第五屆環境荷爾蒙及持久性有機污染物研討會

The 5th Conference on Environmental Hormones and POPs

日　　期：民國98年6月4日(星期四)

地　　點：台灣大學集思會議中心蘇格拉底廳

主 辦 單 位：行政院環境保護署

承 辦 單 位：社團法人中華民國環境分析學會

協 辦 單 位：環境品質文教基金會

國立清華大學化學系

國立台灣師範大學環境教育研究所

嘉南藥理科技大學環境資源管理系

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時 間	內 容	
08:30-08:50	報 到	
08:50-09:00	貴賓開幕致詞 主持人：謝英士董事長 (環境品質文教基金會)	
大會演講	講題	主講人及引言人
09:00-09:50	我國持久性有機污染物 管制策略	主講人：袁紹英處長 (行政院環境保護署環境衛生及 毒物管理處) 引言人：謝英士董事長 (環境品質文教基金會)
09:50-10:10	茶 敘	
10:10-10:50	性別分化與生殖腺發育： 以黑鯛為研究模式	主講人：張清風教授/處長 (國立臺灣海洋大學水產養殖系/ 國科會生物處) 引言人：王碧所長(行政院環境保護署 環境檢驗所)
10:50-11:30	生活環境中環境荷爾蒙 之調查研究	主講人：凌永健教授/理事長 (國立清華大學化學系/中華民國 環境分析學會) 引言人：吳家誠教授/秘書長(國立臺灣師範 大學化學系/消費者文教基金會)
11:30-12:10	我國大學生對於環境 荷爾蒙之認知調查	主講人：葉欣誠教授/常務理事 (國立台灣師範大學環境教育研究所/ 中華民國環境教育學會) 引言人：陳健民教授/學務長(嘉南藥理科技 大學環境資源管理系)
12:10-13:30	午 餐	

時 間	內 容	
專題演講	講題	引言人：劉莉蓮教授 (國立中山大學海洋生物研究所)
13:30-14:00	環境荷爾蒙物質生物效應與生態影響之回顧-從美國佛州阿波卡湖事件至今	主講人：陳健民教授/學務長 (嘉南藥理科技大學環境資源管理系)
14:00-14:30	新興污染物檢測方法 的開發與應用	主講人：丁望賢教授 (國立中央大學化學系)
14:30-15:00	論文海報展示及茶敘	
專題演講	講題	引言人：丁望賢教授 (國立中央大學化學系)
15:00-15:30	戴奧辛生物篩檢法之 評估及應用	主講人：楊喜男博士 (行政院環境保護署環境檢驗所)
15:30-16:00	北台灣水庫中持久性有機污染物之傳輸、來源與累積特性研究	主講人：紀凱獻博士 (中央研究院環境變遷研究中心)
16:00-16:40	綜合討論及頒獎	
16:40-17:00	大會閉幕	

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壁報張貼

大會演講

我國持久性有機污染物管制策略
The Control Strategies for POPs in Taiwan

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[1]行政院環境保護署環境衛生及毒物管理處處長
[2]行政院環境保護署環境衛生及毒物管理處科長
[3]行政院環境保護署環境衛生及毒物管理處技士

摘要

持久性有機污染物(persistent organic pollutants，POPs)兼具生物累積性、高毒性、長距離遷移及在環境中難以分解特性，聯合國遂訂定全球性之「持久性有機污染物斯德哥爾摩公約」，2004 年 5 月 17 日公約生效，旨在減少或消除 POPs 之排放，保護人類健康及環境免受其危害。

目前斯德哥爾摩公約自今(98)年 5 月召開之第四次締約方會議結束後，列管化學物質已由原 12 種增加至 21 種，其中滅蟻樂國內未曾登記使用、十氯酮(克敵康) 已列管為禁用農藥；呋喃及戴奧辛屬工業製程或焚燒副產物，由相關污染防制法規嚴格管制，有關戴奧辛調查、監測、減量工作，本署已積極推動中；其餘多氯聯苯及滴滴涕等 9 種有機氯農藥、五溴二苯醚、靈丹、α-六氯環己烷及 β-六氯環己烷等化學物質，皆已分別依「毒性化學物質管理法」、「環境用藥管理法」、「農藥管理法」管理。

此外我國並將邀集國內環保、衛生、農政及經濟單位，針對已奉核定之「持久性有機污染物斯德哥爾摩公約國家實施計畫」(National Implementation Plan，簡稱 NIP)內容，依公約最新發展滾動修正實施計畫內容，以與國際管理接軌。未來我國仍將積極參加斯德哥爾摩公約國際會議，注意國際動態，同步調整台灣管制方式，以使我國 POPs 管理與國際並駕齊驅

關鍵字：持久性有機污染物(persistent organic pollutants，POPs)、國家實施計畫(National Implementation Plan，NIP)

一、前言

鑑於POPs的具有不易分解與生物累積性，且會對人體及環境產生不可逆反應、長距離傳播問題，聯合國環境規劃署訂定全球性之「持久性有機污染物斯德哥爾摩公約」(Stockholm Convention on Persistent Organic Pollutants，以下簡稱POPs公約)，呼籲以全球運動來減少和消除環境中的 POPs。依據公約第 7 條規定，為使各規範事項得以執行，締約方應制定實施計畫並送交締約方大會審定，我國目前並非 POPs 公約之締約方，但為展現我國重視持久性有機污染物管理，已於 97 年 5 月結合衛生署、農委會及經濟部共同完成本國之「持久性有機污染物斯德哥爾摩公約國家實施計畫」(National Implementation Plan，簡稱 NIP)，並於 97 年 7 月 3 日奉行政院核定，做為國內推動之具體依據。

二、目前公約列管物質及管制策略

為了防範 POPs 對環境所造成的危害，聯合國於 2001 年通過了 POPs 公約，明令禁止阿特靈(aldrin)、氯丹(chlordane)、DDT、地特靈(dieldrin)、安特靈(endrin)、飛佈達（heptachlor)、滅蟻靈（mirex)、毒殺芬（toxaphene)、多氯聯苯（PCBs)、六氯苯（Hexachlorobenzene)、戴奧辛（Dioxins)、及呋喃（furans）等 12 種化學物質的使用，公約對於12種列管物質之管制策略詳如表1，並要求各國必須採取行動，減少環境中該等物質之殘留量，進而確保食品之安全。該公約並於 2004 年 5 月 17 日生效，目前已有 163 個締約會員。

表 1　POPs 公約對於首批列管 12 種化學物質及管制策略

分類		化學物質	管理策略
有意生產或使用之化學物質	附件 A*	阿特靈(Aldrin)、可氯丹(Chlordane)、地特靈（Dieldrin)、安特靈（Endrin)、飛佈達（Heptachlor）、六氯苯（Hexachlorobenzene）、滅蟻樂（Mirex)、毒殺芬（Toxaphene)、多氯聯苯（PCBs）※等 9 種	除特定豁免外，禁止製造及使用，惟 PCBs 使用放寬至 2025 年，2028 年前全面銷毀。
	附件 B*	滴滴涕（DDT）	限制瘧疾疫區使用。

無意生產或使用之化學物質	附件 C*	戴奧辛（Dioxins）和呋喃（Furans） 六氯苯（Hexachlorobenzene） 多氯聯苯（PCBs）◎	1.前驅物質之減廢、再利用、替代品使用等。 2.焚燒技術與廢棄處理技術採最佳可行技術，以進行減量。

註：＊POPs 公約之附件 A 指需消除之物質、附件 B 為需限制之物質、C 為無意生產之物質。

※意指正在使用中之含多氯聯苯設備例如變壓器、容器或含有液體存積量的其他容器。

◎意指無意生產之多氯聯苯物質，如廢物的露天焚燒、掩埋場的焚燒

除上述首批列管之 12 種 POPs 外，最近於 2009 年 5 月 4 日~8 日在瑞士日內瓦召開之斯德哥爾摩公約第四次締約方會議(簡稱 COP4)，經過熱烈討論以及各國間的協商後，又已宣布新增列管 9 種持久性有機污染物(如表 2)，包括 α-六氯環己烷(Alpha hexachlorocyclohexane)、β-六氯環己烷(Beta hexachlorocyclohexane)、六溴二苯醚和七溴二苯醚(Hexabromodiphenyl ether and heptabromodiphenyl ether)、四溴二苯醚和五溴二苯醚(Tetrabromodiphenyl ether and pentabromodiphenyl ether)、十氯酮(克敵康，Chlordecone)、六溴聯苯(Hexabromobiphenyl)、靈丹(Lindane)、五氯苯(Pentachlorobenzene)、全氟辛烷磺酸及其鹽類和全氟辛基磺醯氟(Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride)，至此公約列管化學物質將由原 12 種增加至 21 種。

表 2　POPs 公約第四次締約方會議新增列管 9 種化學物質及管制策略

列管化學物質名稱	公約列管附件[註1]
α-六氯環己烷(Alpha hexachlorocyclohexane)	附件 A
β-六氯環己烷(Beta hexachlorocyclohexane)	附件 A
十氯酮(克敵康，Chlordecone)	附件 A
六溴聯苯(Hexabromobiphenyl)	附件 A
六溴二苯醚和七溴二苯醚(Hexabromodiphenyl ether and heptabromodiphenyl ether)	附件 A
靈丹(Lindane)	附件 A
四溴二苯醚和五溴二苯醚 (Tetrabromodiphenyl ether and pentabromodiphenyl ether)	附件 A
五氯苯(Pentachlorobenzene)	附件 A
全氟辛烷磺酸及其鹽類和全氟辛基磺醯氟 (Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl	附件 B [註2]

fluoride)	

註 1：POPs 公約之附件 A 指「須消除之物質」、附件 B 為「須限制之物質」。

註2：「全氟辛烷磺酸其鹽類和全氟辛基磺醯氟」有條件可使用在：

(1) 可接受用途，包括：照像顯影、滅火泡沫、切葉蟻餌劑。

(2) 例外豁免，包括：金屬電鍍、皮革和服飾、紡織品和室內裝飾、造紙和包裝，以及橡膠及塑料。

針對列為公約附件 B 之 PFOS，在國際間乃由歐盟率先於 2006 年 12 月 27 日公告禁用指令，並於 2008 年 6 月 27 日實施。且自 2008 年 6 月 27 日後，生產商不得向客戶供應含碳氟化合物的滅火泡沫，但於 2006 年 12 月 27 日前已在市場銷售的含碳氟化合物的滅火泡沫，則可繼續儲存及使用，直至 2011 年 6 月 27 日為止。

PFOS 禁用指令，其內容包括：

(1) 上述物質或其在製劑中的濃度等於或超過總體 0.005%(50ppm)，不得於市場銷售。

(2) 上述物質於半成品或半成品部件的濃度等於或超過總體 0.1% (1000ppm)，不得於市場銷售。

(3) 在紡織品或其他塗層物料，上述物質含量必須少於每平方公尺 1 微克(1 μ g/m^2)。

但 PFOS 如運用於光顯影製程、攝影塗料、電鍍鉻抑制劑、水壓流動系統、洗滌劑等製程或用途時，得以豁免。且委員會應審查豁免條件中可用的替代品或技術，以符合下列狀況：

(1) 當有安全的替代品，且技術上、經濟上可行時，便立刻取代 PFOS 的使用。

(2) 繼續使用的前提必須建立在安全的替代品並不存在，且已經有努力開始尋找可用替代品的研究報告。

(3) PFOS 釋放至環境時，必須以最佳可行技術(Best Available Techniques , BAT)將釋放量降至最低。

三、我國針對公約首批列管之 12 種 POPs 管理現況

我國 POPs 管理係由環保、農業、衛生及經濟主管機關分別職掌，針對目前 POPs 公約首批列管 12 種 POPs，除戴奧辛（Dioxins）及呋喃（Furans）外，我國皆以「毒性化學物質管理法」、「農藥管理法」及「環境用藥管理法」進行管制，並分別禁用或未曾登記使用，我國 POPs 管理現況詳如表 3。

此外，為加強部會間橫向聯繫、溝通與協調，環保署與衛生署、農委會亦已建立定期之「衛生署環保署農委會副首長食品安全與環境保護聯繫會議」機制，並亦已共同建立「衛生署農委會環保署環境保護與食品安全通報及應變處理流程」，作為處理環境保護及食品安全事件之標準作業程序，共同協力處理具時效性的食品安全及環境保護議題。

以下即針對首批列管之 12 種 POPs 進行管制現況說明：

表 3　POPs 公約首批列管物質之國內管理現況彙整表

	化學物質	POPs 公約	國內管理現況
1	多氯聯苯 Polychlorinated biphenyl （PCBs）	允許 2025 年之前，在符合不洩漏之條件下，繼續使用。	1.1988 年依毒性化學物質管理法禁止製造、輸入、販賣，並禁止使用於食品業。 2.自 2001 年起依毒性化學物質管理法公告全面禁用。含多氯聯苯之電容器或變壓器，停止使用者應聲明廢棄，並依規定處理。
2	可氯丹（Chlordane）	立即禁止使用	1.1988 年依毒性化學物質管理法公告禁用。 2.1998 年依環境用藥管理法公告禁用。
3	地特靈（Dieldrin）		1.1975 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。 3.1998 年依環境用藥管理法公告禁用。
4	滴滴涕 4,4-Dichlorodiph-enyl trichloroethane（DDT）	禁用但允許防疫使用	1.1973 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。 3.1998 年依環境用藥管理法公告禁用。
5	毒殺芬（ Toxaphene, Campechlor）	立即禁止使用	1.1983 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。

6	安特靈（Endrin）		1.1971 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。
7	飛佈達（Heptachlor）		1.1975 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。 3.1998 年依環境用藥管理法公告禁用。
8	阿特靈（Aldrin）		1.1975 年依農藥管理法禁用於農藥 2.1989 年依毒性化學物質管理法公告禁用。
9	六氯苯（Hexachlorobenzene, HCB）		1993 年依毒性化學物質管理法公告禁用。
10	滅蟻樂（Mirex）		國內未曾登記使用。
11	戴奧辛（Dioxins）	盡最大努力減少排放	空氣污染防制法、廢棄物清理法、土壤及地下水污染整治法、飲用水管理條例已有排放及含量管制標準。
12	呋喃（Furans）		

(一)9 種有機氯劑農藥

1.現況說明

9 種有機氯農藥管理現況詳表 3。1949 年我國首度引進滴滴涕（DDT），以防治衛生及農業蟲害，因其防治面廣，藥效比當時的其他農藥好，且急性毒性低，加以當時尚未發現其殘留毒性，因而被廣泛用於防治作物、森林和牲畜的害蟲，接著陸續使用阿特靈（Aldrin）、地特靈（Dieldrin）、飛佈達（Heptachlor）等有機氯劑農藥。後來由於多種有機氯劑農藥具有環境殘留性及致腫瘤、致畸胎性，因此相繼被公告禁用，包括阿特靈（Aldrin）、可氯丹（Chlordane）、滴滴涕（DDT）、地特靈（Dieldrin）、安特靈（Endrin）、飛佈達（Heptachlor）、六氯苯（Hexachlorobenzene）、毒殺芬（Toxaphene）等 8 種有機氯劑農藥，以降低或消除此 POPs 的排放。另滅蟻樂（Mirex）於國內則未曾登記使用。

2.環境介質與生物基質監控

環境介質方面，依據我國河川調查結果，其底泥、土壤中有機氯劑農藥殘留有明顯逐年下降趨勢；生物基質方面，僅少數魚貝類、食用油脂檢出滴滴涕（DDT），詳表 4 所示。

表 4　9 種有機氯劑農藥環境介質與生物基質監控情形

基質	主管機關	監控結果
環境介質	環保機關	2002~2006 年河川調查結果，河川底泥中濃度已逐漸降低
	農業機關	2004 年調查農田土壤樣品，農藥殘留有逐年下降趨勢
生物基質	衛生機關	➢ 2002 至 2006 年分別針對魚貝類（98 件）、乳製品及嬰幼兒食品（100 件）、蛋類及畜肉（100 件）、食用油脂（100 件）、市售穀類及其製品（50 件）調查： ■ 2002 年魚貝類檢出 5 件含有滴滴涕 ■ 2005 年食用油脂檢出 4 件含有滴滴涕

(二)多氯聯苯（PCBs）

1.現況說明

國內多氯聯苯管理現況詳表 3。目前環保機關主要法源依據係以「毒性化學物質管理法」及「廢棄物清理法」為主，毒管法已公告全面禁用，惟試驗、研究、教育用者不在此限；農業機關以「漁業法」、「動物用藥品管理法」、「飼料管理法」為主，辦理飼料檢驗、監測與重要海域監測工作；衛生機關係以「食品衛生管理法」及「食品中多氯聯苯限量標準」為主，針對乳製品、肉類、蛋類、魚介類、嬰幼兒食品及紙製食品包裝進行監控及管制；經濟機關係以「促進產業升級條例」、「工廠管理輔導法」為主。

由於我國於 1983 年後之電容器、變壓器已不再使用含多氯聯苯添加物，且 PCBs 自 2001 年起依「毒性化學物質管理法」公告全面禁用後，國內目前已無 PCBs 新的運作來源。惟 1980 年 6 月至 1982 年 12 月間產製之電容器因使用中無法檢驗者准繼續使用至報廢，故目前管制目標著重於該類工廠的列管、清查，及其廢棄物處理。國內含多氯聯苯（PCBs）廢棄物之處理，大部

分已於 1998 年前完成清理，其處理方式採輸出至先進國家妥善處理為主。

2.環境介質與生物基質監控

環境介質方面，我國環境中 PCBs 檢測值已降低至安全範圍；生物基質方面，我國養殖水產品、水產飼料及食品檢驗結果均低於國內外相關規定標準值，詳表 5 所示。

表 5　PCBs 環境介質與生物基質監控情形

介質	主管機關	多氯聯苯調查結果
環境	環保機關	➢ 1999 至 2006 年針對 21 條主要河川進行調查，大多數底泥測值低於偵測極限
生物基質	農業機關	➢ 2004 至 2006 年針對畜產及水產飼料調查，均低於國內外相關規定標準值 ➢ 於 2003 至 2006 年針對養殖水產品調查，未檢出殘留，或低於國內外相關規定標準值
	衛生機關	➢ 2002 至 2005 年針對魚貝類（98 件）及蜂蜜（20 件）、乳製品（74 件）及嬰幼兒食品（26 件）、畜肉類（61 件）及蛋類（39 件）、國產食用油（71 件）及進口食用油（29 件）進行調查，均符國內相關衛生標準規定

(三)戴奧辛(Dioxins)及呋喃(Furans)

1.現況說明

因戴奧辛及呋喃均非有意生產，係生產過程之副產物，國內管理方向重點包括源頭減量與清潔生產、管末控制技術及廢棄物再利用輔導。故我國環保主管機關已針對污染源及環境介質訂定戴奧辛管制標準值，至於露天燃燒稻草或廢棄物、金銀紙焚燒等非固定污染源，係採行為禁止方式進行稽查管制；衛生主管機關以「食品衛生管理法」及「食品中戴奧辛處理規範」法規為主，訂定各類食品戴奧辛限量及其相關案件之通報、處理流程、產品處置及健康風險溝通等機制；農業主管機關以「畜牧法」、「漁業法」、「動物用藥品管理辦法」、「飼料管理法」法規為主，辦理農畜水產品檢驗、監測等工作，以防範有害物質污染；經濟主管機關係以「促進產業升級條例」、「工廠管理

輔導法」為主，相關國內戴奧辛管制標準彙整如表 6。

環保署自 2001 年起，推估我國排放入空氣之戴奧辛（Dioxins）排放量，並建立排放清冊逐年更新，若以 2002 年為基準，估計排放入大氣之戴奧辛總量為 327.5 g I-TEQ，估算於 2006 年度排放入大氣之戴奧辛總量已降至 88.9 g I-TEQ，減量成效良好。

衛生署依 2001 至 2006 年所完成之食品中戴奧辛含量背景值分析，配合全國營養調查所得的各類食品攝取量，推估台灣地區成年人的戴奧辛每日攝入量約介於 0.31～1.43WHO-TEQPCDD/F+PCB/day/kg bw，符合世界衛生組織建議值。

表 6　我國戴奧辛管制標準值彙整

空氣類				主管機關
管制對象	適用條件	排放標準 (ng I-TEQ/Nm3)	實施日期	
廢棄物焚化爐	10 ton/hr 或 300 ton/day 以上	0.1	既存 2001.8.8 新設 1997.8.8	環保署
中小型廢棄物焚化爐	4 ton/hr 以上	0.1	新設 2001.1.1 既存 2003.1.1	
	4 ton/hr 以下	0.5	新設 2001.1.1 既存 2004.1.1	
煉鋼業電弧爐	新設	0.5	2002.1.1	
	既存	5.0	2004.1.1	
		0.5	2007.1.1	
鋼鐵業燒結工場	既存	2.0	2006.1.1	
		1.0	2008.1.1	
	新設	0.5	2004.6.16	
鋼鐵業集塵灰高溫冶煉設施	既存高溫冶煉設施	9	2005.10.12	
		1	2006.9.1	
	新設高溫冶煉設施	0.4	2005.10.12	
固定污染源	既存污染源	2.0	2007.1.1	
		1.0	2008.1.1	
	新設污染源	0.5	2006.1.2	
廢棄物類				

