

**Pesticides and the placenta: is there a risk to the fetus?**

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## Outline

- Introduction and Background
  - Pesticides
- *Ex vivo* perfused placenta
  - Method
  - Results
- *In vivo* biodistribution
  - Method
  - Results
- Conclusion & Discussion

## Plutocracy hypotheses

- Exposure to environmental chemicals (xenobiotics) during pregnancy
- Placental transfer may lead to fetal exposure
- Placental accumulation may affect placental function (enzymatic, hormonal, immunological)
- Skewing of *in utero* Th1/Th2 cytokine balance may result in development of allergy in early childhood

List of selected persistent organochlorine compounds

	1,4+1,3-DCB	1,4+1,3-dichlorobenzene	
	1,2-DCB	1,2-dichlorobenzene	<sup>14</sup> C-labelled version available
	1,3,5-TCB	1,3,5-trichlorobenzene	
A	1,2,4-TCB	1,2,4-trichlorobenzene	<sup>14</sup> C-labelled version available
	1,2,3-TCB	1,2,3-trichlorobenzene	
	TeCB	Σ(1,2,3,5+1,2,4,5)tetrachlorobenzene	
	PCB	pentachlorobenzene	
	HCB	hexachlorobenzene	<sup>14</sup> C-labelled version available
	alpha-HCH	alpha-hexachlorocyclohexane	
	beta-HCH	beta-hexachlorocyclohexane	
B	gamma-HCH	gamma-hexachlorocyclohexane	
	delta-HCH	delta-hexachlorocyclohexane	
	p,p'-DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane	<sup>14</sup> C-labelled version available
	p,p'-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene	<sup>14</sup> C-labelled version available
	PCB - 28	(2,4,4'-trichlorobiphenyl)	
	PCB - 52	(2,2',5,5'-tetrachlorobiphenyl)	<sup>14</sup> C-labelled version available
	PCB - 101	(2,2',4,5,5'-pentachlorobiphenyl)	
C	PCB - 118	(2,3',4,4',5'-pentachlorobiphenyl)	
	PCB - 138	(2,2',3,4,4',5'-hexachlorobiphenyl)	
	PCB - 153	(2,2',4,4',5,5'-hexachlorobiphenyl)	<sup>14</sup> C-labelled version available
	PCB - 180	(2,2',3,4,4',5,5'-heptachlorobiphenyl)	

A - chlorinated benzenes, B - organochlorine insecticides, C - polychlorinated biphenyls (indicator congeners)

## Placental transfer

- Transfer kinetics, biodistribution and fetal exposure level of organochlorines unknown
- Responsibility of P1 (Bristol) -
- WP3: Human *ex vivo* placental perfusion study
- WP4: *In vivo* uptake and biodistribution study

## Compounds to investigate

- Limited by availability of <sup>14</sup>C-labelled versions
- Pilot study (4 compounds in total)
- 1,2-dichlorobenzene – DCB (chlorinated benzene)
- p,p'-DDT (organochlorine insecticide)
- p'p'-DDE (organochlorine insecticide)
- 2,2',5,5'-tetrachlorobiphenyl – PCB-52 (polychlorinated biphenyl)

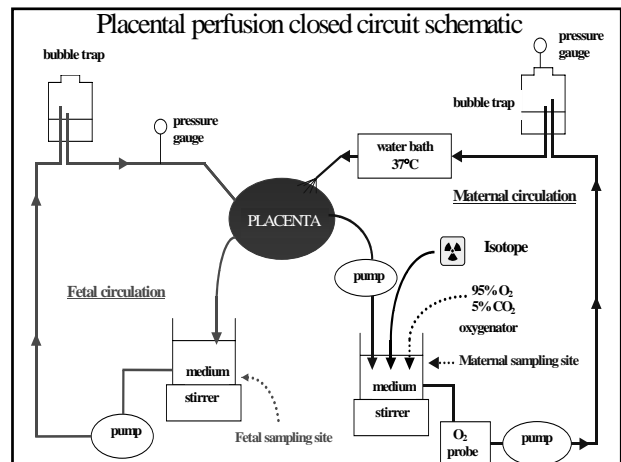
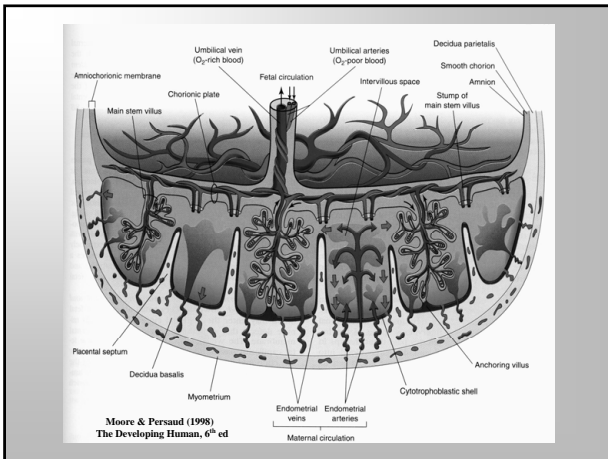
WP3

