystems of the planet. This group of chemicals includes pesticides {e.g., dieldrin, mirex, toxaphene}, industrial compounds and by-products of various industrial processes {e.g. hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs)}. These compounds are biomagnified in food chains and indigenous populations relying on fish or marine mammals for sustenance receive an unusually high dose of OCs (1).

Several OCs display immunotoxic properties in both laboratory animals and humans, the most potent being substances structurally related to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) such as non- and mono-ortho chloro-substituted PCBs as well as 2,3,7,8-chloro-substituted PCDD/Fs. In almost all animal species tested, including primates, PCDD/Fs and PCBs produce myelosuppression, immunosuppression, thymic atrophy, and inhibition of immune complement system components (2). Evidence in laboratory animals suggests that the maturation of the immune system is especially vulnerable to the adverse effects of dioxin-like compounds, chlordane, polycyclic aromatic hydrocarbons and possibly endocrine disrupting compounds such as DDT and kepone (3).

Infants born to Yu-Cheng mothers had more episodes of bronchitis or pneumonia during their first 6 months of life than unexposed infants from the same neighborhood (4). The authors speculated that the increased frequency of pulmonary diseases could result from a generalized immune disorder induced by transplacental or breast milk exposure to dioxin-like compounds, more likely PCDFs (4).

We recently reported that the risk of otitis media in Inuit infants from Nunavik (Northern Quebec) was associated with prenatal exposure to organochlorine compounds (5). This population is exposed to a complex mixture of organochlorine compounds and heavy metal through their traditional diet that includes sea mammal tissues. The present study investigates changes in immune function following transplacental and lactational exposure to a mixture of OCs similar to that found in the arctic aquatic food chain, using the pig as the animal model.

Methods and Materials

Animal treatment

Sixteen Landrace-Yorkshire-Duroc sows were randomly allocated to four treatment groups. Animals in each group were administered different doses of an organochlorine mixture from four months of age until weaning of their first litter. The composition of the organochlorine mixture is described in Table 1 and was designed to approximate that found in the blubber of ringed seal from Northern Québec (Derek Muir, personal communication). OCs were dissolved in corn oil to reach the appropriate concentration and the resulting solution was placed in 2-mL gelatine capsules. The first group received 1 μg, the second group 10 μg and the third group 100 μg PCB/kg body weight/day. Animals in the fourth group (control group) were administered corn oil only. At puberty, females were inseminated with semen from an untreated boar (Duroc). Eight to ten male piglets per dose group were nursed by their sow during three weeks and after weaning received a standard diet. Piglets were vaccinated against *Mycoplasma hyopneumoniae* at 6 weeks of age. Blood samples were collected monthly for organochlorine determination and for immunological measurements.

Table 1. Composition of the organochlorine mixture.

Compound	CAS number	% weight
PCB mixture[1]		32.59
technical chlordane	57-74-9	21.3
p,p'-DDE	72-55-9	19.24
p,p'-DDT	50-29-3	6.79
technical toxaphene	8001-35-2	6.54
α-HCH	319-84-6	6.17
Aldrin	309-00-2	2.52
Dieldrin	60-57-1	2.09
1,2,4,5-tetrachlorobenzene	95-94-3	0.86
p,p'-DDD	72-54-8	0.49
β- HCH	319-85-7	0.46
Hexachlorobenzene	118-74-1	0.35
Mirex	2385-85-5	0.23
γ- HCH	58-89-9	0.20
Pentachlorobenzene	608-93-5	0.18

[1]mixture containing 2,4,4'-trichlorobiphenyl (320 mg), 2,2',4,4'-tetrachlorobiphenyl (256 mg), 3,3',4,4'-tetrachlorobiphenyl (1.4 mg), 3,3',4,4', 5-pentachlorobiphenyl (6.7 mg), Aroclor 1254 (12.8 g) and Aroclor 1260 (19.2 g).

Blood sampling and processing

Blood samples were collected in vacutainers containing heparin as the anticoagulant. Samples were immediately processed for lymphocyte isolation using a Ficoll-Hypaque gradient. Plasma was transferred in glass vials pre-washed with hexane and stored frozen at –20°C. Plasma samples were sent to the *Centre de Toxicologie du* Québec for organochlorine determination.

Organochlorine analysis

A 1:1:3 mixture of ammonium sulfate/ethanol/hexane was first added to the plasma for organochlorine extraction. Extracts were then concentrated and purified on Florisil columns.

Fourteen PCB congeners, chlorinated pesticides and their metabolites (listed in Table 2) were measured in purified extracts by high-resolution dual-capillary column (Hewlett-Packard Ultra I and Ultra II) gas chromatography with dual Ni-63 electron capture detectors. Detection limit for most compounds was 0.02 µg/L.