有害事業廢棄物之焚化處理設施	有害事業廢棄物之焚化處理設施應符合多氯聯苯(PCBs)及 2,3,7,8 四氯戴奧辛、2,3,7,8 四氯聯苯呋喃總破壞去除效率達百分之 99.999 以上規定	1989.5.8	環保署
有害事業廢棄物認定標準	1.0 ng I-TEQ/g (含 2,3,7,8-氯化戴奧辛及呋喃同源物等 17 種化合物)	2007.07.04	
土壤類			
土壤污染管制標準	1000 ng I-TEQ/kg	2001.11.21	環保署
飲用水類			
飲用水水質標準	12 pg-WHO-TEQ/L	2009.1.2	環保署
食品類			
肉類(註 2)	➢ 牛、羊之肉及其製品：3 ➢ 家禽之肉及其製品：2 ➢ 豬之肉及其製品：1 ➢ 肝及衍生產品：6	2006.4.18	衛生署
乳品類(註 2)	液態乳、乳粉、煉乳、調味液態乳、乳油、乳酪、乾酪、發酵乳及乳清粉：3	2006.4.18	
蛋類(註 2)	雞蛋、鴨蛋及其製品：3	2006.4.18	
魚貝類(註 3)	魚及其他水產動物之肉及其製品：4	2006.4.18	
油脂類(註 4)	➢ 牛及羊之油脂：3 ➢ 家禽類之油脂：2 ➢ 豬油：1 ➢ 混合動物油脂：2 ➢ 植物油：0.75 ➢ 魚油：2	2006.4.18	

註：1. 上述所規範之戴奧辛類化合物，僅包括多氯二聯苯戴奧辛(PCDDs)及多氯二聯苯喃(簡稱 PCDFs)，未包括平面多氯聯苯（PCBs）。

2. 脂肪基準(不適用脂肪含量低於 1%者)。單位：pg WHO-$_{PCDD/F}$-TEQ/g fat

3. 總重基準。單位：pg WHO-$_{PCDD/F}$-TEQ/g fresh weight

4. 脂肪基準。單位：pg WHO-$_{PCDD/F}$-TEQ/g fat

2.環境介質與生物基質監控

環保主管機關部分，每年均編列經費，辦理戴奧辛及呋喃環境流布情形、場址監控、相關檢測方法及技術研發，並積極進行不同環境介質之整合工作，期分階段建立戴奧辛流布掌握機制；農業主管機關部分，為加強飼料生產與

衛生安全管理，農委會持續就禽畜及水產飼料中戴奧辛含量進行監測，2004至2006年檢測結果均低於國內外相關規定標準值；衛生主管機關方面，針對戴奧辛對國人是否具有潛在性健康危害議題，進行「國人膳食中戴奧辛暴露評估及風險分析」及「人體血液中戴奧辛背景值調查」等研究，透過總膳食調查，對經過烹調成可食狀態之食物進行污染物分析，並建置國人食品安全之風險評估管理系統，包括建立食物中污染物之背景資料及進行長期監測。相關監控情形詳表7所示。

表7　戴奧辛及呋喃環境介質與生物基質監控情形

種類		監控結果
環境介質	空氣	■ 2002、2006、2007年空氣品質分別為0.083、0.0032及0.0042pg I-TEQ/Nm3，均遠低於日本環境空氣戴奧辛之品質基準0.6 pg TEQ/Nm3
	廢棄物	■ 2005 年針對運轉中 20 座大型一般廢棄物焚化廠進行檢測，底渣含量檢測值在0.0003至0.113 ngI-TEQ/g間，飛灰中間處理衍生物含量檢測值在0.0062至1.950 ngI-TEQ/g間
	水體	■ 2006年調查特定業別原廢水及放流水戴奧辛濃度，以造紙業的紙與紙板製程及紙漿製程排放量最高，年排放量為0.0927 g I-TEQ ■ 2006年進行國內淨水場原水與清水調查，皆小於戴奧辛飲用水管制限值12 pg/L
	土壤	■ 2002年針對全省15處農田水利會所屬農業區以及4處保留區調查，所有土壤樣品戴奧辛值均符合國內土壤戴奧辛管制標準1000 ng I-TEQ/kg d.w.
生物基質		■ 2004至2006年就飼料、水產飼料進行監測，均低於國內外相關規定標準值
		■ 2001至2006年針對台灣 6 個地區之18至45歲人體血液進行背景值調查 ➢ 戴奧辛類化合物總濃度之中間值為 16.4 pg WHO-TEQPCDD/F+PCB/g lipid ➢ 與其他國家比較，顯示台灣地區調查對象血液中戴奧辛類化合物濃度並無偏高情形

四、我國對於公約新增列管之9種POPs因應策略

聯合國斯德哥爾摩公約第四次締約方會議2009年5月8日於瑞士日內瓦國際會議中心落幕，經過熱烈討論以及各國間的協商後，宣布新增列管9種持久性有機污染物。本次9種新增列管之化學物質中，十氯酮(克敵康)係由「農藥管理法」管理，尚未核准登記使用，依農藥管理法不得進口、製造、販賣及使用；靈丹、α-六氯環己烷、β-六氯環己烷及五溴二苯醚環保署已列管為毒性化學物質，除五溴二苯

醚尚可用於阻燃劑等用途外，其他皆已全面禁用。其餘 4 種化學物質及四溴二苯醚(Tetrabromodiphenyl ether)1 項，我國將積極評估國內之運作及管制情形，擴大列管毒化物項目，審慎檢討該等化學物質的管制措施，以符合國際管制趨勢。

此外，我國將因應公約第四次締約國大會增列 9 種化學品之最新發展，擇期邀集國內環保、衛生、農政及經濟單位討論，滾動修正我國已奉核定之「持久性有機污染物斯德哥爾摩公約國家實施計畫」(NIP)內容，以與國際管理接軌。未來仍將積極參加斯德哥爾摩公約國際會議，注意國際動態，同步調整台灣管制方式，以使我國 POPs 管理與國際並駕齊驅

五、結論

我國 POPs 管制之推動工作，涉及層面廣泛，必須由環保、農業、衛生、經濟等主管機關依據權責辦理，各相關機關應妥善執行管制策略及法規，方可落實訂定之政策目標。除由政府部門制定確實可行之環保政策外，尚需企業界負起減少污染與減少損耗資源之責任，及環保團體之號召、民眾之配合參與，在結合社會力量形成社會共識後，方可消除或降低環境中的 POPs 及有害化學品之排放與減少對全球之危害。

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性別分化與生殖腺發育：以黑鯛為研究模式
Protandrous Black Porgy Fish as a Model to Study Sex Differentiation and Gonadal Development

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Abstract

Protandrous black porgy fish, *Acanthopagrus schlegeli,* have a striking life cycle with a mono-male sex differentiation at the juvenile stage and male-to-female sex change at 3 years of age. Male sex differentiation occurred at 4 - 5months of age, while bisexual gonad (ovotestis) exists till sex change. This sex pattern provides an excellent model for studying the molecular mechanism of sex differentiation and sex change in fish. Our data demonstrated the dual roles of *cyp19a1a* in testicular development during early sexual differentiation as well as in ovarian development during the later natural sex change and also the importance of *Sf-1* for the early male sex differentiation, *wnt4* in the early ovarian development, and *foxl2/cyp19a* in the late ovarian growth in black porgy. *Sf-1 and dax-1* showed a cooperative function for the testicular development and an antagonistic interaction for oocyte development. Plasma luteinizing hormone (LH) is closely associated with the development of male phase. Estradiol (E2, 6 mg/kg feed) administration in juvenile fish resulted in complete suppression of testicular development and induced sex change (from undifferentiated gonad to female gonad). The E2 treatment resulted in induced-sex change in juvenile fish, with *Dmrt1, dax-1,* and *Sf-1* transcripts being decreased during the period of E2-administration. Higher gonadal aromatase activity was observed in the E2 group as compared to control. Induced sex change was followed by a natural reversible sex change with the withdrawal of E2-adminstration. Plasma LH levels were higher in the E2-terminated group during the period of reversible sex change.

The expression of sex steroid receptors (androgen [*ar*] and estrogen [*esr1* and *esr2a*] receptors) and other related genes (*Sf-1, dax-1, star, cyp11a1, hsd3b1, cyp17a1* and *cyp19a1b*) in black porgy brain indicated the presence of the requisite machinery required for *de novo* synthesis of E2. A synchronous peaked expression of *star*, the key neurosteroidogenic enzyme genes (*cyp11a1, hsd3b1, cyp17a1* and *cyp19a1b*) and estrogen receptors at 4 months of age indicate that black porgy brain may synthesize and respond to E2. Brain aromatase activity and estradiol levels also increased significantly in forebrain and hypothalamus, respectively, at 4-months of age. *sf-1* showed no significant increase in expression till 4-months of age. On the other hand, *dax-1* exhibited a significant decrease in expression with advancement in age, especially at 4 months compared to 2 months of age. It remains plausible that *dax-1* exerts a repressive function for a subset of *sf-1* regulated genes (*star* and *cyp19a1b*) that somehow regulate the timing of peak steroidogenesis and hence brain sex differentiation. Testicular regression was shown to be important for the initiation of ovarian development in the bisexual gonad. The successful induction of an early sex change in the testis-excised fish suggests that testicular tissue may exert some inhibitory effects on the advanced ovarian (vitellogenic) development in bisexual gonad. Our results provide new insight into the development of advanced oocytes during sex differentiation and sex change in black porgy.

1. Introduction

Teleosts exhibit diverse mechanism for sex differentiation and development, which are complex and multi-level phenomena. A coordinated interplay of signals is required to regulate the proliferation, differentiation, development and organization of the reproductive tissues. Sex differentiation, formation of a functional testis or ovary from a bipotential gonad, in lower vertebrates including fish is labile and sensitive to the external environment and in many cases a high degree of plasticity of phenotypic sex persists throughout the whole life

span. In fish, a combination of genetic and environmental factors determines sex. Although studies identified several genes essential for early gonadal development in mammals, the exact roles of these genes remain to be elucidated (Swain and Lovell-Badge, 1997; Koopman, 2001). *SRY/Sry* is considered to act as a switch to initiate the transcription of a cascade of other genes, which contribute to testicular development in mammals (Gubbay et al., 1990). In fish, however, *Sry* or its equivalent gene has not been found except the putative testis-determining factor, *dmy* gene, in the medaka, *Oryzias latipes* (Matsuda et al., 2002). Genes containing a DM domain (*Dmrt1*, DM-related transcription factor 1, a [Double-sex/-Mab-3]-domain gene) are involved in sex determination in both vertebrates and invertebrates (Raymond et al., 1998), which is a unique conservation of function between phyla, not seen in any other gene involved in sex determination.

Sex development in fish could be divided into gonochorism and hermaphroditism. A species is defined as hermaphrodite when substantial proportion of individuals in a population function as both sexes, either at the same time (synchronous hermaphrodite) or at different times (sequential hermaphrodites i.e., protogyny (in which some or all individuals function first as females and later in life function exclusively as males such as in grouper) and protandry (sex change is from male to female (such as black porgy). The gonads of sequential hermaphrodites possess the genetic information to produce both male and female reproductive organs, but only the dominant gene is expressed at any give time. Different cues – varying from species to species – may induce sex changes. Thus, hermaphroditic fishes form unique models to study sex differentiation and sex change. Natural sex change occurs as a spontaneous phenomenon in many teleosts (Bruslé-Sicard and Fourcault, 1997). But, the underlying molecular mechanism of sex change is still unclear in hermaphrodite species.

The regulation of sexual differentiation and sex change processes involves coordinated interactions between the genetic and hormonal. The number of genes known to be involved in

sexual differentiation and sex change in teleosts is increasing. Steroid hormones mediate both the natural and induced sex change in teleosts. During the critical period of differentiation, treatments with exogenous sex hormones often induce sex reversal (Yamamoto, 1969; Hunter and Donaldson, 1983). Consequently, endogenous androgens and estrogens have been assumed to act as the natural inducers of testicular and ovarian differentiation, respectively. The hormonal balance between estrogens and androgens appears to be crucial in the process of sexual differentiation in developing fish. This balance relies on the availability and activity of the steroid synthesizing enzymes, and in particular on the cytochrome P450 aromatase complex (P450arom; also called estrogen synthase). From studies in mammals, it is known that sex steroids play a key role in the sexual differentiation of the brain, thereby influencing all aspects of reproduction, from gonadal sexual development to sexual behaviour (Balthazart and Ball, 1998). Nevertheless, the mechanism of action of exogenous hormones and the role of endogenous sex hormones in sex differentiation and sex change remains unclear.

Black porgy, *Acanthopagrus schlegeli* (Bleeker, 1854), a marine protandrous monogynous hermaphroditic sparid fish characterized by the presence of bisexual gonads (ovotestis), with only one type of female (monogynous i.e., secondary females are only derived from male sex change) and a natural male to female sex change occurring at around 2-3 years old was chosen to analyze the role of differential expression of gene transcripts and steroid levels in sex differentiation and sex change.

2. Morphological changes in gonad during sex development in black porgy

Black porgy, *A. schlegeli* has a multiple spawning pattern and can tolerate very wide ranges of salinity (euryhaline fish). The spawning period lasts from late winter to early spring (January to March). Fish are functional males for the first two years of life but begin to sexually reverse to females during the third year (Chang et al., 1994; Chang and Yueh,

1990). This sex pattern provides a very good model to study the mechanism of sex development in fish. About 30–50% of three-year-old black porgy will change to females. There is no significant difference in the body size and length between three year- old males and females in black porgy. However, three-year-old females have a larger body size than two-year-old males. Therefore, a size-advantage model (Iwasa, 1991) is likely to explain the evolutional force for the sexual development in the protandrous black porgy. Size difference was also observed in the protandrous *Rhabdosargus sarba* (Yeung and Chan, 1987).

The development of a bisexual gonad is a dynamic process that depends on season and age. Bisexual gonad with testicular and ovarian tissue (with the appearance of oogonia and primary oocytes in around the central cavity of ovarian tissue) separated by connective tissues was found in black porgy before sex change. Undifferentiated gonad tissue with early germ cell was observed in 3 month-old fish. Central cavity (ovarian cavity) with oogonia appeared in gonad of juvenile fish at 4-5 months of age. Blood vessel organization was clearly observed at the side of the ovarian cavity. Putative ovarian tissue developed from the inner side of central cavity while putative testicular tissue appeared in the distal part of the central cavity. The connective tissue was at the outer side of the ovarian tissue, and it separated the ovarian and testicular tissues at later stage of development (Lee et al., 2008). Spermatogonia were observed in 5-month-old fish. Lobular testicular tissue with late spermatogonia and spermatocytes appeared at 6-to7-months-of age. Active spermatogenesis was observed at 8- to 11-months and mature sperms were found during the spawning period (11-12 month-old). Ovarian tissue formed a very small portion in the bisexual gonad in 0+- to 1-yr-old fish only with few primary oocytes in central cavity of the bisexual gonad (Lee et al., 2008; Wu et al., 2008a,b).

During the first and second spawning season, the testicular tissue was dominant with only a few primary oocytes, which made the fish functional males. Gonadal histology

revealed that the ovarian portion of gonad increased together with the regression of testicular tissue during the post-spawning season, resulting in the ovarian tissue forming the dominant tissue in the bisexual gonad during the non-spawning period in 1^{+}-yr-old fish. Testicular tissue regenerated together with the reduction in ovarian tissue during late non-spawning season. Testicular tissue became the main tissue in bisexual gonad during the pre-spawning season and further developed to a functional testis during the second spawning season. Similarly, the ovarian portion of the gonad increased with the regression of testicular tissue during the post-spawning period and ovarian tissue maintained dominance in the bisexual gonad during the non-spawning period in 2^{+}-yr-old fish. Ovarian tissue further developed to female function (natural sex change) during the third spawning season. Thus, the changes occurring in gonad tissue during the process of natural sex change are from a "bisexual gonad" to "full ovarian gonad" with primary oocytes and regressed testicular tissue, and then finally to an "advanced (vitellogenic) ovary" (Lee et al., 2008). Vitellogenic oocytes did not appear in the ovary until the complete regression of testicular tissue. The development of vitellogenic oocytes is an important and critical event for the success of natural sex change. It seems that some unknown signals are lacking, which let the fish remain in the male phase in the first two years of life even though the ovarian tissue tends to further develop in a larger proportion than testicular tissue during the nonspawning season. Similar findings on the gonadal profiles were also observed in protandrous *Rhabdosargus sarba*, *Sparus aurata* and yellowfin bream, *Acanthopagrus australis* (Pollock, 1985; Yeung and Chan, 1987).

3. Gene Profiles during Sex Differentiation and sex change in black porgy

The gonad is unique among all organs because of its bipotential nature. Testicular and ovarian organogenesis involves a pathway of developmental genes, which are differentially regulated in males and females. The number of other genes known to be involved in sexual

differentiation in mammals is increasing. On the other hand only a few of these genes have been identified in fish. The functions of these genes have not been fully elucidated in fish and both conserved and divergent functions between mammals and fish have been suggested. The changes in the profiles of certain genes involved in sex development were analyzed in black porgy.

Sex differentiation: Histological analysis showed that gonad sex differentiation occurred at the age of 4 - 5 months in black porgy (Wu et al., 2008a,b; Lee et al., 2008). Numerous studies have established the fact that sex steroids play important role in the process of sex differentiation in fish (Guiguen et al., 1999). Aromatase, the key enzyme that converts testosterone to estrogen, has been reported to be involved in ovarian differentiation in all fish species investigated (Guiguen et al., 1999; Kitano et al., 2000). Teleosts are characterized by the fact that they possess two aromatase genes, *cyp19a1a*, mainly expressed in the gonads, and *cyp19a1b*, mainly expressed in the brain (Tchoudakova et al., 1998; Chiang et al., 2001; Kwon et al., 2001; Valle et al., 2002). A second brain aromatase gene (*cyp19a1bII*) has been found in rainbow trout *Oncorhynchus mykiss* (Dall Valle et al., 2005). *cyp19a1a* transcripts levels and Cyp19a1a protein are significantly higher in undifferentiated gonads as compared to early male differentiated gonads in black porgy (Wu et al., 2008 a,b). Gonad *dmrt1* transcripts, a differentiated testis-specific gene in some teleosts including black porgy (Wu et al., 2008a) was differentially expressed in the gonad tissue of black porgy at 4- 5-months of age. Gonadal *dmrt1* transcripts increased significantly in the differentiated gonad as compared undifferentiated gonad. Treating birds with the aromatase inhibitor fadrozole lead to elevated *Dmrt1* levels indicating that *Dmrt1* may be down regulated by aromatase (Smith et al. 2003). This indicates that *dmrt1* may have an important role in testis determination in teleosts, since alteration of aromatase levels during sex differentiation can cause sex reversals.

The orphan nuclear factors, *Sf-1* (steroidogenic factor 1; or adrenal 4 binding proten,

Ad4BP) and *dax-1* (dosage-sensitive sex reversal, adrenal hypoplasia congenital critical region on the X-chromosome, gene 1), has been implied as an important factor for steroidogenesis and sex differentiation in animals (Ikeda et al., 1993; 1994, Zazopoulos et al., 1997; Lalli et al., 1998; Wang et al., 2001). *Sf-1* expression was low in the undifferentiated gonad but increased significantly during testicular sex differentiation in black porgy. *dax-1* was not significantly expressed during early male sex differentiation in black porgy but it increased in the differentiated gonad. *dax-1* is probably more important in the testicular spermatogenesis and development than sex differentiation in black porgy. *dax-1* is reported to regulate the expression of multiple steroidogenic enzymes in mice (Lalli et al., 1998; Wang et al., 2001; Zazopoulos et al., 1997) and also suppress *Sf-1* mediated *cyp19a* expression in medaka (Nakamoto et al., 2007). *Sf-1* expression was high in testicular tissue than in ovarian tissue during sex differentiation in rat (Hatano et al., 1994), pig (Pilon et al., 1998) and turtle (Fleming et al., 1999) while it was high in the differentiating ovary compared to testicular gonad in chicken (Smith et al., 1999), American alligator (Western et al., 2000) and American bullfrog (Mayer et al., 2002). *dax-1* was expressed at higher levels in male than in female gonad of frog (Sugita et al., 2001) but higher in females in chicken during late gonadal development (Smith et al., 2000). No sex difference in the expression of *dax-1* during gonadal differentiation was found in tilapia (Smith et al., 2000), American alligator (Western et al., 2000), and sea turtle (Torres Maldonado et al., 2002). The expression of *cyp11b2*, the key steroidogenic enzyme for the production of 11-ketotestosterone, was also positively correlated to the expression of *dax-1*. It is suggested that *Sf-1* and *dax-1* may cooperatively and positively regulate *cyp11b2* enzyme expression in the testis of black porgy. *Sf-1* may also act at the *cyp19a* promoter and enhances *cyp19a* expression in fish (Tchoudakova et al., 2001). Low and constant levels of *wnt-4* expression together with gradually increased but still low levels of *fig*α expression were also found in the gonad from 4- 6-month-age periods. No

significant changes of *foxl2* expression were observed during sex differentiation in black porgy.