Human perfused placenta model

- Cannot use human subjects
- Developed *ex vivo* human perfused post-partum placenta model to aid screening of compounds and extrapolation of animal data to humans
- Compare extent of uptake and transfer of xenobiotics across human placenta
- Assist exposure-dose determination

Perfused placenta - method

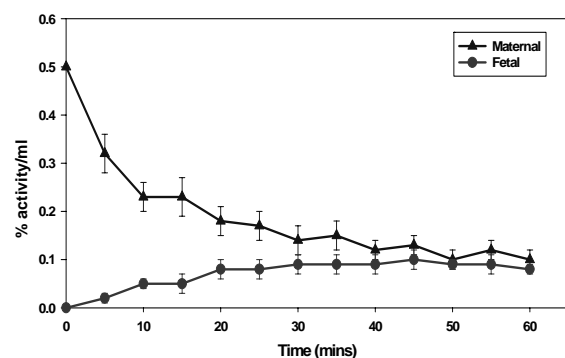
- Fresh placentas obtained from Caesarean delivery without adverse pathology
- Medium 199 supplemented with Heparin, Dextran, BSA and sodium bicarbonate
- Placenta maintained at 37°C, pH7.4 and fetal and maternal sides cannulated and perfused with supplemented medium, 50% O<sub>2</sub>, 45% N<sub>2</sub>, 5% CO<sub>2</sub>
- <sup>14</sup>C-pesticide (Rx):10μCi (0.37MBq)
- Rx added to maternal side and samples removed from maternal and fetal circulation at time intervals up to 120 minutes



Experimental criteria

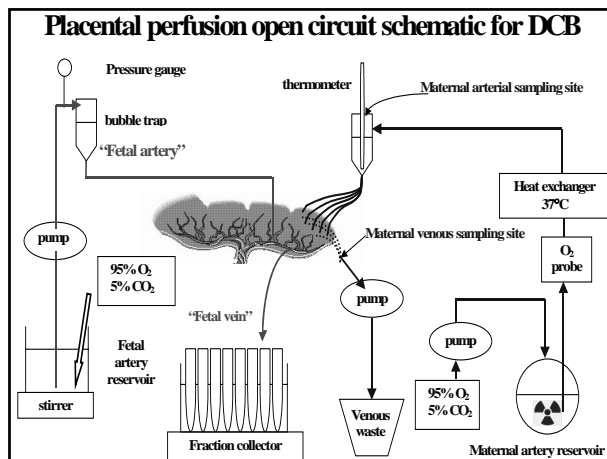
- Elective section – no adverse pathology
- Collection within 10 minutes of delivery
- Fetal pressure <40mm Hg
- Fluid loss < 3.3% (flow rate 6ml/min)
- Temp 37°C (± 0.5), pH 7.4 (± 0.1)
- Constant glucose consumption and lactate production
- Adequate transfer and equilibrium levels of perfusion marker – antipyrine (creatinine)

Concentration of DCB, closed circuit (n = 11, all)



### Closed circuit - solutions

- Silicone tubing replaced by Flourel
- Pre-gassing – direct oxygenation of perfusate avoided
- Extra albumin added (+ 0.4%, total 1.1%)
- Rapid transfer and nature of DCB (recirc. losses) – more suited to open circuit design



### Open circuit

- Non-recirculating
- Clearance:
 
$$Cl = [\text{Recipient vein conc}/\text{Donor art. conc}] \times \text{recipient flow rate (ml/min)}$$
 - the greater the value, the greater the transfer
- Clearance index:
 
$$Cl_I = Cl (\text{test compound})/Cl (\text{ref. compound})$$
 expressed as ratio