Immune system parameters
Vaccination response: monthly antibody titers (*Mycoplasma hyopneumoniae*) were determined by an ELISA method (BIOVET, St-Hyacinthe, Québec). Phagocytosis: polymorphonuclear cells were incubated with increasing concentrations of fluorescent bacterial particles and the number of functional cells were determined by flow cytometry using the Vybrant Phagocytosis Assay Kit (Molecular Probes) (1, 5, 8 months). Lymphocytes surface markers: cells were stained with the appropriate FITC-conjugated mAb markers and analyzed by flow cytometry for determination of the following subsets: CD4+, CD8+, CD4+CD8+, SLA-DR and CD8+DR (1, 2, 4, 6 and 8 months). Complement system: C2 component activity was determined in plasma samples collected at 1, 5 and 7 months by an hemolytic complement assay.

Results and Discussion
Weight gain of sows during treatment was similar in all groups (data not shown) and there were no sign of toxicity. Tables 2 presents mean concentrations of the most abundant organochlorines in plasma samples collected after 30 weeks of treatment. Organochlorine compounds were not found in plasma samples from sows belonging to the control group. Concentrations of the main OCs in samples from sows in the 100 µg/kg dose group are 7 to 10 fold greater than those measured in samples from animals in the 10 µg/kg group. Only the most abundant and persistent compounds were detected in plasma samples from the low dose group (1 µg/kg group).

There was no statistically significant difference between groups with regard to birth weight, length or ano-genital distance of male piglets (data not shown). No difference between groups was observed for phagocytosis, surface markers or C2 component activity measurements (data not shown). Table 3 shows the percentage of male piglets with plasma antibody levels against *Mycoplasma hyopnemoniae* equal to or greater than 7 mg/L. Exposure to OCs did not modify substantially the percentage of animals responding to the vaccine during the first months of life. However, at 6, 7 and 8 months of age, a lower percentage of animals in the high dose group (100 µg/Kg bw) reached a 7-mg/L antibody concentration (seroconversion level) compared to the other groups. For exemple, at 8 months of age, only 10% (1/10) of animals in the high dose group were above this level, compared to 80% (8/10) in the middle dose group ($p = 0.005$; Fisher exact test), 70% (7/10) in the low dose group ($p = 0.02$) and 50% (3/6) in the control group ($p = 0.12$).

These results indicate that the high dose of the organochlorine mixture can interfere with immune system function in this animal model, as suggested by the low percentage of animals with proper antibody response following vaccination. Total PCB concentration in maternal plasma during gestation reached approximately 20 mg/Kg lipids, a concentration approximately 10-fold higher than that previously documented in Inuit women (1). Studies are currently underway to investigate the efficacy of vaccination programs currently implemented in the Inuit population of Northern Québec.

Table 2. Concentration of organochlorines (μg/L) in plasma samples collected from sows treated during 30 weeks with the organochlorine mixture and from control animals.

	Controls	1 μg/kg	10 μg/kg	100 μg/kg
ß-HCH	ND	ND	0.05 ± 0.01[b]	0.30 ± 0.02
p,p'-DDE	0.07 ±0.06	0.21 ± 0.03	2.40 ± 0.31	19.0 ± 2.2
p,p'-DDT	ND	0.07 ± 0.01	0.68 ± 0.07	4.3 ± 0.2
hexachlorobenzene	ND	ND	0.07 ± 0.02	0.58 ± 0.10
mirex	ND	ND	0.06 ± 0.01	0.43 ± 0.04
oxychlordane	ND	ND	0.07 ± 0.01	0.62 ± 0.02
trans-nonachlor	ND	ND	0.11 ± 0.04	0.55 ± 0.10
α-HCH	ND	0.03 ± 0.01	0.27 ± 0.09	1.80 ± 0.20
dieldrin	ND	ND	0.22 ± 0.09	1.20 ± 0.12
ΣPCBs[3]	ND	0.12 ± 0.03	2.03 ± 0.26	14.4 ± 0.6

[1]ND = not detected; [2]arithmetic mean ± standard deviation; [3]sum of 14 PCB congeners (IUPAC nos. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187)

Table 3. Percentage of male piglets showing plasma antibody[1] levels greater than 7 mg/L.

Group/Age	1 mo.	2 mo.	3 mo.	4 mo.	5 mo	6 mo.	7 mo.	8 mo.
Control	0	75.0	50.0	62.5	50.0	87.5	62.5	50.0
1 μg/Kg bw	0	92.3	75	27. 3	50	55.6	50.0	70.0
10 μg/Kg bw	20.0	80.0	66. 7	16. 7	36.4	70.0	50.0	80.0
100 μg/Kg bw	0	66. 7	66. 7	30.7	58.3	30.0	20.0	10.0

[1] *Mycoplasma hyopnemoniae* antibodies. Vaccine administered at 6 weeks of age.

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