Sexual differentiation of brain structures and the role of way sex steroids in differentiation is an active research area. In all vertebrates, steroid hormones participate in the development/organization of brain areas important in the production of sex-specific reproductive behaviors. Perinatal exposure to estrogens in mammals and birds is also known to permanently affect neuronal/glial morphology and brain function in males (Arnold and Gorski, 1984), and sex differences in the brain also arise from aromatization during a critical developmental window (Morris et al., 2004). The expression of sex steroid receptors (androgen receptor (*ar*), estrogen receptor alpha (*esr1*) and estrogen receptor beta (*esr2a*)) receptors) and other related genes (*Sf-1, dax-1, star, cyp11a1, hsd3b1, cyp17a1* and *cyp19a1b*) in black porgy brain indicated that black porgy brain possesses the requisite machinery required for the *de novo* synthesis of E2. A synchronous peaked expression of *star*, the key neurosteroidogenic enzyme genes (*cyp11a1, hsd3b1, cyp17a1* and *cyp19a1b*) and *esr*s at 4 months of age in brain of black porgy indicate that they may synthesize and respond to E2 (Tomy et al 2007; 2009). *Sf-1 showed* no significant increase in expression till 4-months of age. On the other hand, *dax-1* exhibited a significant decrease in expression with advancement in age, especially at 4 months compared to 2 months of. It remains plausible that *dax-1* exerts a repressive function for a subset of *Sf-1* -regulated genes (*star* and *cyp19a1b*) that somehow regulate the timing of peak steroidogenesis and hence brain sex differentiation (Tomy et al., 2009). Brain aromatase activity and E2 levels also increased significantly in forebrain and hypothalamus, respectively, at 4-months of age (Tomy et al., 2007; 2009). The biological significance for elevated aromatase levels, and thus the high production of neural estrogens in teleosts, is still unresolved. The increases in the expression of the genes involved in the estrogen biosynthesis (*cyp19a1b*) and sex steroid signaling (sex steroid receptors) at

4-months of age are in agreement with the central importance of sex steroids as positive feedback regulators of the HPG axis in fish. The early expression of the genes, concurrent to gonadal sexual differentiation, provide evidence that these genes are functional during early postnatal development and sex differentiation, and have distinct contribution to different aspects of sex hormone-dependent brain differentiation.

Sex change: The ovarian portion of the gonad increases in size significantly during the post-spawning season, further developing into a full ovary during the third spawning season in naturally sex-changed fish. *esr1*, *ar*, *Sf-1, dax-1* and *cyp11b2* transcripts were significantly higher in bisexual testis than bisexual ovary in 1+ and 2+-year-old fish. In contrast, increased ER alpha transcripts were detected in the bisexual ovary and sex-changed ovary of 3-year-old fish as compared to those in bisexual ovary of 1-and 2-year-old fish. On the other hand, ovarian *Sf-1 transcripts* were low in 1^{+}- and 2^{+}-yr-old fish and became high in the vitellogenic ovary of 3-year-old sex changed female. In contrast, *dax-1* transcripts were high in ovarian tissue in 1+-yr-old male and then dramatically decreased to low levels in ovarian tissue of both 2+-yr-old males and 3-yr-old females. *Sf-1, dax-1* and *cyp11b2* expressions were at low levels in the regressed testicular tissue of 3-yr-old female. In the bisexual gonads of 1-year-old fish low expressions of the Cyp19a1a proteins are detected both in the testis and ovary, but during natural sex change *cyp19a1a* expression increases in the ovarian part of the ovotestis. Ovarian *cyp19a1a* transcripts remained low during the non-spawning and pre-spawning seasons even though the ovarian tissue increased in proportion in the gonad (Lee et al., 2008; Wu et al., 2008 a,b) Ovarian *foxl2* transcripts were low in the second spawning season, significantly increased during the pre-spawning season, and reached the highest levels at the third spawning season. High expression of *wnt4* was observed only in active ovary when the testis regressed and the fish was proceeding to natural sex change in

2^{+}-yr-old fish indicating that *wnt4* expression in ovarian tissue is negatively regulated by factors releasing from testicular tissue (Wu et al., unpublished data).

4. GnRH, gonadal aromatase activity and plasma steroid profiles during sex change

Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus in the brain of vertebrates, including fish, and regulates the synthesis and secretion of gonadotropins (GTH) in the pituitary. The GTHs, in turn, regulate gonadal steroidogenesis, gametogenesis, and gonadal growth, thereby directly influencing reproductive function. Thus GnRH and GTH are important factors regulating the reproductive process and sex steroid production in teleosts (Amano et al., 1997). Three forms of GnRH, seabream (sb) GnRH, salmon (s) GnRH, and chicken (c) GnRH-II in brain, and two forms of GnRH (sbGnRH and sGnRH) in the pituitary were detected in black porgy. sbGnRH is suggested as the physiological and main form of GnRH in the relation to plasma LH concentrations in black porgy. The mRNA expressions of sGnRH, sbGnRH and cGnRH-II were found to be higher in mature testis and ovary, compared to gonads at different stages of maturity suggesting their important roles in the regulation of hypothalamic-pituitary-gonadal axis, and also in gonadal development and sex change in black porgy (An et al., 2008). As in other vertebrates, in most of teleost species (Elizur et al., 1996; Hassin et al., 1995) two distinct gonadotropins- GtH I and GtH II (FSH and LH)- are involved in reproductive processes. FSH and LH have distinct temporal expression and release patterns (Meiri et al., 2004; Schulz et al., 2001; Dickey and Swanson, 1998) with different regulatory mechanisms, but precise mechanisms underlying the control of the release of each gonadotropin remain unclear. Tightly regulated communication between the ovary and brain ensures that the neural signal for ovulation occurs when ovarian follicles are mature. At the onset of puberty an increase in GnRH secretion stimulates gonadotropin secretion and subsequent gonadal maturation. In several species, the signal to initiate LH and,

presumably, LHRH release at puberty onset involves a decrease in responsiveness to negative feedback by gonadal steroids.

In black porgy, higher levels of plasma LH during the nonspawning season (May-August) favor the development of testicular tissue in bisexual gonad. Higher sex steroid receptors, FSH receptor and LH receptor are present in the bisexual testicular tissue than in the ovarian tissue suggesting that the bisexual testicular tissue is more sensitive than the ovarian tissue. Elevated plasma levels of LH and E2 during the pre-spawning and spawning season were correlated with the natural sex change in 3-yr-old black porgy (Chang et al., 1994; Chang and Yueh, 1990; Lee et al., 2000). However, plasma E2 is maintained at low levels when ovarian tissue develops to become the dominant tissue during the post-spawning and non-spawning seasons in 1^{+}- and 2^{+}-year-old fish (Du et al., 2005). Plasma 11-KT levels were also associated with the testicular function in black porgy. Plasma 11-KT levels decreased significantly but no changes in plasma testosterone were detected in the naturally sex-changing females. The levels of plasma vitellogenin were parallel to those of plasma E2 in both males and reversing females. Plasma vitellogenin levels were very low before November and then significantly increased in November (right after the elevation of plasma E2) in natural reversing females. The data indicate that the occurrence of plasma E2 and vitellogenin correlates with the natural sex change.

Sex steroids (E2, T, 11-KT) and cortisol were demonstrated to act directly on brain cells and modulated GnRH release, probably through a direct action on GnRH neurons (Lee et al., 2004). Administration of sex steroids stimulates LH expression in several species, such as salmonids (Gielen and Goos, 1983), platyfish (*Xyphophorus maculates*) (Schreibman et al. 1986), eel (*Anguilla anguilla*) (Dufour et al., 1983; Huang et al., 1997), black carp (*Mylopharyngodon piceus*) (Gur et al., 1995), goldfish (*Carassius auratus*) (Kobayashi *et al.,* 1989) and black porgy (Lee et al., 2000). Long-term oral administration of E2 produced high

levels of plasma LH and gonadal aromatase activity, resulting in sex change in black porgy (Lee et al*.,* 2000; Du et al., 2001) thus providing a link between E2 and the endocrine mechanism of sex change in protandrous black porgy. Androgens (T or DHT) had little or no effect on plasma LH (Du et al., 2001). E2 treatment increased mRNA expression levels of three gonadotropin GTH subunits (GTHalpha, FSHbeta, and LHbeta) and two GTH receptors (FSHR and LHR) from pituitary and gonads of black porgy and plasma E2levels, indicating that E2 stimulated the increases in GTH subunit and GTH-receptor mRNAs. These data indicate that E2 plays an important regulatory role in the brain-pituitary-gonad axis of immature black porgy (An et al., 2009).

Gonadal aromatase activity has also been shown to have a relationship with ovarian development in animals. Gonadal aromatase activity in black porgy was low in the midspawning period and then significantly increased in February–April (postspawning in 3-year-old fish) and September (prespawning season in 3-year-old fish) as compared to October–January (prespawning season and spawning season in 2-year-old fish). The development of the ovarian tissue during the postspawning season in fish after 2 years of age and during the prespawning season in the 3-year-old fish caused the increase of gonadal aromatase activity. Like in other vertebrates, brain aromatase activity (Borg et al., 1987; Callard et al., 1981; Gonzalez and Piferrer, 2003; Mayer et al., 1991; Pasmanik and Callard, 1988) and mRNA (Forlano et al., 2005; Gelinas et al., 1998, Kazeto et al., 2003; Kazeto and Trant, 2005) expression levels fluctuate seasonally and with reproductive state in teleosts, implying an important role in reproductive physiology. In black porgy inhibition of brain aromatase prevents natural sex change to female (Lee et al., 2001). In the blue banded goby (*Lythrypnus dalli*) females have significantly higher brain aromatase activity levels than males, but within minutes to hours of sex change, a significant increase in aggressiveness is coincident with a dramatic drop in brain aromatase activity (40%) (Black et al., 2005). Thus,

brain estrogen levels likely shift prior to changes in gonadal structure and circulating steroids that may then maintain the sexual phenotype (Forlano et al., 2006).

5. Effect of exogenous hormone treatment on sex differentiation and sex change

Endogenous estrogens are considered to be key steroids and aromatase a key enzyme mainly for ovarian differentiation and development in marsupials (Coveney et al., 2001), reptiles (Merchant-Larios et al., 1997; Pieau and Dorizzi, 2004), birds (Scheib, 1983; Villalpando et al., 2000) and teleosts (Kobabyshi et al., 2003; Kobayashi and Iwamatsu, 2005). Treatment with aromatase inhibitor to block aromatase action also resulted in the production of phenotypic male from genotypic female in birds (Elbrecht and Smith, 1992; Hudson et al., 2005), reptiles (Wibbels and Crews, 1994; Belaid et al., 2001), and teleosts such as tilapia and rainbow trout (Guiguen et al., 1999) and medaka (Kobayashi et al., 2003). Role of estrogen during sexual differentiation in fish has been poorly investigated, probably because of the size of fish and of their gonads at early developmental stages. The significance of estradiol for testicular growth/development in black porgy was investigated by administering estradiol and aromatase inhibitor. Oral administration of estradiol (E2; 4 mg per kg of feed) to 1- and 2-year-old black porgy for 5–6 months (starting from September) showed complete suppression of spermatogenesis and spermiation and successfully induced the precocious sex change with vitellogenic oocytes and large primary oocytes. Lower levels of plasma 11-KT and higher levels of plasma vitellogenin were shown in the E2 group. Higher gonadal aromatase activity in concordance with elevated plasma levels of LH was observed in the E2 group. The highest aromatase activity in the forebrain in the control group was observed in January. Higher aromatase activity in forebrain, midbrain, and hindbrain was also detected in the E2 group than the control group. The change of aromatase activity in brain was more susceptible than that in gonad. The data showed that the increase of plasma LH levels and

gonadal aromatase activity may be important to the controlled sex change by administration in black porgy (Lee et al., 2000). Oral administration with aromatase inhibitors (AI; fadrozole and 1,4,6-androstatriene-3,17-dione) to 2-year-old black porgy resulted in all AI-fed fish remaining as functional males with active spermiation at 3 years of age in contrast to control fish which were females (35.7%), bisexual, and functional males (64.3%) were found. We further found that by giving AI to the fish from September to January was enough to block the natural sex change. The involvement of aromatase in the sex change of black porgy is thus clearly demonstrated (Lee et al., 2002).

Administration of E2 (6 mg E2 per kg of feed for a period of 3 months) and aromatase inhibitor treatment (20 mg AI per kg of feed; for a period of 7 months) induced an early sex change with the regression of testicular tissue in concomitance with ovarian development resulting in female sex differentiation. Exogenous AI administration resulted in decreased *cyp19a1a* transcripts, lower plasma estradiol levels, and active proliferation and development of oogonia to primary oocytes (Wu et al., 2008b) while exogenous estradiol resulted an increase in plasma estradiol levels, *cyp19a1a* and *wnt4* transcripts, gonadal aromatase and low and constant low levels of *Sf-1* together with decreased levels of *dax-1* transcripts. Both high dose of exogenous E2 and AI induced testicular regression within 1-2 months of administration. Although AI administration also induced ovarian development, the diameters of primary oocytes in AI-induced females were smaller than those in E2-induced female (Wu et al., 2008b). Plasma E2 concentrations in 6-month-old black porgy was higher in the E2 administered fish compared to the control fish. Thus, E2 administration resulted in high levels of plasma E2 and induced female sexual development. On the other hand, AI treatment resulted in lower levels of plasma E2. We further found that E2 was able to induce a reversible sex change in the juvenile black porgy when E2 administration was terminated before the spawning season change with the E2-induced ovary reverting to a bisexual gonad,

consisting of regenerating testicular tissue and regressing ovarian tissue. Low *Sf-1 and cyp19a1a*, but high *dax-1* expression levels were detected in the regressing ovarian tissue during the reversible sex change. *Sf-1* was highly expressed in the regenerating testicular tissue during the reversible sex change. E2-administration affected the expression profiles of *dax-1* and *cyp19a* in a reciprocal manner, and further decreased gene expression in steroidogenic pathway.

On the other hand, long-term, low dose of E2 (0.25 mg/kg feed) administration clearly enhanced testicular development, as revealed by the growth of a large and active testis together with high levels of plasma 11-KT (a key sex steroid closely associated with testicular function in fish) (Chang et al., 1995) suggesting that aromatase expression is important and necessary for testicular differentiation and development in protandrous black porgy. Thus results from steroid hormone treatment on the gonad development in black porgy indicated that the effects of E2 on the gonadal development was dependent on the E2 dosage, low dose (0.25 – 1.0 mg/kg feed) favored testicular growth and high dose (4 – 6 mg/kg feed) favored ovarian development.

6. The effects of testis-excision in the ovarian development in bisexual gonad of 1^+-yr-old fish

Surgical operation to remove testicular tissue in 1^+-yr-old fish induced an early sex change in the fish similar to the natural sex change in 2^+- to 3-yr-old fish. The development of vitellogenic oocytes was observed after removal of testicular tissue in the bisexual gonad. Quantitative real-time PCR showed that transcripts of *Sf-1, wnt4* and genes in steroidogenic pathway such as *star*, *cyp11a1*, *cyp17a1*, *hsd3b1* and *cyp19a1a* significantly increased ($P < 0.05$) in female ovary in the testis-excised group as compared to ovarian tissue in the control group. In contrast, *dax-1* transcripts decreased in the female ovary as compared to the control

fish. Furthermore, ovarian aromatase activity and plasma E2 concentrations increased significantly in the testis-excised as compared to the control group.

7. Conclusions

Sex differentiation and sex change in fish are considered as an integrative, complex and multi-level phenomenon that may be caused by specific environmental stimuli, reaching certain neuroendocrine systems and resulting in predictably anatomical changes in the reproductive system of a hermaphrodite fish protandrous black porgy is a unique model fish to study male sex differentiation and development. Our data demonstrated the dual roles of *cyp19a1a* in testicular development during early sexual differentiation as well as in ovarian development during the later natural sex change and also the importance of *Sf-1 for* the early male sex differentiation, *wnt4* in the early ovarian development, and *foxl2/cyp19a1a* in the late ovarian growth in black porgy. The study also suggests a precedence or concurrence of neural differentiation over gonadal development in black porgy. Endocrine factors (aromatase, E2, *ar*, *esr*s, FSH receptor, and LH receptor in gonad; pituitary LH) may play important roles in the sex change. Plasma LH is closely associated with the development of male phase. A higher sensitivity in the bisexual testicular tissue than in the ovarian tissue is suggested. High *Sf-1*and *dax-1* favored the maintenance of testicular development in male phase. In contrast, low *Sf-1 and* high expression were found in ovarian tissue before sex change. Therefore, our data suggests that *Sf-1* and *dax-1* have a cooperative function for the testicular development and an antagonistic interaction for oocyte development. The expression levels of *dax-1* probably regulate the timing of oocyte development and vitellogenesis for sex change in the protandrous black porgy. Testicular regression was shown to be important for the initiation of ovarian development in the bisexual gonad. The possible endocrine mechanisms of sex change in protandrous black porgy is further suggested. The successful induction of an early

sex change in the testis *dax-1*-excised fish further suggests for the first time that testicular tissue may exert some inhibitory effects on the advanced ovarian (vitellogenic) development in bisexual gonad. Our results provide new insight into the development of advanced oocytes during the natural sex change in black porgy.

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生活環境中環境荷爾蒙之調查研究
Environmental Hormones in Our Living Environment

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摘要

1962 年出版的寂靜春天(Silent Spring)，提及美國五大湖使用農藥，導致幼鳥無法孵化，大量鳥類死亡，1996 年出版的失竊的未來(Our Stolen future)，提及內分泌干擾化學物質(Endocrine Disrupting Chemicals, EDCs)導致野生動物的生殖器官和環境生態異常的現象，1998 年出版的雌性化自然，提及蘇格蘭醫生發現人類精子在 50 年內降了 50%，丹麥教授發現睪丸癌罹患率是 50 年前的 3 倍，睪丸不降症、尿道下裂等相關病症發生率也以每年 2~3%的比率增加，人類的生殖力逐年下降，和野生動物異常的變化類似，同樣的，過去 40 年女性乳癌的罹患率也一直在增加，這都和可能接觸到雌激素(oestrogen)有關。1998 年日本井口泰權教授提出環境荷爾蒙名詞，一般民眾對此一課題有更深的認識。1999 年美國國家科學院將 EDCs 改為荷爾蒙活性物質(Hormonally Active Agents, HAAs)。各國政府對此一攸關人類存亡的問題，多數廠商也以商業機密為由，加上受限於欠缺雌激素影響人類生殖的直接證明，政府立法以保護大眾免受到環境荷爾蒙危害的進展有限，然而隨著越來越多的科學研究，許多的證據提出生活環境中的少數化學物質，表示人們可能是接觸到這些化學物質而致病。

環境荷爾蒙係指外因性干擾生物體內分泌之化學物質，也稱為內分泌干擾素(endocrine disruptors)，其可模擬類似為生物體既有的荷爾蒙，欺騙身體而造成體內對荷爾蒙的過度作用，妨礙生物體內荷爾蒙的正常活動。此外環境荷爾蒙也可藉由強化、干擾或抑制等作用形式，去影響生物體內荷爾蒙的運送、調節、結合、訊號產生、或細胞代謝等反應機制。如果從廣義的角度來看，只要外界化學物質能改變體內正常荷爾蒙作用過程的任一環節產生干擾荷爾蒙的作用，都可算是環境荷爾蒙。有時極低濃度的環境荷爾蒙暴露量，即可對生化與細胞作用機制造成巨大的改變，尤其對懷孕期胚胎形成與

嬰幼兒成長初期影響最大,危害效應輕則造成嬰兒神經發育不全、免疫與生殖系統不良，重則引發致突變性。1977 年台灣中部地區發生的多氯聯苯毒油事件，遭到毒害之婦女的下一代，多有發展遲緩，智力降低等問題，意味著人類的智慧、情感、能力可能在無聲無息中就喪失掉，文明的社會將不再存在。

環境荷爾蒙對人體健康的危害，既長遠又嚴重。多氯戴奧辛/呋喃與多氯聯苯在目前的環境荷爾蒙黑名單中分別排名第一和第二。2004 年生效的斯德哥爾摩持久性有機污染物公約(Stockholm Convention on Persistent Organic Pollutants)中，多氯戴奧辛、多氯呋喃與多氯聯苯更與其他 9 種含氯物質(艾氏劑 Aldrin、氯丹 Chlordane、狄氏劑 Dieldrin、滴滴涕 DDT、異狄氏劑 Endrin、七氯 Heptachlor、六氯代苯 Hexachlorobenzene、滅蟻靈 Mirex、毒殺芬 Toxaphene)被聯合國環境規劃署列為優先管制的 12 種持久性有機污染物，可見戴奧辛對人體的健康影響深遠。新興污染物如多溴聯苯醚 (Polybrominated diphenyl ethers，PBDEs)則是被列在候選名單中第一順位。2006 年生效的歐盟電子電機禁用有害物質指令(RoHs)，規範電機電子產品鉛、汞、鎘、六價鉻多溴聯苯及多溴聯苯醚等有害物質之限量。2007 年生效的歐洲議會和歐盟理事會「化學物質註冊、評估、許可和限制法」(REACH)法規，規劃此類似化學物質之管制，2008 年 10 月 歐盟危險物質法令名單(DSD 的第 30 次修正版)新增了 800 多種物質，其中將近 290 種物質被鑒定為致癌、可導致基因突變和有生殖影響的物質。