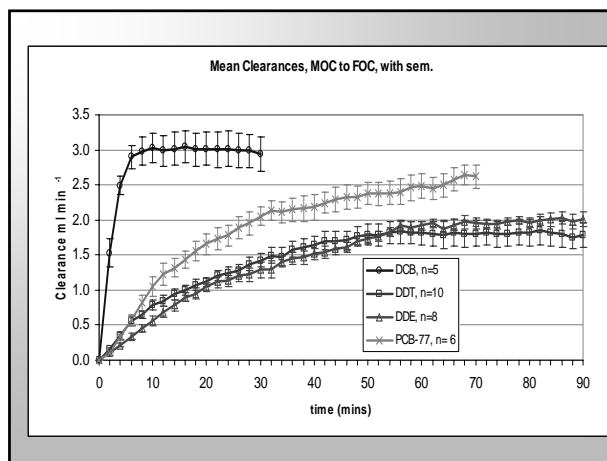


Table 1a Placental transfer of pesticides *ex-vivo*

Pesticide	Maternal to fetal transfer		Fetal to maternal transfer	
	n	Mean clearance index (s.e.m.)	n	Mean clearance index (s.e.m.)
DCB	5	0.98 (0.02)	5	0.92 (0.03)
DDT	10	0.61 (0.01)	10	0.61 (0.01)
DDE	8	0.61 (0.01)	8	0.59 (0.03)
PCB-77	8	0.73 (0.02)	4	0.73 (0.03)

Table 1b Accumulation in perfused area of placenta *ex-vivo*

Pesticide	Maternal to fetal transfer		Fetal to maternal transfer	
	n	Mean % uptake (s.e.m.)	n	Mean % uptake (s.e.m.)
DCB	5	4.2 (0.6)	5	3.7 (0.7)
DDT	10	23.4 (2.2)	10	20.2 (0.9)
DDE	8	18.0 (1.5)	8	18.9 (1.6)
PCB-77	8	22.5 (1.3)	4	22.3 (2.1)