本報告將從歸類為環境荷爾蒙的化學物質談起，簡介其特性及使用；其次，從暴露途徑談起，說明環境荷爾蒙進入體的途徑，簡介身體負荷級生物監測之觀念，以為個人防制環境荷爾蒙的參考；接著，回顧國內環境及產品中環境荷爾現況及相關活動，提出因應策略之個人看法。

我國大學生對於環境荷爾蒙之認知調查

Survey of College Students' Understanding on EDCs in Taiwan

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摘要

環境荷爾蒙(Environmental Hormones)近年來在環境專業領域已是眾人耳熟能詳的名詞，我國環保署近年也開始注意到這些內分泌干擾物質(Endocrine Disrupting Chemicals, EDCs) 對於環境、生態與人體健康的影響。在我國已公告的毒性化學物質中，就有超過二十種具有疑似環境荷爾蒙的性質。然而，一般社會大眾而言對於環境荷爾蒙究竟有多少瞭解，恐怕不是終日接觸相關訊息的專業人士可以判斷的。事實上，一般社會人士對於環境問題多為一知半解，且受到新聞媒體的影響相當明顯。環境荷爾蒙在一般的環境問題報導中偏向專業性質，且並非急毒性物質，不易引發重大環境事件，因此若以科學傳播與教育的觀點來看，可以研判一般人對於環境荷爾蒙與其相關議題的瞭解不甚深入。由於大學生可以作為一般較接觸各種知識來源的代表群體之一，因此，本研究透過對於大學生的調查，以理解我國一般大學生對於環境荷爾蒙的認知程度。依據對於環境荷爾蒙與相關議題之文獻回顧，研究者先整理出認知變項之主要類別，包括「環境荷爾蒙的基礎概念」、「生活環境中環境荷爾蒙的分佈」、「免於環境荷爾蒙危害的行動策略知識」三大類，在各類別下再各以二項內涵，分別設計三至五題是非或選擇題，編制成一雙向細目表，在發展為試測問卷。同時，基於許多大學生的各方面學習成就與對社會的關懷程度低落，在回答上述問題前，先請填答者表示是否「聽說過」若干專有名詞，以先釐清與篩選大學生的最基本認知。同時，問卷也調查大學生對於相關資訊的主要來源管道為何，也瞭解其一般的環境行動經驗。問卷初稿經過數位該領域專家之審查，以確定專家效度後，對北中南三地共三百餘名大學生進行施測，並以 SPSS 進行統計分析。調查結果顯示，大多數受測大學生甚至連「環境荷爾蒙」一詞都沒有聽說過！較多人聽說過戴奧辛與多氯聯苯。然而，多數受測大學生瞭解環境荷爾蒙會對生物體造成不良影響，但對於來源與途徑等較為專業的問題則不甚瞭解。此外，受測大學生中對於國內外因應對策與個人因應方法有還算良好的判斷能力。這也顯示長期以來，台灣的大學生對於環境污染與污染物可能對人體造成的影響已具有相當的認知程度，縱使對於專業領域不甚瞭解，但也能對於環境污染與防護知道有還算正確的判斷。本研究結果對於本領域未來之研究、科學教育與政府單位之宣導政策等，均具有相當之參考價值。

關鍵字：環境荷爾蒙、問卷調查、雙向細目表、專家效度、認知程度、SPSS。

專題演講

環境荷爾蒙物質生物效應與生態影響之回顧
-從美國佛州阿波卡湖事件至今

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摘要

近二、三十年來，許多生態學者、流行病學家、內分泌學家和環境毒理學家皆呼籲環境中一些具有類似生物體內激素作用之化學物質可能對人類健康與生態造成危害。這些被統稱為環境荷爾蒙(environmental hormones)或內分泌干擾物(endocrine disrupting chemicals)是因為具有類似生物體內荷爾蒙之功能，能抑制其作用，進而改變生物體內免疫、神經與內分泌系統之正常運作。這類化學物質可能產生的人類健康影響包括：女性乳癌和子宮內膜異常增生(endometriosis)、男性前列腺癌及睪丸癌、不正常的性發育、降低男性生殖力、腦下垂體及甲狀腺功能改變、免疫力抑制和神經行為作用等。

除潛在的人類健康效應外，許多報告亦顯示類似作用之物質能使多種水中和野生生物體內分泌系統的功能失常。這些具環境累積性之有機物質包括：多氯聯苯、戴奧辛、DDT與其他有機氯殺蟲劑、烷基酚類、bisphenol-A 等物質。其產生不良的影響包括：魚類和鳥類不正常的甲狀腺功能和發育；減少貝、魚、鳥類和哺乳類動物的生殖力；降低魚類、鳥類和爬蟲類動物的孵化率；造成魚、鳥、爬蟲類動物和哺乳類動物的去雄性化(demasculinization)和雌性化(feminization)；軟體動物、魚類、鳥類去雌性化(defeminization)和雄性化(masculinization)；減少後代存活力；改變鳥類和海洋哺乳類動物的免疫力和行為等。這些在野生動物中觀察到的異常現象皆被認為與生物體內之內分泌受影響有關。Colborn 博士於 1996 年出版之失竊的未來(Our Stolen Future)一書中明確的指出，目前環境中環境荷爾蒙的含量已經對人類與不同地區的生態系造成某些程度的影響。

環境荷爾蒙成為近年來熱門的環保議題起源於 1980 年的美國佛羅里達州阿波卡湖(Lake Apopka)。當地鱷魚曾受 DDT 與其他有機氯殺蟲劑的毒害而引起環境生態學家之重視。Apopka 事件是由於一殺蟲劑製造廠外洩 DDT、DDE、大克 (dicofol)等有機氯殺蟲劑並

進入 Apopka 湖水體，造成當地雄性鱷魚的去雄性化，以及雌性鱷魚受仿雌激素影響而產生超級雌性化(super-feminization)的現象，並導致當地鱷魚族群之數量和孵化成功率的下降。經過了將近 30 年，許多的研究數據都顯示我們一直都忽略了與我們共存共生的野外生物，而其實他們更容易受到環境賀爾蒙的影響，因為他們常常毫無保護的就直接暴露在其中。這種慢性的屠殺，就如同人類其他迫害自然環境的行為一樣，唯有我們的自覺能夠阻止。

個人將近年來有關此類物質對野生生物(包括魚類)影響的研究結果彙整並將於大會報告。

Abstract

In recent years, scientists from different fields including ecologist, epidemiologists, endocrinologists, and environmental toxicologists have provided some solid evidences which indicated that some chemicals may mimic natural hormones and pose threats to both human and wildlife. These so-called environmental hormones or endocrine disrupting compounds may inhibit or through different mechanisms to influence the normal functions of not only endocrine but also neural and immune systems in higher organisms. These speculations include increases of breast cancers and endometriosis in women and prostate and testis cancers in men, abnormal sexual development, decreases of fertilization success, immune function suppression, increases in ADHD, autism and other neural behavior disorders.

Wildlife has not been left alone from the influences of these chemicals. Reports have been indicated anomalies in both aquatic and terrestrial animals and others lower organisms. Those include abnormal thyroid and development functions in avian, decreases of reproductively in shellfish, fish, avian, and mammals, decreases of hatching success in oviparous species, demasculinization or feminization in some reptiles and mammals of either sexes, defeminization or masculinization in some mollusks or fish, decreases of survivorship

of the offspring for some species, and alteration of behaviors and immune functions in some avian and marine mammals. These also have been outlined in Theo Colborn's " Our Stolen Future", which also stated that the levels of some environmental hormones in certain areas already affect the ecological systems to some extent.

The whole issue of environmental hormones becoming aware in both scientists and general public started from 1980 when Lake Apopka, Florida, was spilled by DDT and DDT-like pesticide, Dicofol. Followed by the spill, researchers found that local alligators were either demasculinized in male or super-feminized in female which resulted in an sharp decreases in the population of the local alligators. After almost 30 years, more and more evidences showing that we still kept ignoring wildlife which coexists with us. They are even more vulnerable to pollutants to which they are usually exposed directly (and unknowingly). Like other human activities leading to environmental damages, this kind of chronic eliminations of lives on earth can only be stopped by our self-consciousness.

I will report some advances in the effects of environmental hormones on wildlife including fish.

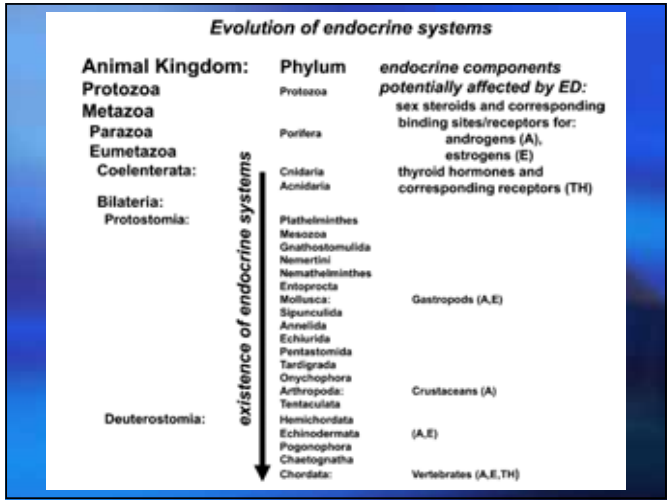

Table 1. Composition of sex chromosome and determination and differentiation of sex in different classes of vertebrates

Class	Composition of sex chromosome	Factors affecting determination and differentiation of sex	Description
Fishes	(+)	Hormone, growth temperature, rank in population	Sex conversion possible (M→F and F→M)
Amphibians	(+)	Hormone, temperature	Sex conversion possible (M→F or F→M)
Reptiles		Hormone, temperature, environmental factors	No sex conversion
Crocodiles	(–)	"	"
Lizard	(–)	"	"
Turtles	(+)	"	"
Snake	ZZ-ZW	No effect of environmental factors	No sex conversion
Birds	ZZ-ZW	No effect of environmental factors	No sex conversion
Mammals	XX-XY	No effect of environmental factors	No sex conversion

(+): XX-XY type or ZZ-ZW type) in some species.
(–): Unclear differentiation of hetero-sex chromosome.

Natural and anthropogenic environmental estrogens: the scientific basis for risk assessment, Miyamotot and Klein, Pure and Appl. Chem 70(9), 1829-1845, 1998

A review on environmental hormones' effects on wildlife-from the Lake Apopka event till now

環境荷爾蒙物質生物效應與生態影響之回顧-從美國佛州阿波卡湖事件至今

Dept. of Environmental Resources and Management
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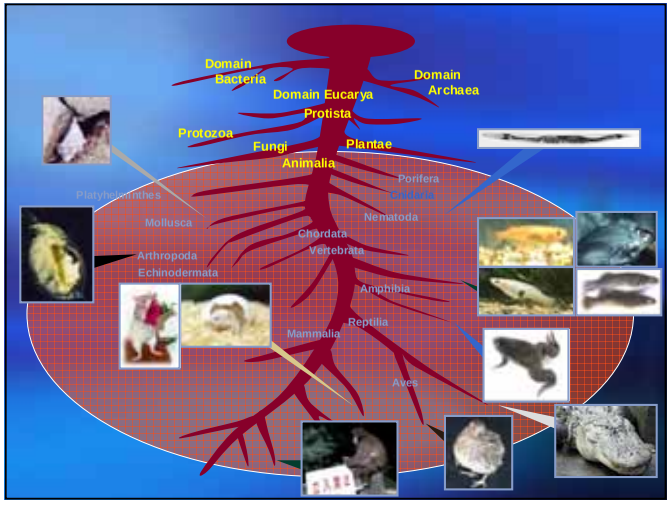


Action	Examples	Species or system	Reference
Enzyme Induction			
•P450 aromatase	Atrazine	American alligator	Crain *et al.*, 1997
Altered Hormone Metabolism			
•testosterone	DDT, Dieldrin	'Birds'	Peakall, 1967
	Endosulfan	CD-1 mice	Wilson & LeBlanc, 1998
	Vinclozolin	CD-1 mice	Wilson *et al.*, 1998
	Nonylphenol polyethoxylate	Daphnia	Baldwin *et al.*, 1998
	Tributyltin	Periwinkle	Ronis and Mason, 1996
	Pulp Mill Effluent	Fathead minnow	Parks and LeBlanc, 1998
	2,3,7,8-Dioxin	Great Blue heron	Sanderson *et al.*, 1997
•progesterone	DDT, Dieldrin	'Birds'	Peakal, 1967
•thyroxine	DDT	Laboratory rat	Bastomsky, 1974
	PCBs	Laboratory rat	Byrne *et al.*, 1987

The story begins

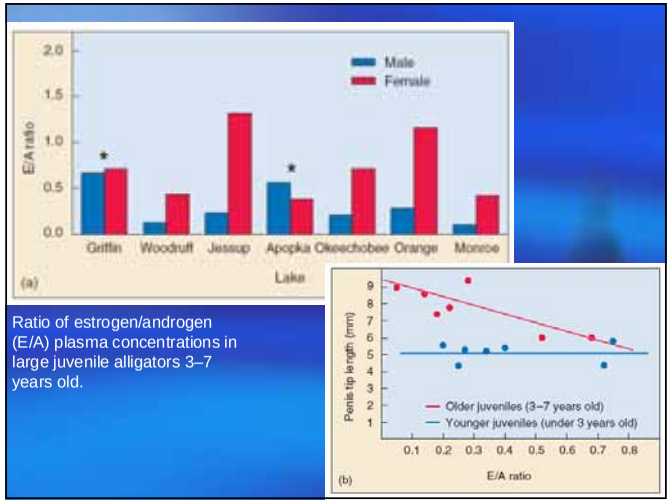
- 1940's marshes of lake Apopka drained for agricultural use (19,000 acres)
- 1950-1990 extensive eutrophication and numerous fish / alligator kills
- 1992 alligator / turtle population crash
  - Reproduction problems, gender defintion
  - 1980 Dicofol spill (90% gator die-off)

Chemicals in Environment: 61 chemicals measured

Polychlorinated biphenyl (PCBs)	Hexacychlorohexane	Trifluralin
Polybrominated biphenyl (PBB)	DDT	Amitrol
Octachlorostyrene	DDE & DDD	Nitrofen
Tributyltin	Chlordane	Carbendazim
Triphenyltin	Oxychlordane	Methomyl
Alkylphenols	trans-Nonachlor	Carvaril (NAC)
Bisphenol A	Heptachlor	Dibromochrolopropane
Styrene dimers and trimers	Heptachlor epoxide	Malathion
DEHP	Aldrin	Ethylparathion
Butylbenzyl phthalate	Endrin	Fenvalerate
Di-n-butyl phthalate	Dieldrin	Permethrin
Dicyclohexyl phthalate	Endosulfan	Cypermethrin
Diethyl phthalate	Methoxyhlor	Manzeb
Dipentyl phthalate	Kelthane	Maneb
Dihexyl phthalate	Hexachlorobenzene (HCB)	Zineb
Dipropyl phthalate	Pentachlorophenol (PCP)	Ziram
Di-(2-ethylhexyl)adipate	2,4,5-Trichloropehnoxy a.a.	Vinclozolin
Benzo(a)pyrene	2,4-Dichlorophenoxy a.a.	
Benzophenone	Arachlor	
2,4-Dichlorophenol	Atrazine	
4-Nitrotoluene	Symazine (CAT)	
n-Butylbenzene	Methribuzin	

Detected chemicals
High detection rate

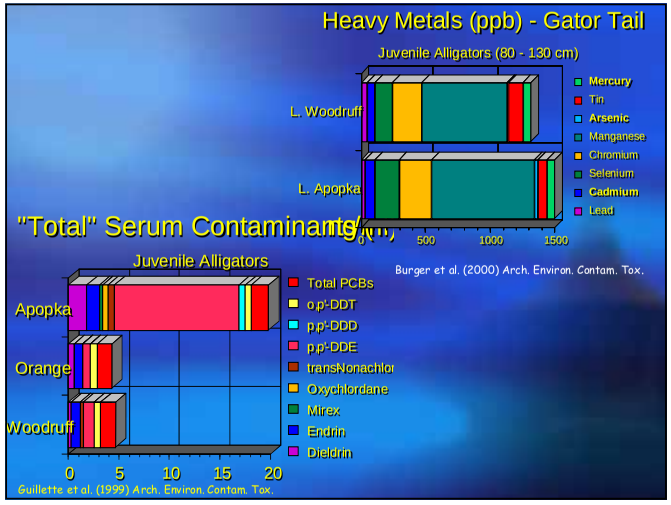
Action	Examples	Species or system	Reference
Hormone Agonist			
•Estrogen	4-Nonylphenol	Rainbow trout	White *et al.*, 1994
	o,p'-DDT	Red-eared turtle	Palmer and Palmer, 1995
	hydroxy-PCBs	Red-eared turtle	Bergeron *et al.*, 1994
	o,p'-DDT	Western gull	Fry and Toone, 1981
	p,p'-DDD	American alligator	Crain, 1997
	p,p'-DDE	Tiger salamander	Clark *et al.*, 1998
	p,p'-DDE	American alligator	Matter *et al.*, 1998
	o,p'-DDE	American alligator	Matter *et al.*, 1998
	Kepone	Seatrout	Thomas and Khan, 1997
•Androgen	Pulp Mill Effluent	Least killifish	Davis and Bortone, 1992
	Pulp Mill Effluent	Mosquitofish	Davis and Bortone, 1992
	4-Nonylphenol	Daphnia	Baldwin *et al.*, 1997
•Corticosterone	DDT	Senegal walking frog	Hayes *et al.*, 1997
Hormone Antagonist			
•anti-Estrogen	DDT	Tiger salamander	Clark *et al.*, 1998
	p,p'-DDE	American alligator	Matter *et al.*, 1998
•anti-Androgen	*p,p'*-DDE	Laboratory rat	Kelce *et al.*, 1995
	Vinclozolin	Laboratory rat	Gray *et al.*, 1993
•anti-Progestin	Methoxychlor	African clawed frog	Pickford and Morris, 1999

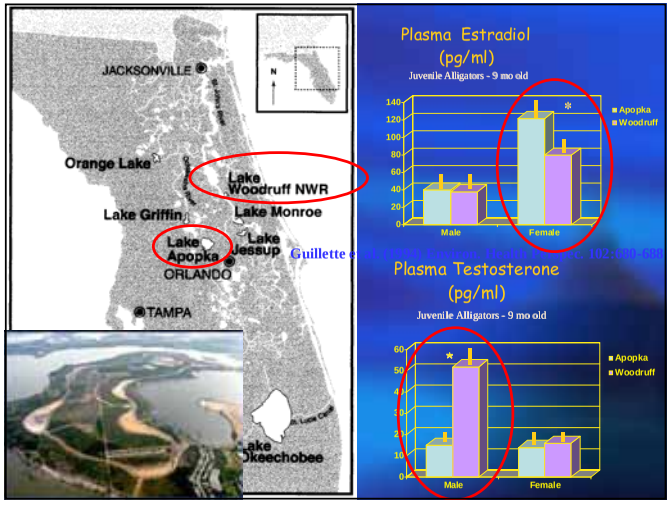


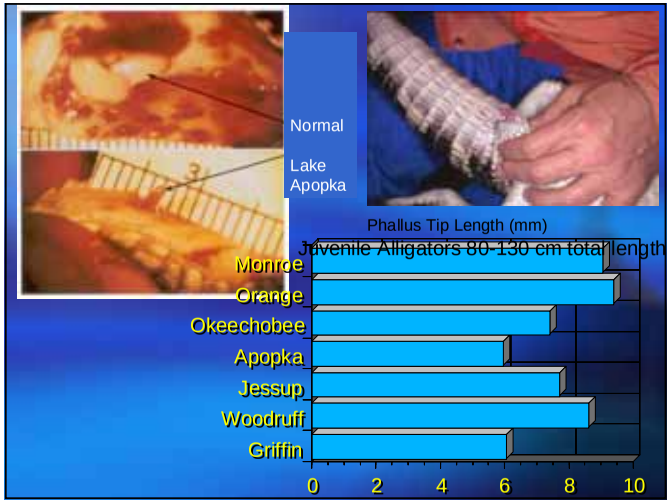
Ratio of estrogen/androgen (E/A) plasma concentrations in large juvenile alligators 3–7 years old.

Endocrine alterations reported in hatchling and juvenile alligators from the Lake Apopka

Hormone	Measure	Age group	Reference
Testosterone	reduced plasma conc.	Hatchling males	Guillette *et al.*, 1994
	reduced plasma conc.	Juvenile males	Guillette *et al.*, 1994; 1996b; 1997; 1999b; Crain *et al.*, 1998; Pickford *et al.*, 2000
	elevated ovarian synthesis	Hatchling females	Guillette *et al.*, 1995c
	elevated hepatic metabolism	Juvenile females	Gunderson, LeBlanc and Guillette, unpubl.
Estradiol-17β	elevated plasma conc.	Hatchling females	Guillette *et al.*, 1994
	reduced plasma conc.	Juvenile females	Pickford *et al.*, 2000; Guillette *et al.*, 1999b
	reduced ovarian synthesis	Hatchling females	Guillette *et al.*, 1995c
Dehydrotestosterone	elevated plasma conc.	Juvenile females	Pickford *et al.*, 2000
Thyroxin	elevated plasma conc.	Juvenile males	Crain *et al.*, 1998







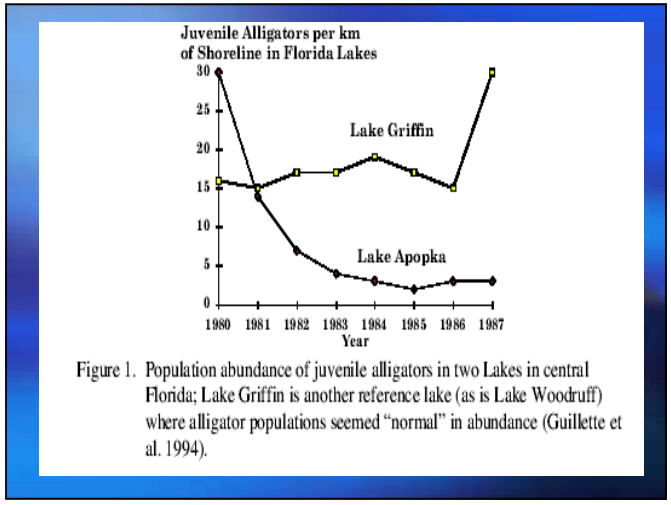
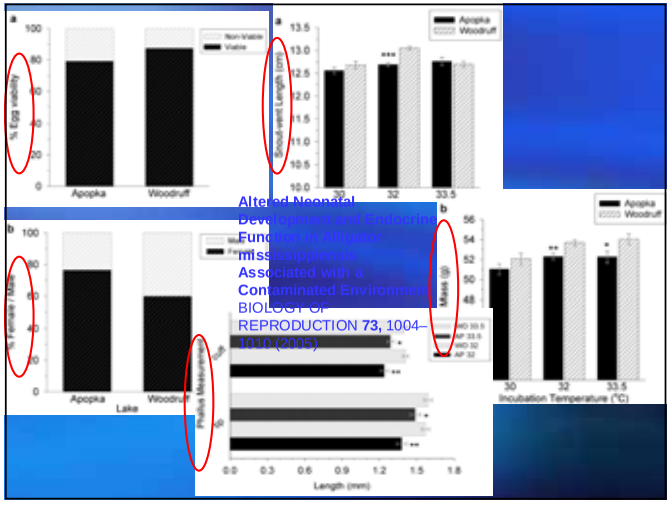
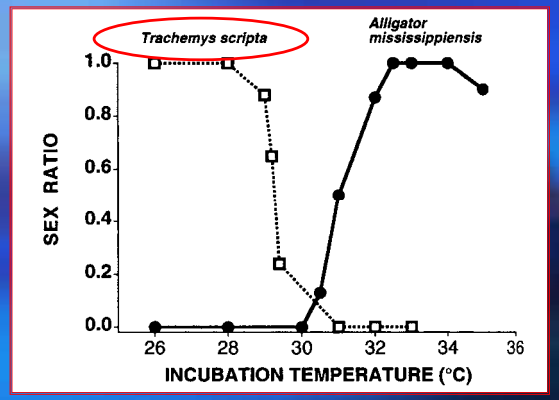


Figure 1. Population abundance of juvenile alligators in two Lakes in central Florida; Lake Griffin is another reference lake (as is Lake Woodruff) where alligator populations seemed "normal" in abundance (Guillette et al. 1994).



Altered Neonatal Development and Endocrine Function in Alligators Associated with a Contaminated Environment BIOLOGY OF REPRODUCTION 73, 1004–1010 (2005)

Alligator-warm males, cool females



All eggs incubated at 28.68C, a male-biased sex ratio.

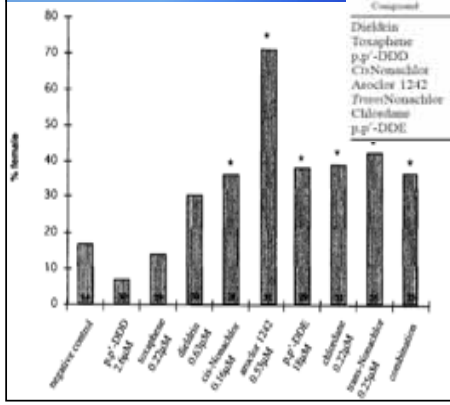
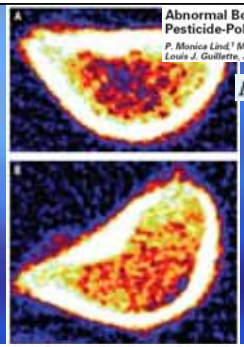


TABLE 1. *Compounds identified by Heinz et al. (1991) in alligator eggs from Lake Apopka, Florida and applied to red-eared slider turtle eggs to assess their effects on sex ratios (Willingham and Crews, 1999).**

Compound	Amount (ng) / 10g egg	Concentration (µM)
Dieldrin	1.197	0.63
Toxaphene	0.415	0.22
p,p'-DDD	4.2	2.6
CisNonachlor	0.356	0.18
Aroclor 1242	0.848	0.53
TransNonachlor	0.556	0.25
Chlordane	0.451	0.22
p,p'-DDE	28.2	18

Willingham, E. J. and D. Crews. 1999. Organismal effects of environmentally relevant pesticide concentrations on the red-eared slider turtle. Gen. Comp. Endocrinol. 113:429–435.



Abnormal Bone Composition in Female Juvenile American Alligators from a Pesticide-Polluted Lake (Lake Apopka, Florida)

P. Monica Lind,[1] Matthew R. Milnes,[2] Rebecca Lundberg,[1] Dieldrich Bermudez,[2] Jan Orberg,[3] and Louis J. Guillette, Jr.[2]

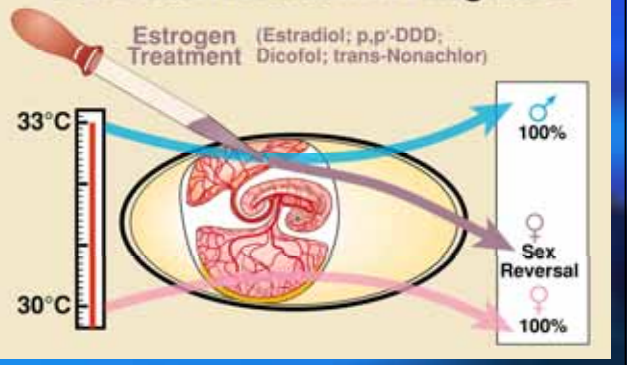
Environ Health Perspect 112:359–362 (2004)

Figure 2. Images from pQCT scans of proximal tibial metaphysis. (*A*) Lake Woodruff female. (*B*) Lake Apopka female. The white outer shell is cortical bone with a high BMD, and the inner spongy structures are trabecular bone with a much lower BMD. The blue areas contain bone tissue with a lower mineral density compared with the yellow and red areas.

Table 4. Femur, metaphyseal measure point.

Measures	Lake Woodruff (n = 8)	Lake Apopka (n = 9)
Total cross-sectional area (mm^2)	67.4 ± 12.3	89.9 ± 10.7
Trabecular area (mm^2)	37.9 ± 6.4	46.8 ± 5.9
Trabecular bone mineral content (mg/mm)	7.1 ± 1.6	10.1 ± 1.3
Trabecular BMD (mg/cm^3)	171.7 ± 15.9	218.3 ± 7.3*
Total BMD (mg/cm^3)	507.3 ± 23.9	567.0 ± 13.3*

Sex Determination in Alligators



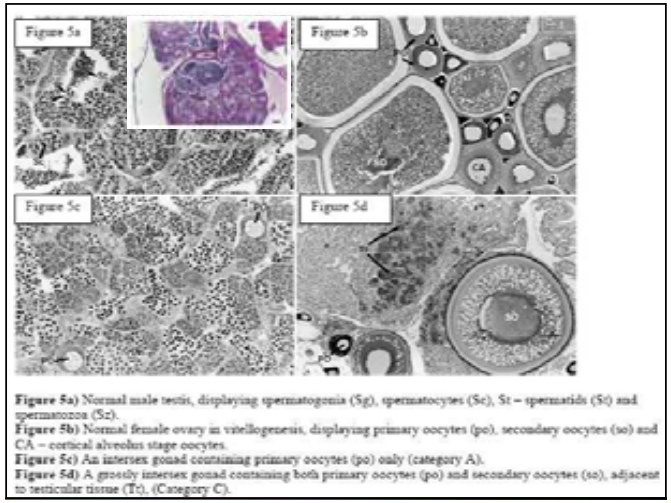
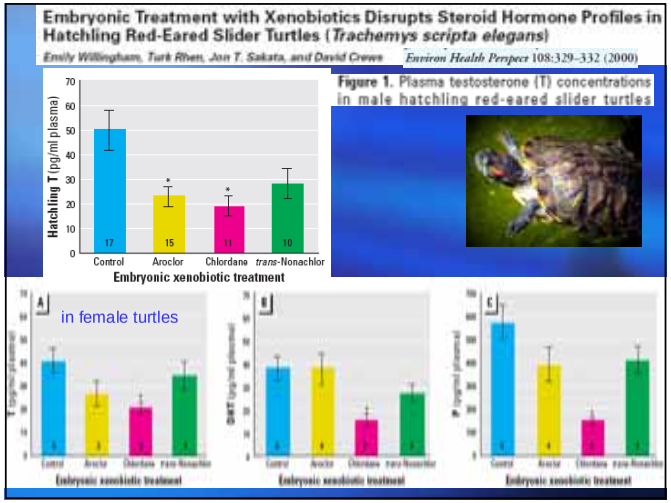
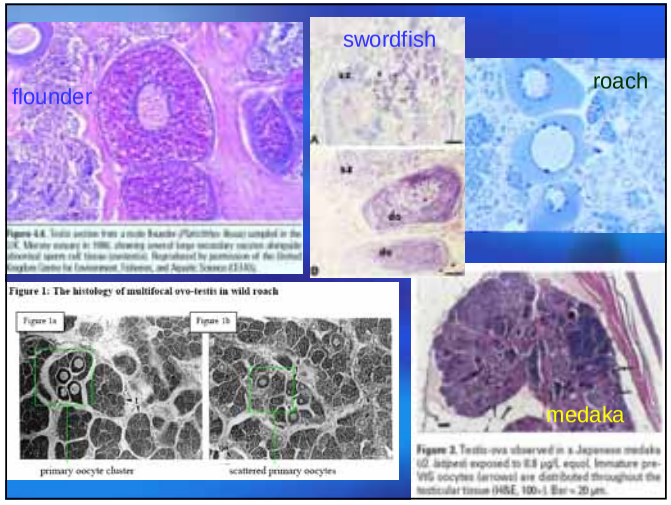
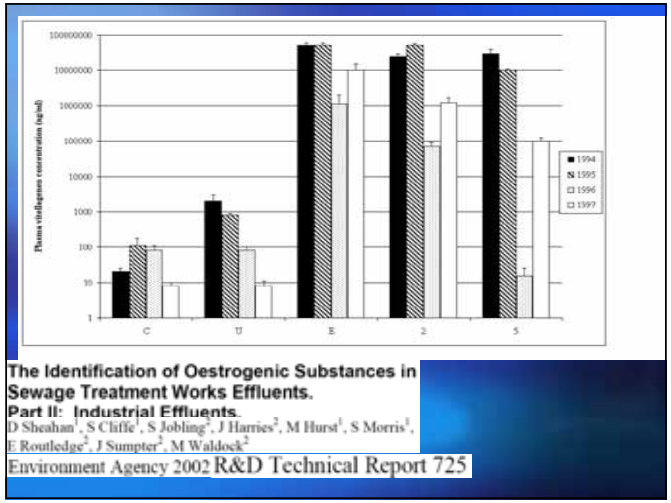
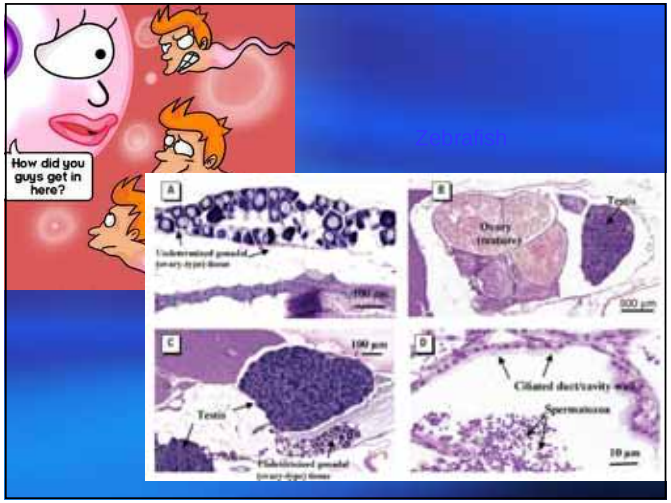


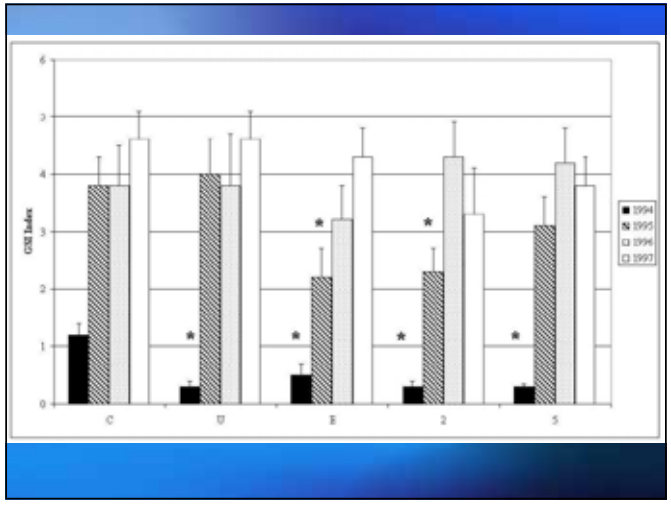
Figure 5a) Normal male testis, displaying spermatogonia (Sg), spermatocytes (Sc), St – spermatids (St) and spermatozoa (Sz).
Figure 5b) Normal female ovary in vitellogenesis, displaying primary oocytes (po), secondary oocytes (so) and CA – cortical alveolus stage oocytes.
Figure 5c) An intersex gonad containing primary oocytes (po) only (category A).
Figure 5d) A grossly intersex gonad containing both primary oocytes (po) and secondary oocytes (so), adjacent to testicular tissue (Tt), (Category C).











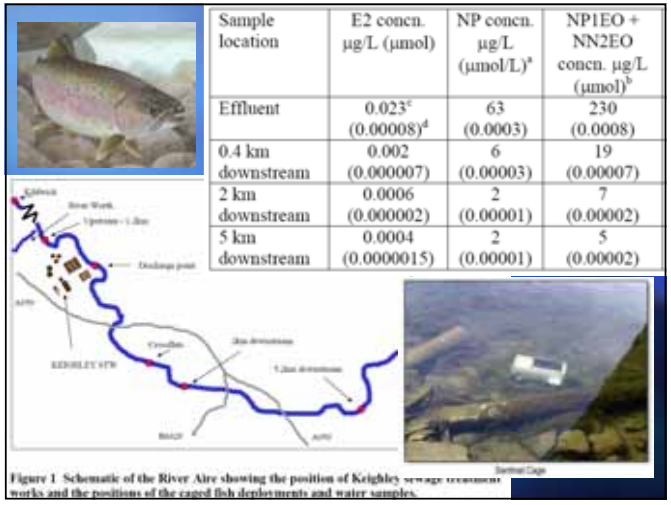


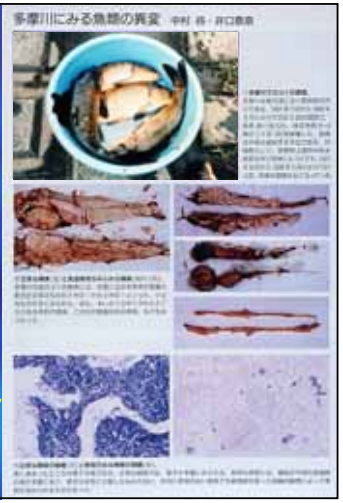
Figure 1 Schematic of the River Aire showing the position of Keighley sewage treatment works and the positions of the caged fish deployments and water samples.

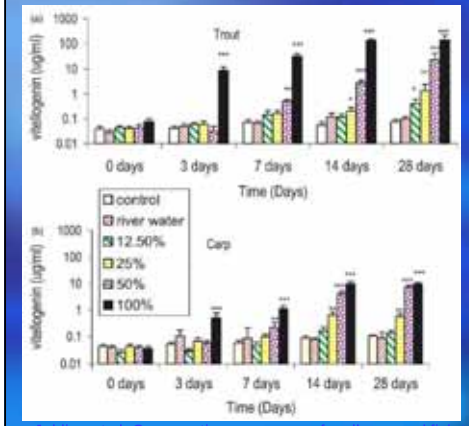
Studies related to VTG induction in fish

Test Species	sex	VTG level	References
carp (*C. carpio*)	♀ ♂	↗	Purdom *et al*., 1994
trout (*O. mykiss*)	♂	↗	Harries *et al*., 1996; 1997
roach (*R. rutilius*)	♂	↗	Rodger-Gray *et al*., 2000
roach (*R. rutilius*)	♂	↗	Sheahan *et al*., 2002
sunfish (*L. megalotis*)	♂	↗	Poter and Janz, 2003

White Sucker in Lake Superior, Canada,

Carps in Tama river, Japan,





Jobling et al.,Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquatic Toxicology 66 (2004) 207–222

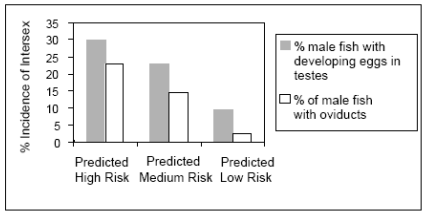


Figure 1. Incidence of the intersex condition in male roach taken from 57 sites in the national spatial fisheries survey (2002/2003) predicted to be at high, medium and low risk of oestrogenic effects.

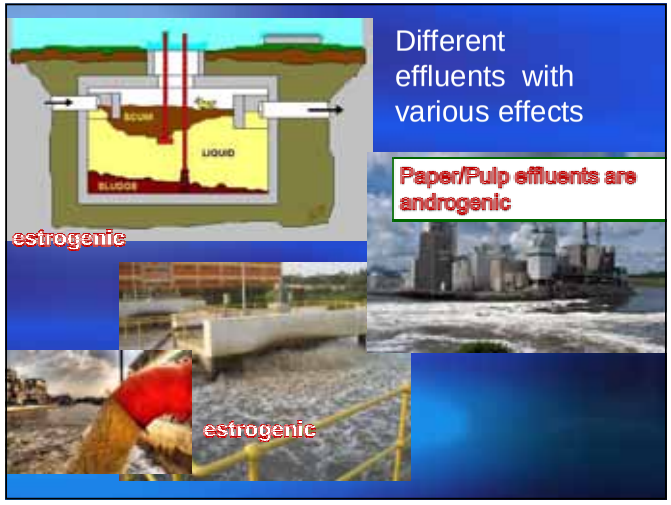
Causes and consequences of feminisation of male fish in English rivers, Science Report SC030285/SR Environment Agency July 2004

Alkylphenols in Effluents of STPs

Location	Concentration (μg/L)	
	NP	OP
Canada	0.8-15.1 (1.9)	0.12-1.7 (0.69)
UK	<0.2-5.4 (0.5)	
Switzerland	5-11	
Spain	6-343	
Japan	0.08-1.24	0.02-0.48
USA	0.171-37 (1.02)	<LOD-0.673 (0.072)
Germany	<LOD-0.77 (0.111)	<LOD-0.073 (0.014)
Italy	0.7-4 (1.8)	

EDCs in Sewage Treatment Plant (STP) Effluent

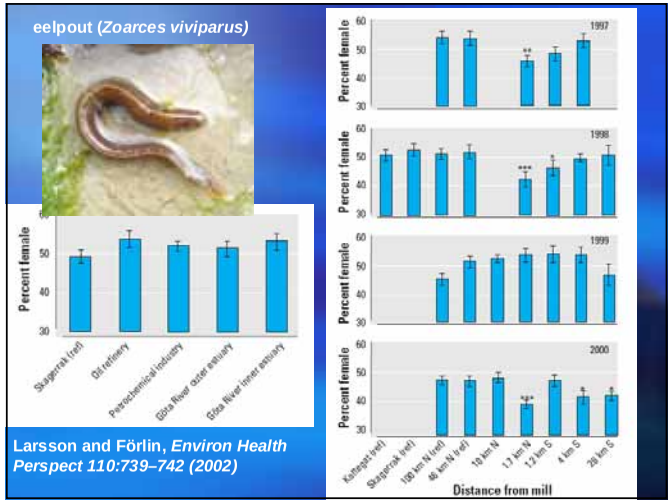
Compound	Concentration in Effluent	Reference
17β-estradiol	1-50 ng/L	Desbrow *et al.* 1998
Estrone	1.4-76 ng/L	Desbrow *et al.* 1998
17α-ethinylesradiol	0.2-7 ng/L	Desbrow *et al.* 1998
Testosterone	50 ng/L	Lim *et al.* 2000
Alkylphenol polyethoxylates	15-76 μg/L	Blackburn *et al.* 1999
Nonylphenol	<1-330 μg/L	Blackburn and Waldock 1995
Octylphenol	0.2 μg/L	Nasu *et al.* 2001
Bisphenol A	<1 μg/L	Rudel *et al.* 1998