Pesticide	Maternal to fetal transfer		Fetal to maternal transfer	
	n	Mean % uptake/g (s.e.m.)	n	Mean % uptake/g (s.e.m.)
DCB	5	0.18 (0.01)	5	0.17 (0.01)
DDT	10	1.21 (0.11)	10	1.22 (0.08)
DDE	8	0.96 (0.14)	8	0.96 (0.14)
PCB-77	8	1.75 (0.15)	4	1.91 (0.27)

### WP4 Biodistribution study Objectives

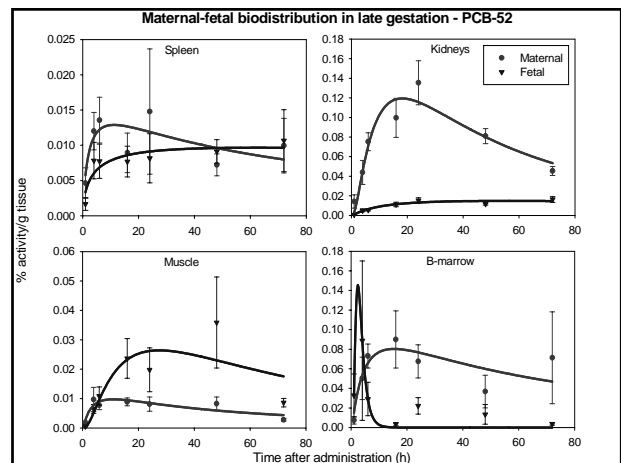
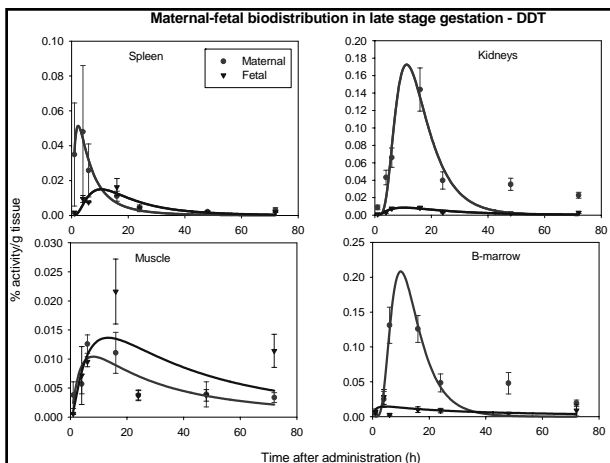
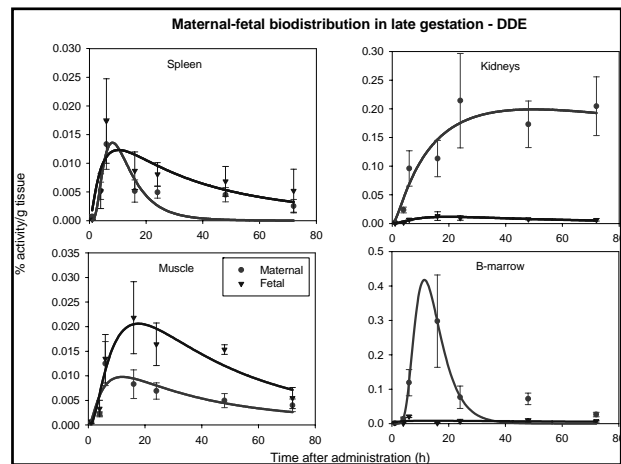
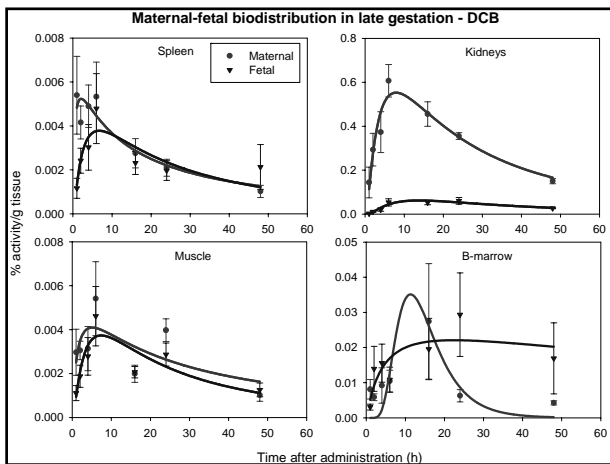
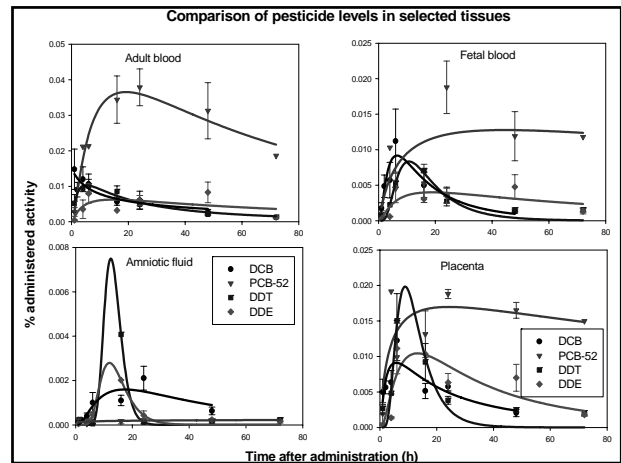
- Measure placental transfer of environmental chemicals to which women may be exposed during pregnancy
- Compare maternal and fetal biodistribution in suitable model
- Determine key target organs