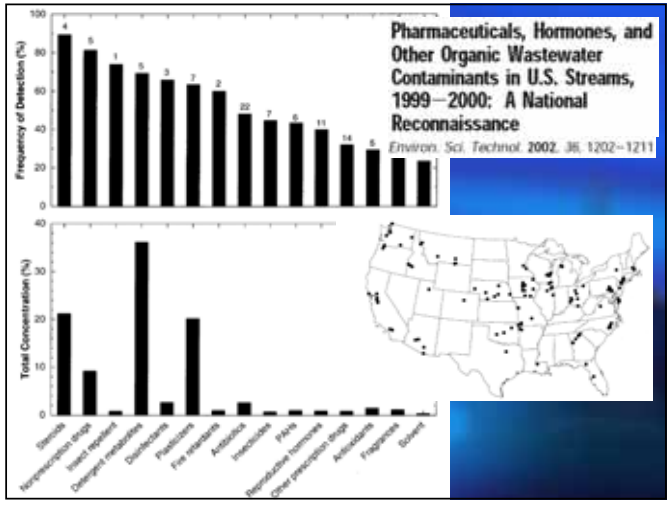


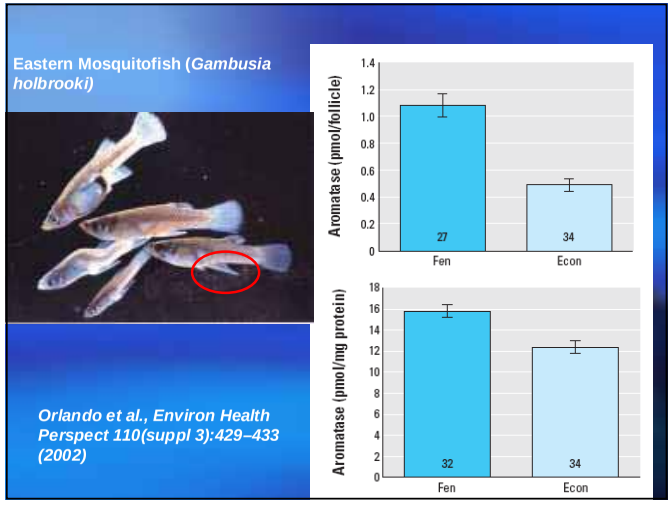
Hormones in Effluents of STPs

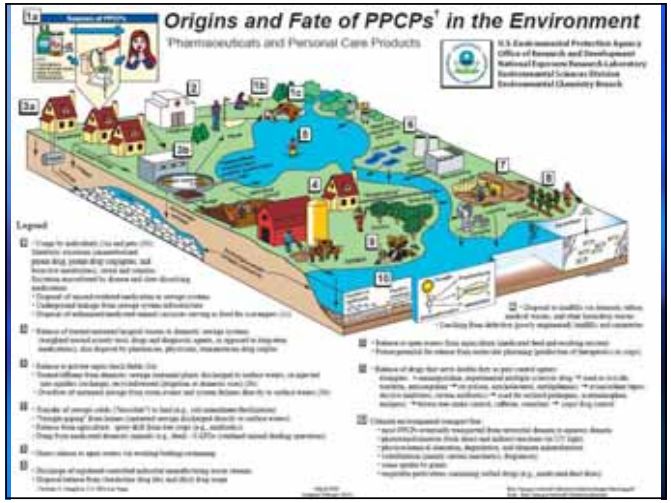
Location	Concentration (ng/L)	
	17β-estradiol (E2)	Ethinylestradiol (EE2)
Italy	0.44-3.3 (1.0)	<LOD-1.7 (0.45)
Netherlands	<0.1-5.0 (<LOD)	<0.2-7.5 (<LOD)
Germany	<LOD-3 (<LOD)	<LOD-15 (1)
Canada	<LOD-64 (6)	<LOD-42 (9)
UK	2.7-48 (6.9)	<LOD-7 (<LOD)
Japan	<LOD-55 (14)	
USA	0.477-3.66 (0.9)	<LOD-0.759 (0.248)
Germany	<0.15-5.2 (0.4)	<0.10-8.9 (0.7)

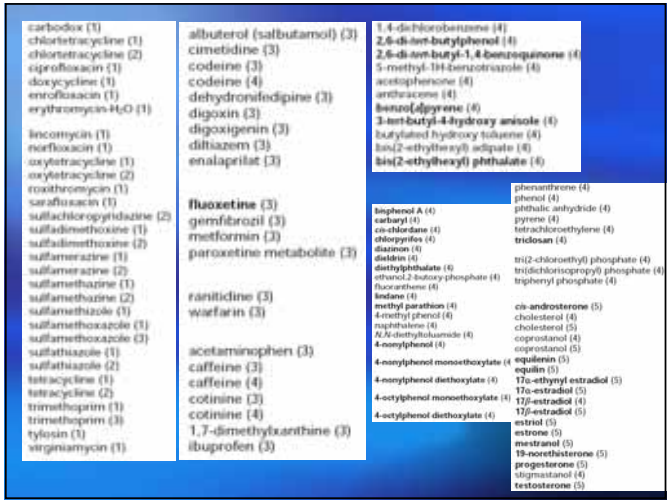

CAFOs, estrogenic and/or androgenic?
Pharmaceuticals
& Personal Care Products (PPCPs)
PPCPs need more studies

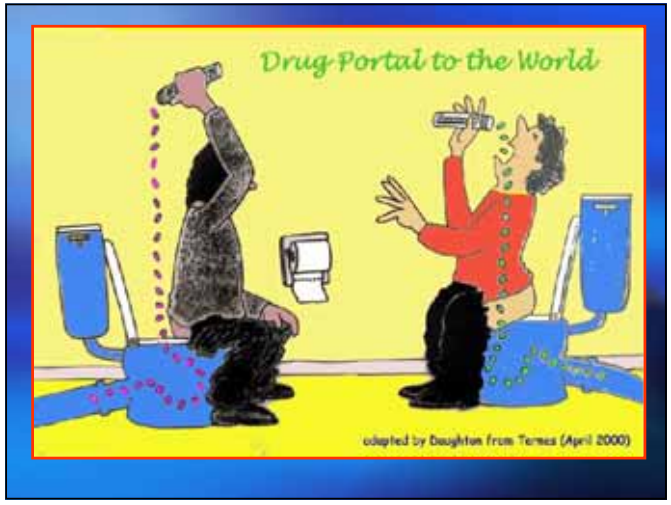

eelpout (Zoarces viviparus)
Percent female
Skagerrak (ref)
Oil refinery
Petrochemical industry
Gota River outer estuary
Gota River inner estuary
1997
1998
1999
2000
Kattegat (ref)
Skagerrak (ref)
100 km N (ref)
46 km N (ref)
18 km N
1.7 km S
1.2 km S
4 km S
28 km S
Distance from mill
Larsson and Förlin, Environ Health Perspect 110:739–742 (2002)

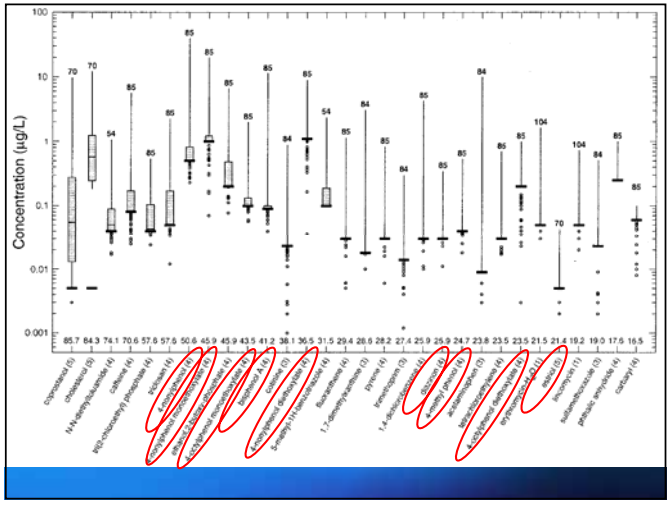

Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance
Environ. Sci. Technol. 2002, 36, 1202–1211
Frequency of Detection (%)
Total Concentration (%)
Sterols
Nonprescription drugs
Insect repellent
Detergent metabolites
Disinfectants
Plasticizers
Fire retardants
Antibiotics
Insecticides
Fibers
Reproductive hormones
Other prescription drugs
Antioxidants
Fragrances
Solvent


Eastern Mosquitofish (Gambusia holbrooki)
Aromatase (pmol/follicle)
Aromatase (pmol/mg protein)
27
34
32
34
Fen
Econ
Orlando et al., Environ Health Perspect 110(suppl 3):429–433 (2002)


Origins and Fate of PPCPs[1] in the Environment
Pharmaceuticals and Personal Care Products
U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Environmental Sciences Division
Environmental Chemistry Branch
Legend


carbodox (1)
chlortetracycline (1)
chlortetracycline (2)
ciprofloxacin (1)
doxycycline (1)
enrofloxacin (1)
erythromycin-H2O (1)
lincomycin (1)
norfloxacin (1)
oxytetracycline (1)
oxytetracycline (2)
roxithromycin (1)
sarafloxacin (1)
sulfachloropyridazine (2)
sulfadimethoxine (1)
sulfadimethoxine (2)
sulfamerazine (1)
sulfamerazine (2)
sulfamethazine (1)
sulfamethazine (2)
sulfamethizole (1)
sulfamethoxazole (1)
sulfamethoxazole (3)
sulfathiazole (1)
sulfathiazole (2)
tetracycline (1)
tetracycline (2)
trimethoprim (1)
trimethoprim (3)
tylosin (1)
virginiamycin (1)
albuterol (salbutamol) (3)
cimetidine (3)
codeine (3)
codeine (4)
dehydronifedipine (3)
digoxin (3)
digoxigenin (3)
diltiazem (3)
enalaprilat (3)
fluoxetine (3)
gemfibrozil (3)
metformin (3)
paroxetine metabolite (3)
ranitidine (3)
warfarin (3)
acetaminophen (3)
caffeine (3)
caffeine (4)
cotinine (3)
cotinine (4)
1,7-dimethylxanthine (3)
ibuprofen (3)
1,4-dichlorobenzene (4)
2,6-di-tert-butylphenol (4)
2,6-di-tert-butyl-1,4-benzoquinone (4)
5-methyl-1H-benzotriazole (4)
acetophenone (4)
anthracene (4)
benzo[a]pyrene (4)
3-tert-butyl-4-hydroxy anisole (4)
butylated hydroxy toluene (4)
bis(2-ethylhexyl) adipate (4)
bis(2-ethylhexyl) phthalate (4)
bisphenol A (4)
carbaryl (4)
cis-chlordane (4)
chlorpyrifos (4)
diazinon (4)
dieldrin (4)
diethylphthalate (4)
ethanol,2-butoxy-phosphate (4)
fluoranthene (4)
lindane (4)
methyl parathion (4)
4-methyl phenol (4)
naphthalene (4)
N,N-diethyltoluamide (4)
4-nonylphenol (4)
4-nonylphenol monoethoxylate (4)
4-nonylphenol diethoxylate (4)
4-octylphenol monoethoxylate (4)
4-octylphenol diethoxylate (4)
phenanthrene (4)
phenol (4)
phthalic anhydride (4)
pyrene (4)
tetrachloroethylene (4)
triclosan (4)
tri(2-chloroethyl) phosphate (4)
tri(dichlorisopropyl) phosphate (4)
triphenyl phosphate (4)
cis-androsterone (5)
cholesterol (4)
cholesterol (5)
coprostanol (4)
coprostanol (5)
equilenin (5)
equilin (5)
17α-ethynyl estradiol (5)
17α-estradiol (5)
17β-estradiol (4)
17β-estradiol (5)
estriol (5)
estrone (5)
mestranol (5)
19-norethisterone (5)
progesterone (5)
stigmastanol (4)
testosterone (5)


Drug Portal to the World
adapted by Daughton from Temes (April 2000)


Concentration (µg/L)
100
10
1
0.1
0.01
0.001
coprostanol (5)
cholesterol (5)
N,N-diethyltoluamide (4)
tri(2-chloroethyl) phosphate (4)
caffeine (4)
diazinon (4)
triclosan (4)
4-nonylphenol diethoxylate (4)
bisphenol A (4)
4-octylphenol diethoxylate (4)
4-nonylphenol monoethoxylate (4)
4-octylphenol monoethoxylate (4)
cotinine (3)
5-methyl-1H-benzotriazole (4)
fluoranthene (4)
1,7-dimethylxanthine (3)
pyrene (4)
ibuprofen (3)
1,4-dichlorobenzene (4)
cotinine (4)
phenol (4)
acetophenone (4)
tri(dichlorisopropyl) phosphate (4)
4-octylphenol diethoxylate (4)
triphenyl phosphate (4)
caffeine (3)
isophorone (1)
sulfamethoxazole (3)
gallaxolide (4)
carbaryl (4)

Toxic effects by chemicals on cormorants

USA (Great Lakes)
- ➢ Embryo mortality
- ➢ Edema
- ➢ Deformity
- ➢ Abnormal reproduction
- ➢ Immune toxicity
- ➢ Eggshell thickness (DDE)

The Netherlands (Region in Rhine river)
- ➢Reduction of thyroid hormone

Japan (Tokyo Bay)
- ➢Lack of right eye, cross-bill
- ➢Deformity the spinal cord of embryo
- ➢Thyroid hyperplasia

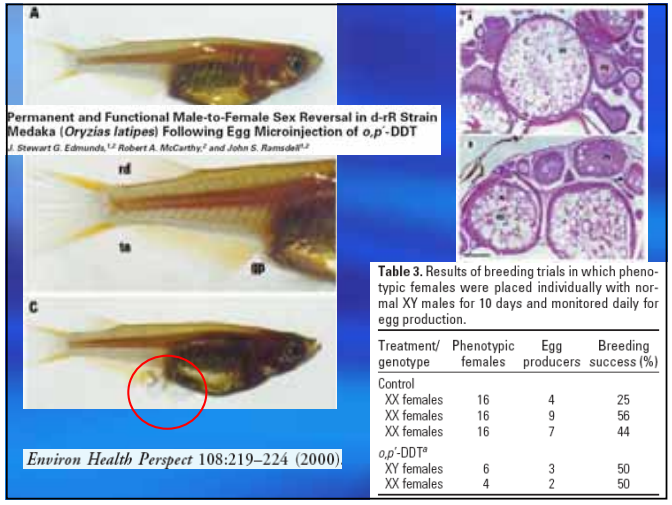
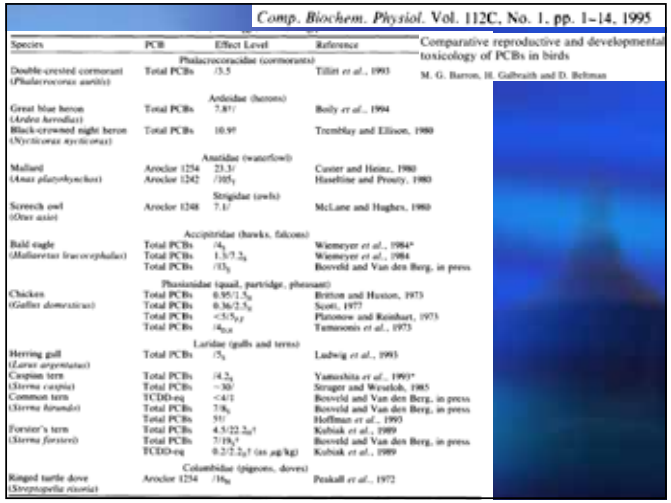


Table 3. Results of breeding trials in which phenotypic females were placed individually with normal XY males for 10 days and monitored daily for egg production.

Treatment/ genotype	Phenotypic females	Egg producers	Breeding success (%)
Control			
XX females	16	4	25
XX females	16	9	56
XX females	16	7	44
o,p'-DDT[a]			
XY females	6	3	50
XX females	4	2	50



Comp. Biochem. Physiol. Vol. 112C, No. 1, pp. 1–14, 1995

Comparative reproductive and developmental toxicology of PCBs in birds

M. G. Barron, H. Galbraith and D. Beltman

Species	PCB	Effect Level	Reference
Phalacrocoracidae (cormorants)			
Double-crested cormorant (*Phalacrocorax auritus*)	Total PCBs	/3.5	Tillitt *et al.*, 1993
Ardeidae (herons)			
Great blue heron (*Ardea herodias*)	Total PCBs	7.8/	Body *et al.*, 1994
Black-crowned night heron (*Nycticorax nycticorax*)	Total PCBs	10.9/	Tremblay and Ellison, 1980
Anatidae (waterfowl)			
Mallard	Aroclor 1254	23.3/	Custer and Heinz, 1980
(*Anas platyrhynchos*)	Aroclor 1242	/105	Haseltine and Prouty, 1980
Strigidae (owls)			
Screech owl (*Otus asio*)	Aroclor 1248	7.1/	McLane and Hughes, 1980
Accipitridae (hawks, falcons)			
Bald eagle	Total PCBs	/4	Wiemeyer *et al.*, 1984*
(*Haliaeetus leucocephalus*)	Total PCBs	1.3/7.2	Wiemeyer *et al.*, 1984
	Total PCBs	/13	Bosveld and Van den Berg, in press
Phasianidae (quail, partridge, pheasant)			
Chicken	Total PCBs	0.95/1.5	Britton and Huston, 1973
(*Gallus domesticus*)	Total PCBs	0.36/2.5	Scott, 1977
	Total PCBs	<5/5	Platonow and Reinhart, 1973
	Total PCBs	/4	Tamasonis *et al.*, 1973
Laridae (gulls and terns)			
Herring gull (*Larus argentatus*)	Total PCBs	/5	Ludwig *et al.*, 1993
Caspian tern	Total PCBs	/4.2	Yamashita *et al.*, 1993*
(*Sterna caspia*)	Total PCBs	~30/	Struger and Weseloh, 1985
Common tern	TCDD-eq	<4/1	Bosveld and Van den Berg, in press
(*Sterna hirundo*)	Total PCBs	7/8	Bosveld and Van den Berg, in press
	Total PCBs	5/1	Hoffman *et al.*, 1990
Forster's tern	Total PCBs	4.5/22.2	Kubiak *et al.*, 1989
(*Sterna forsteri*)	Total PCBs	7/19	Bosveld and Van den Berg, in press
	TCDD-eq	0.2/2.2 (in μg/kg)	Kubiak *et al.*, 1989
Columbidae (pigeons, doves)			
Ringed turtle dove (*Streptopelia risoria*)	Aroclor 1254	/16	Peakall *et al.*, 1972

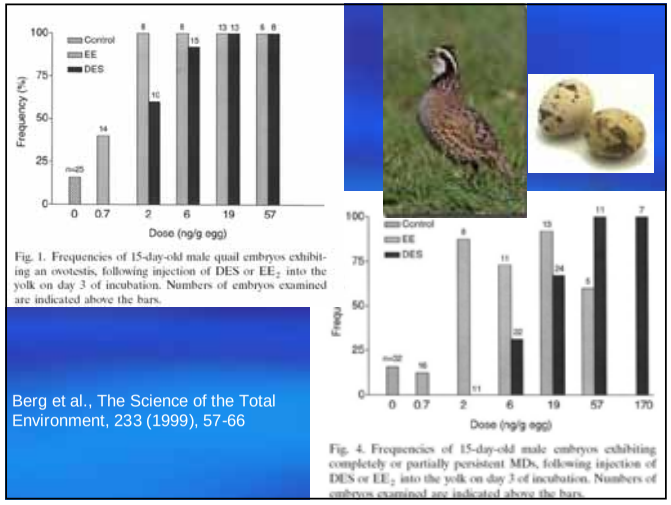
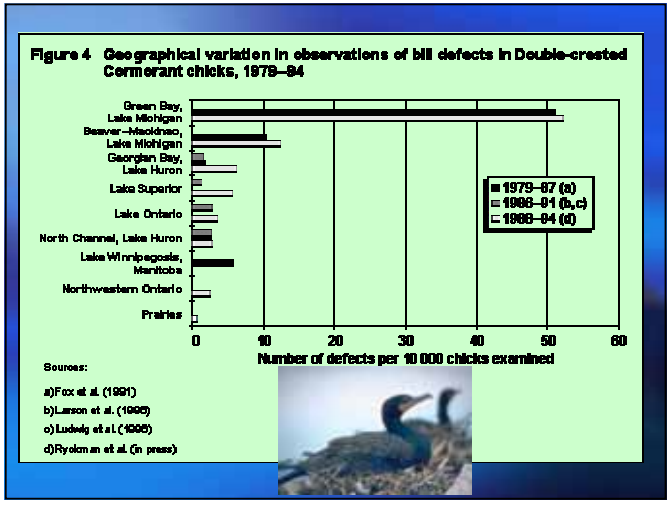
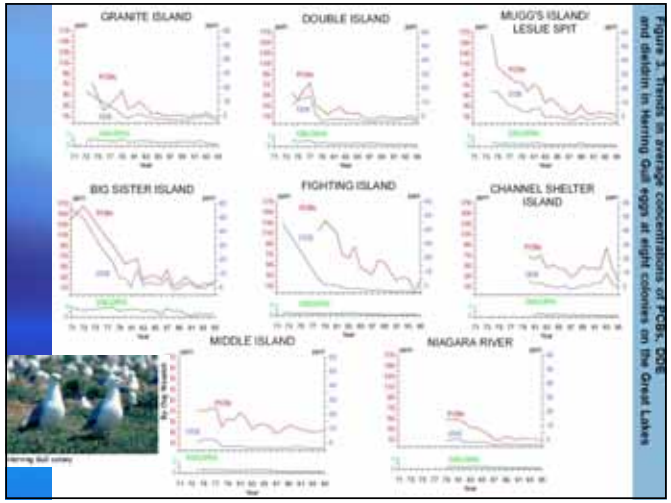


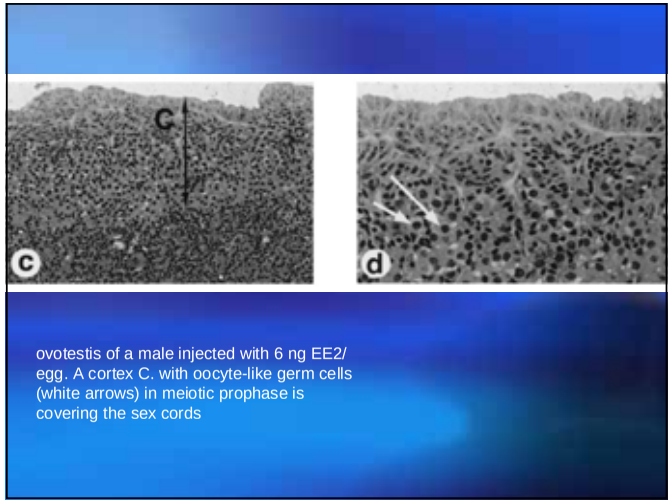
Fig. 1. Frequencies of 15-day-old male quail embryos exhibiting an ovotestis, following injection of DES or EE_2 into the yolk on day 3 of incubation. Numbers of embryos examined are indicated above the bars.

Berg et al., The Science of the Total Environment, 233 (1999), 57-66

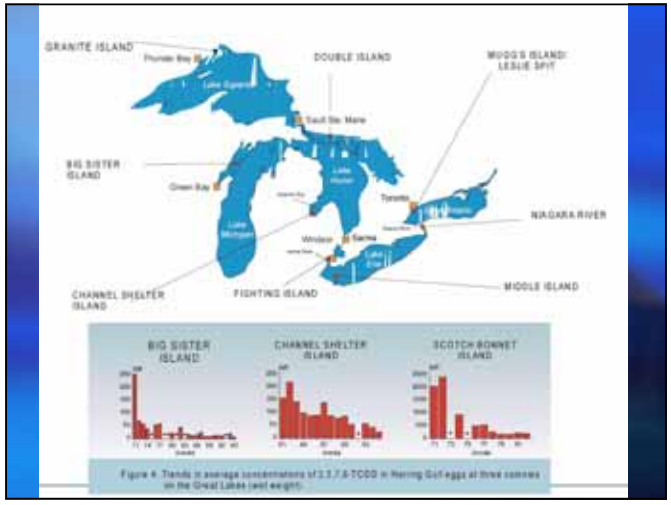
Fig. 4. Frequencies of 15-day-old male embryos exhibiting completely or partially persistent MDs, following injection of DES or EE_2 into the yolk on day 3 of incubation. Numbers of embryos examined are indicated above the bars.

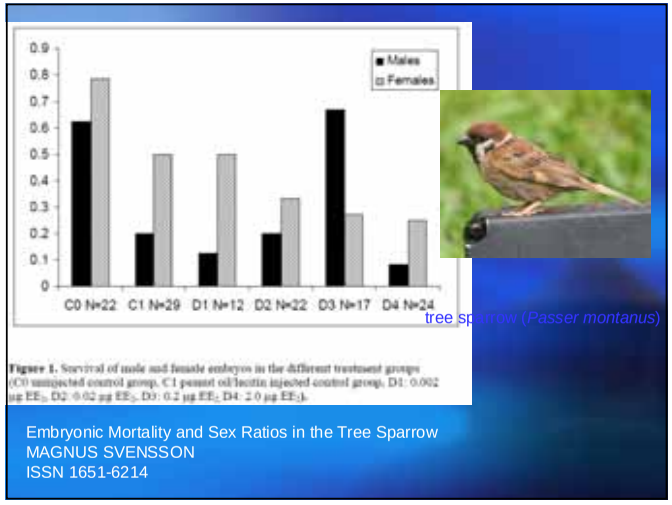






ovotestis of a male injected with 6 ng EE2/ egg. A cortex C. with oocyte-like germ cells (white arrows) in meiotic prophase is covering the sex cords





tree sparrow (*Passer montanus*)

Figure 1. Survival of male and female embryos in the different treatment groups (C0 uninjected control group, C1 peanut oil/lecithin injected control group, D1: 0.002 μg EE_2, D2: 0.02 μg EE_2, D3: 0.2 μg EE_2, D4: 2.0 μg EE_2).

Embryonic Mortality and Sex Ratios in the Tree Sparrow
MAGNUS SVENSSON
ISSN 1651-6214

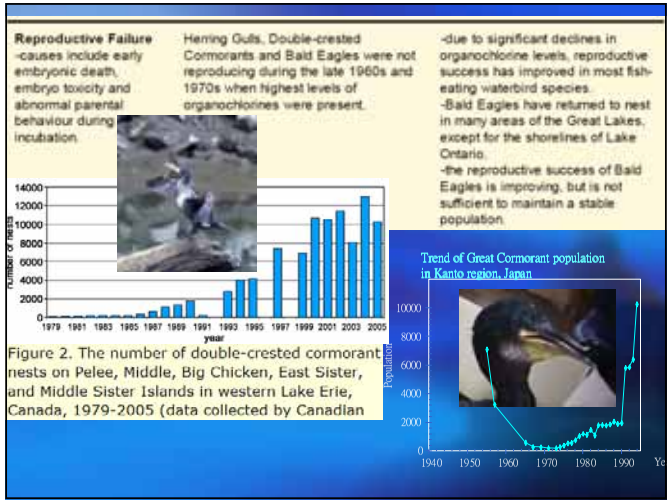
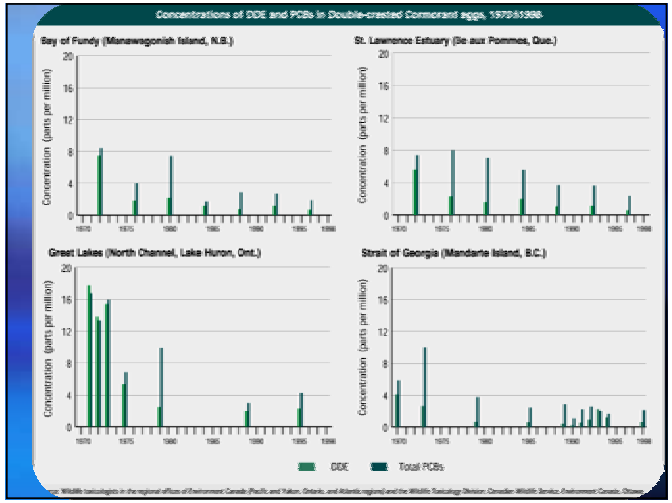


Figure 2. The number of double-crested cormorant nests on Pelee, Middle, Big Chicken, East Sister, and Middle Sister Islands in western Lake Erie, Canada, 1979-2005 (data collected by Canadian



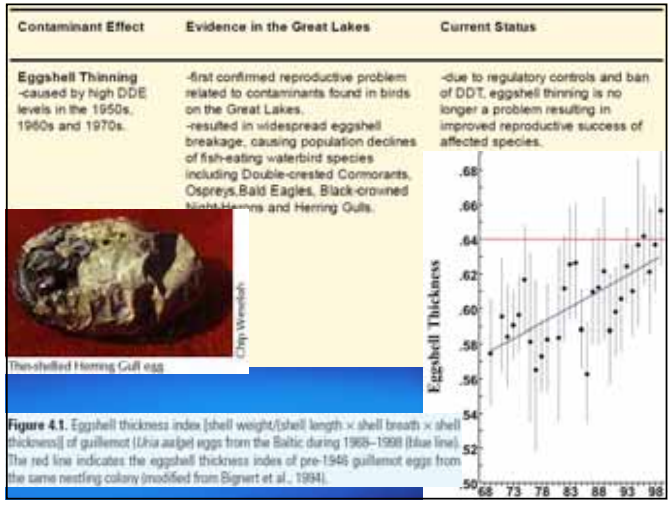


Figure 4.1. Eggshell thickness index [shell weight/(shell length × shell breath × shell thickness)] of guillemot (*Uria aalge*) eggs from the Baltic during 1968–1988 (blue line). The red line indicates the eggshell thickness index of pre-1946 guillemot eggs from the same nesting colony (modified from Bignert et al., 1994).

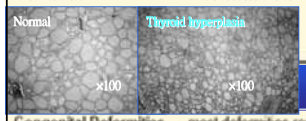
TABLE 2

VARIATION IN CALCULATED PREVALENCE OF FIVE- AND SIX-EGG RING-BILLED GULL CLUTCHES IN COLONIES WITH PORTION OF RANGE, GROWTH STATUS, SIZE, AND TIME

Portion of range	Colony location	Growth	Year	Size	Prevalence per 1000 nests	Source[a]
Long established	Miquelon Lake, Alta.	STBL[b]	1965	435	2.3	(1)
	Lozinsky's Slough, Sask.	STBL	1958	1200	1.7	(2)
	Bachelor's Is., Man.	STBL	1979	1596	5.6	(3)
	Rogers City, L. Huron	STBL	1979	8000	3.0	(4)
	Mohawk Is., L. Erie	DECN[c]	1972	1000	5.7	(5)
	Muggs Is., L. Ont.	INCR[c]	1972	2375	19.0	(5)
		SAT'D[d]	1980	6069	0.5	(6)
	Gull Island, L. Ont.	SAT'D	1980	27,500	0.8	(7)
	Ile de la Couvee, St. Lawrence R.	INCR	1978	11,000	24.1	(8)
	Eastern Headland, L. Ont.	INCR	1980	68,000	1.0	(6)
Expansion	Honey Lake, Calif.	INCR	1941	75	26.7	(9)
		INCR	1953	717	34.9	(10)
	Banks Lake, Wash.	INCR	1978	2700	16.1	(11)
	Sprague Lake, Wash.	INCR	1978	850	18.0	(11)
	Potholes Reservoir, Wash.	DECN	1978	11,000	1.9	(11)
	Okanagan Lake, B.C.	INCR	1972	98	122.4	(12)
	Vrooman Is., L. Superior	INCR	1979	167	305.4	(13)
	Gravel Is., L. Superior	SAT'D	1979	378	5.3	(14)
	Granite Is., L. Superior	INCR	1977	1600	121.0	(15)
		INCR	1979	2400	41.0	(16)
		INCR	1980	2699	28.0	(16)

Fox AND Boersma, Wilson Bull., 95(4), 1983, pp. 552-555

Contaminant Effect	Evidence in the Great Lakes	Current Status
Enlarged Thyroid -linked to exposure to certain contaminants (e.g. PCBs, DDE, dieldrin).	-most prevalent in Herring Gulls from contaminated sites including Saginaw Bay (Lake Huron), Green Bay (Lake Michigan), western Lake Erie and Lake Ontario.	-in response to decreased contaminant levels in the Great Lakes aquatic food chain, the severity of enlarged thyroid has decreased in Herring Gull populations.
Congenital Deformities	-most deformities reported in the early to mid-1970s from contaminated sites on Lake Ontario and in the 1980s on Lake Michigan. -crossed bills, jaw defects, extra limbs, and malformed feet, joints and eyes were found in Herring Gulls and at least eight other species of fish-eating waterbirds.	-waterbirds continue to display higher rates of deformities (e.g. bill defects in Bald Eagles) compared to clean sites outside of the Basin. -greatest incidence in areas of high contamination such as Green Bay (Lake Michigan) and Saginaw Bay (Lake Huron). -studies continue on the links between contaminants and developmental problems in certain waterbird species.



Double-crested Cormorant with a deformed bill
Chip Weseloh

Abnormal courtship and nursing behaviors

Herring gulls (*Larus argentatus*) from Lake Ontario include aberrant parental behavior involving a failure to sit on eggs or defend nests (Fox et al., 1978)

Ring doves (*Streptopelia risoria*) fed a mixture of DDE, PCBs, mirex, and photomirex failed to respond to male courtship behaviors in the normal fashion; pairs receiving the highest dosage spent less time feeding their young. (McArthur et al., 1983)

The administration of a PCB mixture to adult breeder doves resulted in aberrant incubation and courtship behaviors. (Peakall and Peakall, 1973 ; Tori and Peterle, 1983)

Alterations in Behavior: Sex ratio skew and Supernormal clutch

- **Adult western gull population on Santa Barbara Island between 1972 and 1978, the sex ratio was estimated to be 0.67 males/female. Female-female pairing was 15%.(Hunt et al. *Auk* 97, 473–479, 1980).**

- **A supernormal clutch incidence of 0.6 to 1 % was observed in herring gulls inhabiting northeastern Lake Michigan during the period from1978 to 1981. (Shugart, et al. *Condor* 90, 933–935, 1988; Fitch and Shugart. *Behav. Ecol. Sociobiol.* 14, 1–7, 1983)**

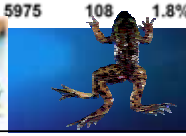
Super normal clutch size

- **Ring-billed Gulls (*Larus delawarensis*) on Granite Island, northern Lake Superior. In 1979 the colony had 2,400 nesting pairs, with a total of 99 nests containing 5-7 eggs. In 1980 the colony had increased in size to 2,600 nests and contained a total of 71 superclutches. Hatching success for superclutches was 34% in 1979 and 30% in 1980, whereas for normal-sized clutches it was 77% in 1979 and 61% in 1980. (**Conover et al. 1983)**

- **The occurrence of super-normal clutches was 1.8%, 4.1% and 0.3% in the Caspian Gull, Mediterranean Gull and Whiskered Tern in Poland. (**Betleja et al. **Waterbirds** Volume 30(4)624-629, 2007)

Malformed Frogs 1998 - 2001

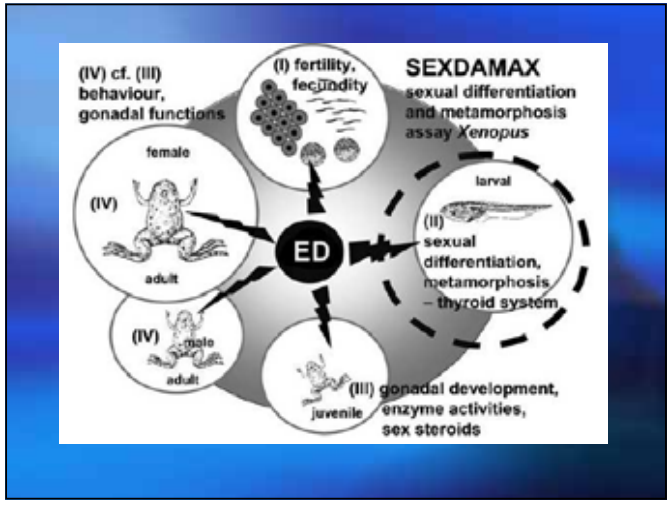
Species	N	Malf	Prev. (%)
Rana pipiens	3226	46	1.4
Hyla spp.	988	22	2.2
R. clamitans	768	14	1.8
R. sylvatica	433	13	2.9
Bufo americanus	242	5	2.1
R. septentrionalis	144	4	2.8
Pseudacris crucifer	119	4	3.4
Pseudacris triseriata	32	0	0
R. catsbeiana	1	0	0
unknown	12	0	0
Total	**5975**	**108**	**1.8%**

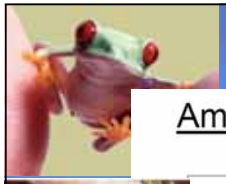


Problems remain, just shifting of the situation

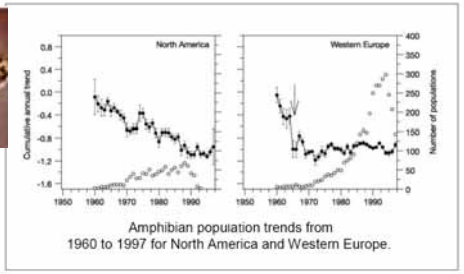
Table 1. MOST SENSITIVE EFFECTS OF 2,3,7,8-TCDD IN ANIMALS

Effect	Species	Exposure (LOEL or LOAEL)	Maternal body burden (increment to background)*
Adverse effects			
Developmental effects - neurotoxicity (object learning)	Rhesus monkey	~160 pg/kg/d	42 ng/kg**
Reproductive toxicity - decreased sperm count - vaginal threads	rat	64,000 pg/kg*** 200,000 pg/kg***	28 ng/kg 73 ng/kg
Immunotoxicity	rat	100,000 pg/kg***	50 ng/kg
Immunological (viral sensitivity)	mouse	10,000 pg/kg***	10 ng/kg
Hormonal (endometriosis)	Rhesus monkey	~160 pg/kg/d	42 ng/kg**





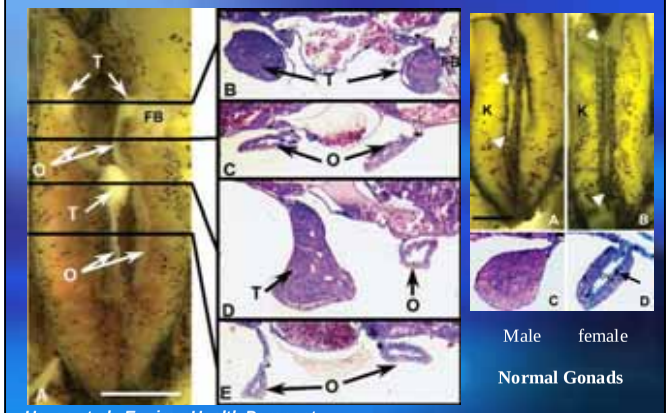
Amphibian Population Declines



Amphibian population trends from
1960 to 1997 for North America and Western Europe.

Houlahan et al., 2000, Nature 404:752

A mixed hermaphrodite at NF stage 66, treated with 0.1 ppb atrazine.



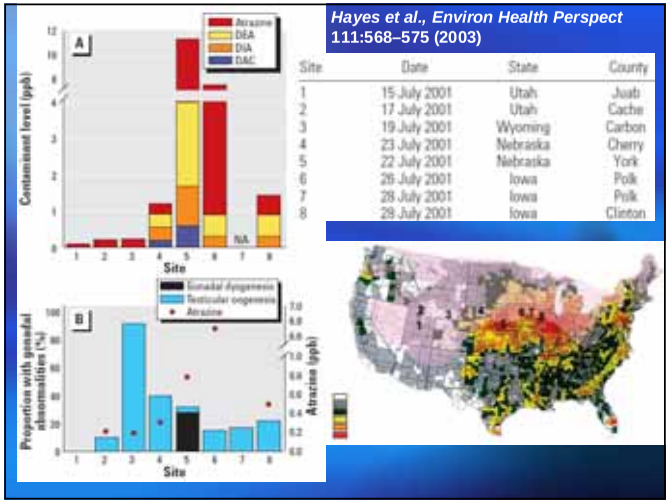
Male female

Normal Gonads

Hayes et al., Environ Health Perspect
114(suppl 1):134–141 (2006).

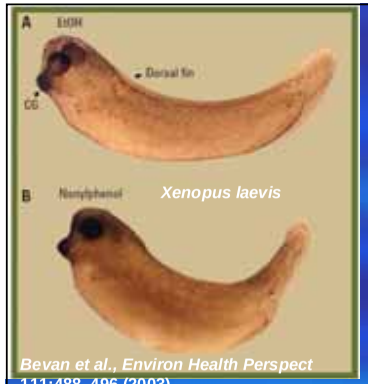
African clawed frogs (*Xenopus laevis*)

Hayes et al., *Environ Health Perspect*
114(suppl 1):40–50 (2006).



Hayes et al., Environ Health Perspect
111:568–575 (2003)

Site	Date	State	County
1	15 July 2001	Utah	Juab
2	17 July 2001	Utah	Cache
3	19 July 2001	Wyoming	Carbon
4	23 July 2001	Nebraska	Cherry
5	22 July 2001	Nebraska	York
6	26 July 2001	Iowa	Polk
7	28 July 2001	Iowa	Polk
8	29 July 2001	Iowa	Clinton

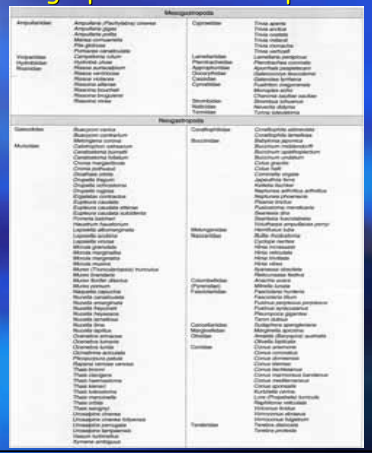


Bevan et al., Environ Health Perspect
111:488–496 (2003).