# CHILD HEALTH AND THE ENVIRONMENT: RESULTS FROM EU FRAMEWORK 5

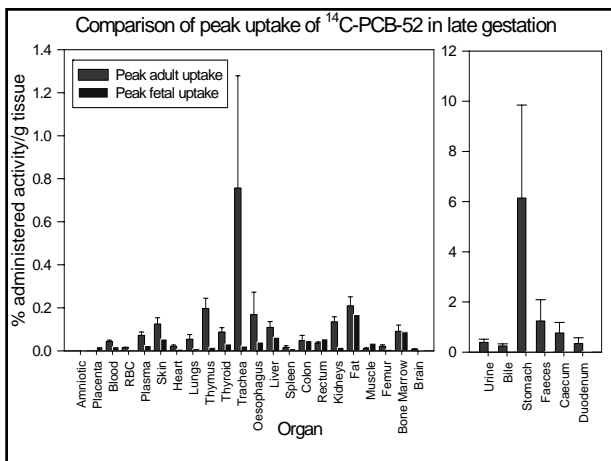
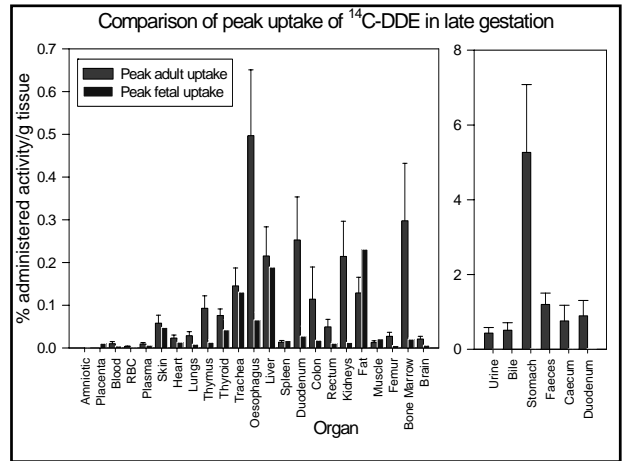
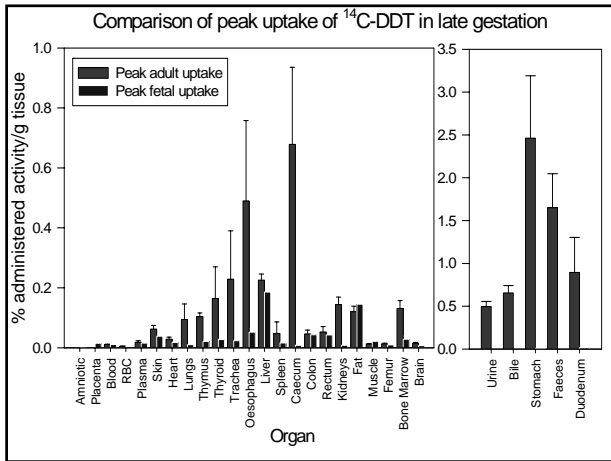
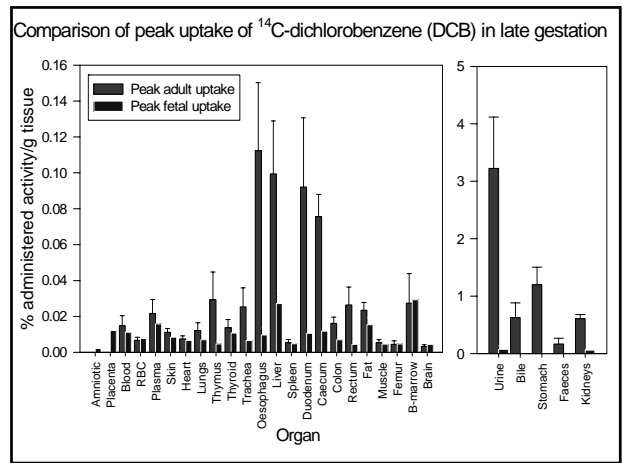
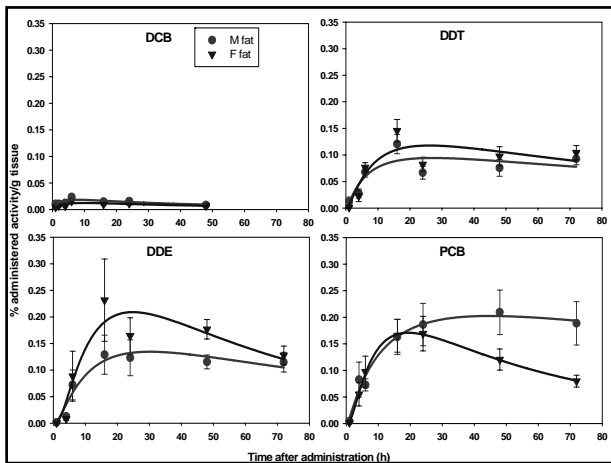
## Placental structure

Haemotrichorial	Rat/mouse	3 layers Maternal BS/fetal endothelium
Haemodichorial	Rabbit	2 layers
Labyrinthine Haemomonochorial	Guinea pig Chipmunk	1 layer over labyrinthine fetal vascular bed (countercurrent)
Villous Haemomonochorial	Human Armadillo	1 layer over villous fetal vascular bed (leaf-like: intervillous space)

Enders 1965



# CHILD HEALTH AND THE ENVIRONMENT: RESULTS FROM EU FRAMEWORK 5



*Ex vivo* pesticide results summary

- No significant difference between DDT and DDE transfer
- Highly significant differences between all other compounds
- DCB > PCB-77 > DDE > DDT extent of transfer
- M-F similar to F-M – no backflow
- Passive diffusion in both directions
- Significant accumulation of DDT, DDE, PCB-77 but not DCB in placental tissue

### *In vivo* results - pesticides

- Rapid transfer across the placenta - all compounds
- Peak fetal uptake within 24 hours
- Fetal concentrations can exceed maternal levels:  
Blood (plasma and erythrocytes), Spleen, Muscle, Femur,  
Brain, Bone marrow, Liver, Fat
- Implications for development due to increased  
sensitivity of fetus?
- Exact levels unknown but implications for  
carcinogenesis, neurotoxicity etc.
- Spin-off study : *in vitro* cell barrier model

### Conclusions

- Some concordance between models
- Rapid placental transfer
- Fetal organ uptake
- Different pattern of biodistribution
- Potential detriment: immunological, neurological  
and hepatic function
- EDCs and neurotoxicants (reproductive and  
neurological damage)
- Useful base for interpretation of clinical findings
- More information needed re. *in utero* exposure

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