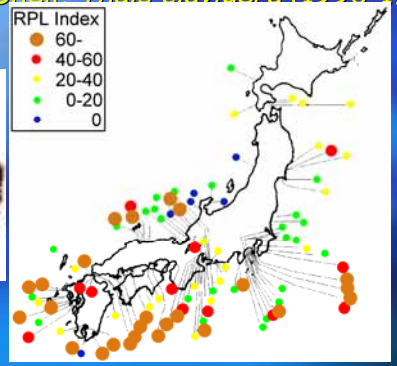
Figure 5. Representative photomicrographs of the dorsal aspect of the trunk of two stage 37 tadpoles. (*A*) Embryo maintained in saline demonstrating the presence of the dorsal fin populated by small melanocytes and larger dendritic melanocytes along the surface of the spinal cord. (*B*) Embryo treated with 1 μM methoxychlor demonstrating a thin, poorly developed dorsal fin devoid of melanocytes, spotty melanocytes atop the spinal cord, crooked spine, and poorly defined somites. (Both embryos had also been processed for TUNEL staining.) Magnification 100×.

Imposex-Exhibiting Species of Gastropods

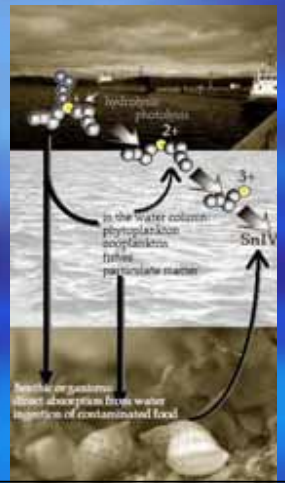
- Over 140 species of gastropods have been observed to be affected by imposex (up to December 1998) in the world.
- In Japan, 39 species of snails show imposex.

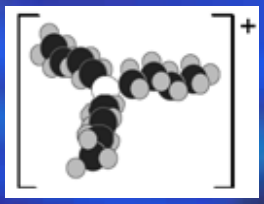


Geographical Distribution of Imposex in the Rock Shell *Thais clavigera* (1996-1999)

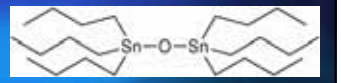


Relative Penis Length Index（RPL Index）
September 1996～January 1999

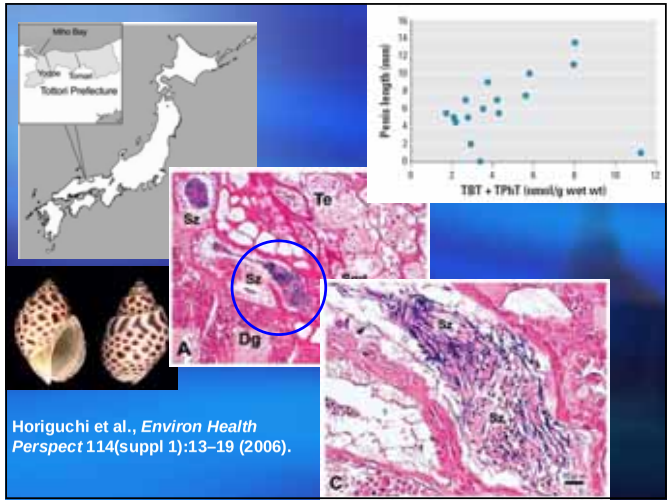




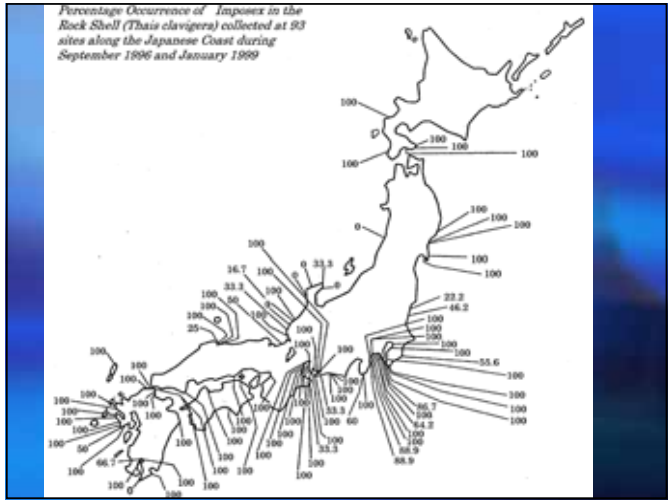
有機錫

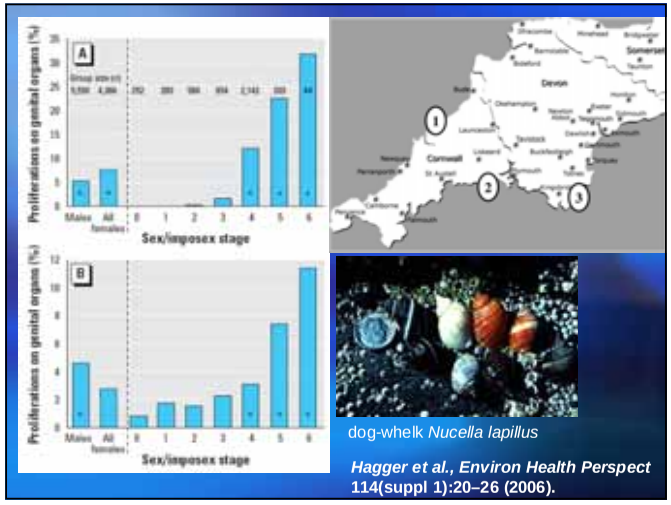


Tributyltin oxide



Horiguchi et al., *Environ Health Perspect* 114(suppl 1):13–19 (2006).

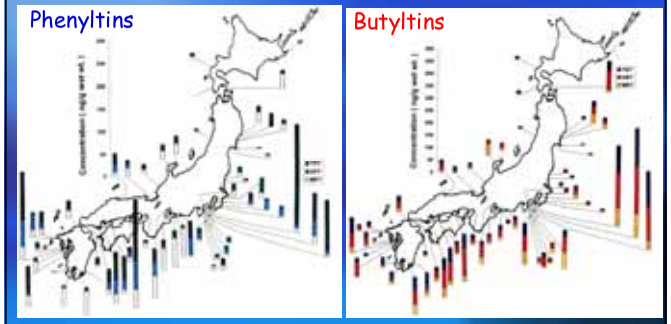




dog-whelk *Nucella lapillus*

***Hagger et al., Environ Health Perspect* 114(suppl 1):20–26 (2006).**

Contamination by Orgaotin Compounds in the Rock Shell, *Thais clavigera* (1996-1999)



Organotin concentrations in tissue (September 1996～January 1999)

Site	Date	Sex	Imposex[b] (%)
Shiangsan	Aug. 1998	Male	
		Female	67.1
	Jan. 1999	Male	
		Female	100
Lukang	Aug. 1998	Male	
		Female	59.3
	Jan. 1999	Male	
		Female	100
Chiku	Aug. 1998	Male	
		Female	36.7
	Jan. 1999	Male	
		Female	80.0

Hung et al., Environmental Pollution 112 (2001) 145~152

The Rock Shell, *Thais clavigera* (Muricidae: Neogastropoda)

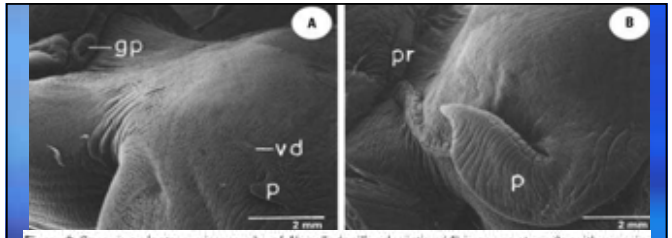
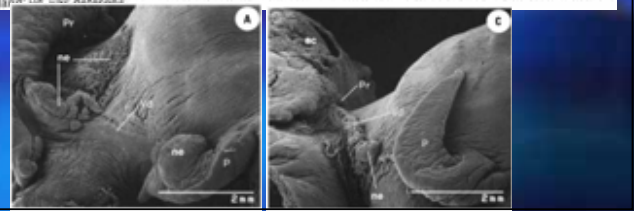
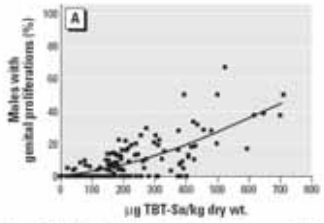


Figure 2. Scanning electron micrographs of *Nucella lapillus* depicting (*A*) imposex stage 3a with a penis and an anterior section of the vas deferens and (*B*) imposex stage 5a with a fully developed penis, vas deferens, and a prostate gland supplanting the vagina. Abbreviations: gp, genital papilla; p, penis; pr, prostate gland; vd, vas deferens.



Distributions of organotin compounds (ng/g, dry weight) in *Thais clavigera* collected from the northern (SS), central (LK) and southern (CK) oysters mariculture areas in Taiwan in August 1998 to January 1999[a]

	August 1998			November 1998			January 1999		
	SS (*n* = 27)	LK (*n* = 27)	CK (*n* = 27)	SS (*n* = 27)	LK (*n* = 27)	CK (*n* = 27)	SS (*n* = 27)	LK (*n* = 27)	CK (*n* = 27)
MBT	ND (ND)	39.9±12.9 (24.3–60.1)	ND (ND)	ND (ND)	ND (ND)	ND (ND)	50.2±5.2 (ND–59.4)	50.4±20.6 (ND–80.4)	36.0±3.9 (ND–39.4)
DBT	ND (ND)	76.5±16.9 (51.2–96.7)	ND (ND)	32.4±6.3 (ND–32.6)	ND (ND)	ND (ND)	84.4±7.6 (74.2–97.1)	31.4±3.6 (ND–35.4)	41.6±2.8 (ND–43.7)
TBT	321±40 (268–381)	285±20 (264–313)	117±27 (91.8–155)	714±39 (671–768)	415±31 (382–457)	106±16 (84–128)	1816±143 (1608–1949)	680±84 (605–795)	172±28 (140–216)
MPhT	ND (ND)	53.7±22.3 (ND–75.9)	ND (ND)	39.4±8.4 (ND–49.7)	66.3±4.9 (ND–73.4)	ND (ND)	ND (ND)	33.1±2.1 (ND–35.4)	ND (ND)
DPhT	29.2±5.4 (ND–35.1)	81.6±17.5 (64.2–108)	ND (ND)	ND (ND)	77.8±29.9 (42.6–116)	26.8±2.3 (ND–29.1)	ND (ND)	ND (ND)	ND (ND)
TPhT	244±37 (197–286)	435±41 (385–486)	70.9±16.9 (ND–88.2)	88.3±43.2 (29.7–137)	173±35 (125–207)	40.7±5.4 (ND–44.8)	34.9±3.1 (ND–37.8)	58.1±1.3 (ND–59.2)	ND (ND)
ΣBTs	321±40	402±50	117±27	746±39	415±31	106±16	1951±156	770±109	249±35
ΣPhTs	273±42	570±81	70±17	145±52	302±89	67±8	35±3	91±3	ND



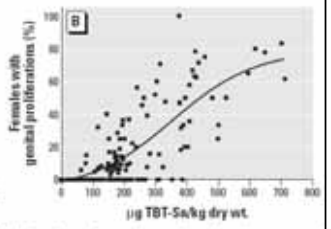
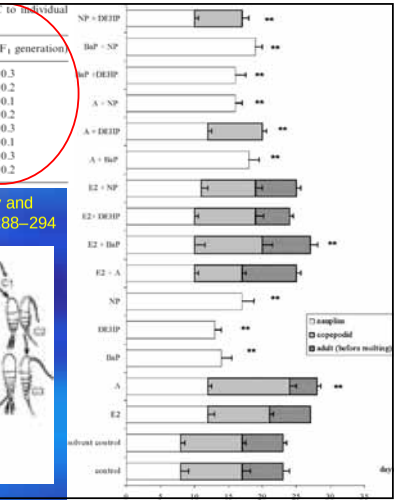


Figure 7. *Nucella lapillus*. Relationship between TBT body burden and prevalence of proliferations on genital organs such as penis and vas deferens in males (*A*) and females (*B*). Values for 149 samples analyzed with 2,945 males and 2,321 females are shown, including calculated regressions: (*A*) $y = 6.82 + (77.7 + 6.82) / (1 + 10^{(601 - x) \times 0.0019})$; $r = 0.745$; $p < 0.0005$. (*B*) $y = 6.85 + (79.0 + 6.85) / (1 + 10^{(338 - x) \times 0.0032})$; $r = 0.788$; $p < 0.0005$.

Sex ratio in *E. affinis* exposed for 30 days at 18 ± 1 °C to individual contaminants or binary mixtures of compounds

Treatment	F:M (F_0 generation)	F:M (F_1 generation)
Control	1.00 ± 0.2	1.0 ± 0.3
Solvent control	1.00 ± 0.1	1.0 ± 0.2
E2	1.00 ± 0.2	1.25 ± 0.1
A	0.96 ± 0.3	1.11 ± 0.2
E2 + A	1.02 ± 0.1	1.28 ± 0.3
E2 + BaP	1.02 ± 0.2	1.22 ± 0.1
E2 + DEHP	1.00 ± 0.2	1.43 ± 0.3
E2 + NP	1.00 ± 0.3	1.43 ± 0.2



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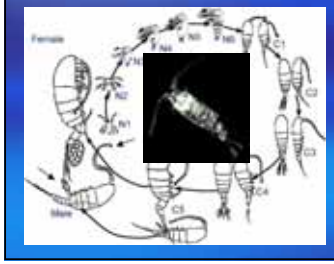

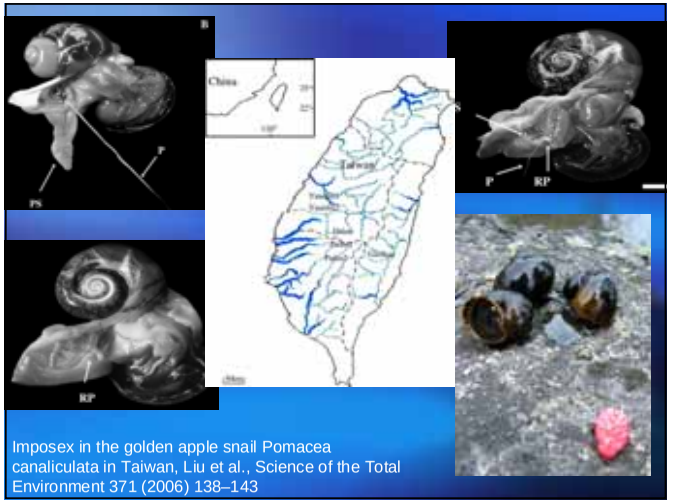

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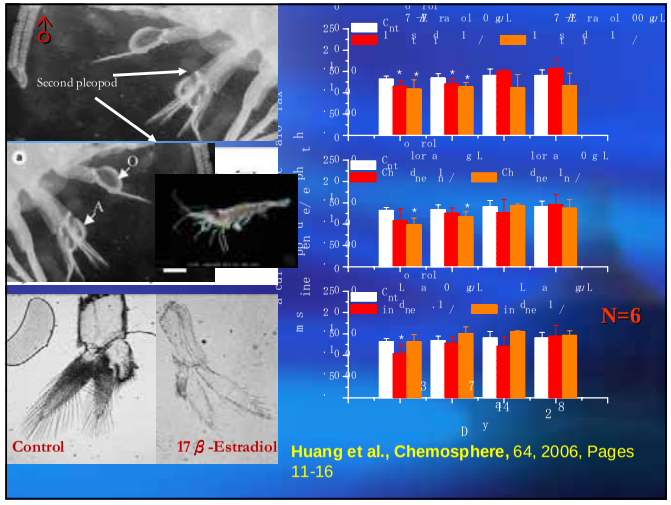
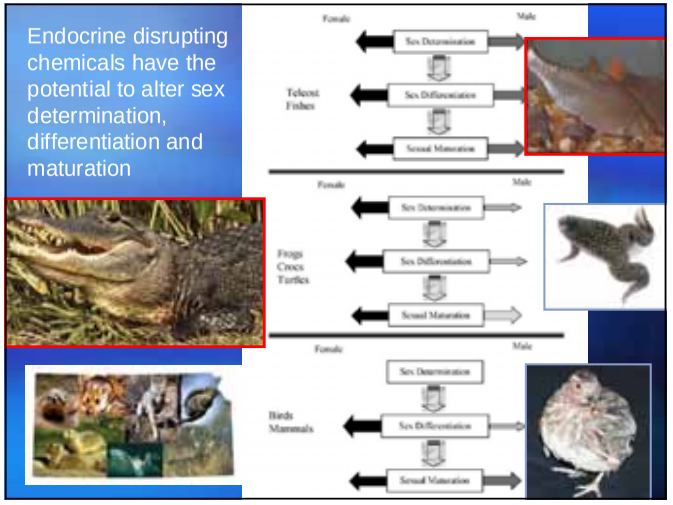
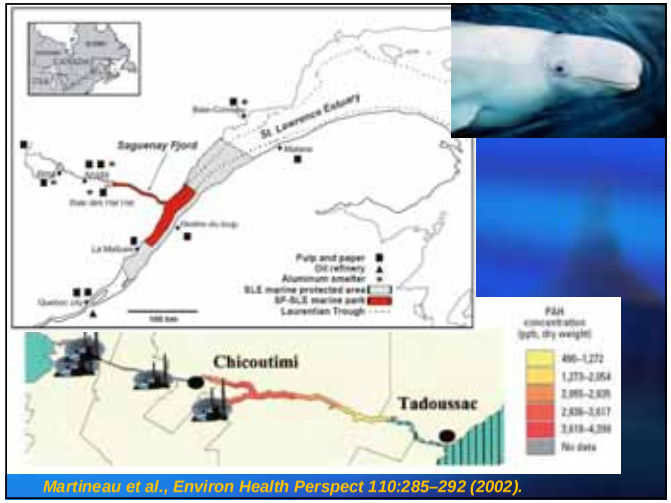


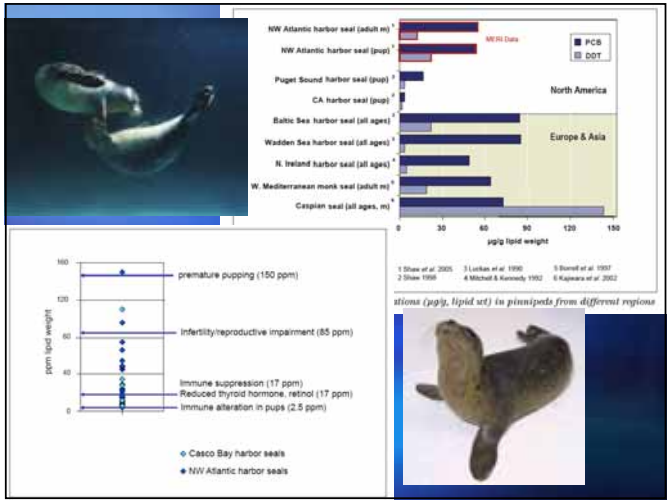
Huang et al., Chemosphere, 64, 2006, Pages 11-16

Endocrine disrupting chemicals have the potential to alter sex determination, differentiation and maturation





Martineau et al., Environ Health Perspect 110:285–292 (2002).



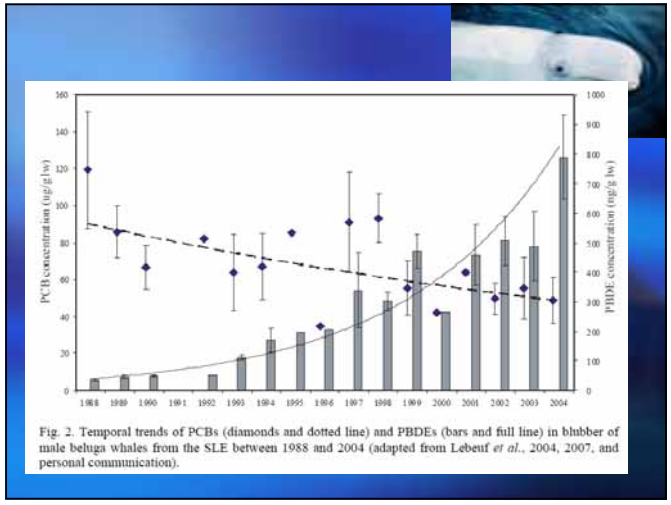
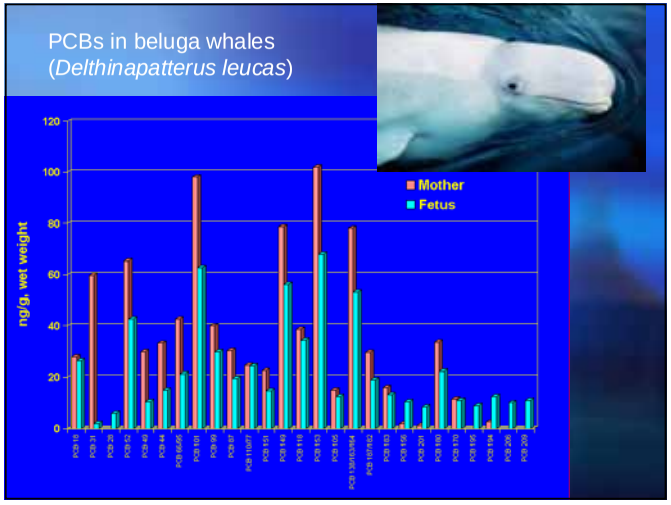


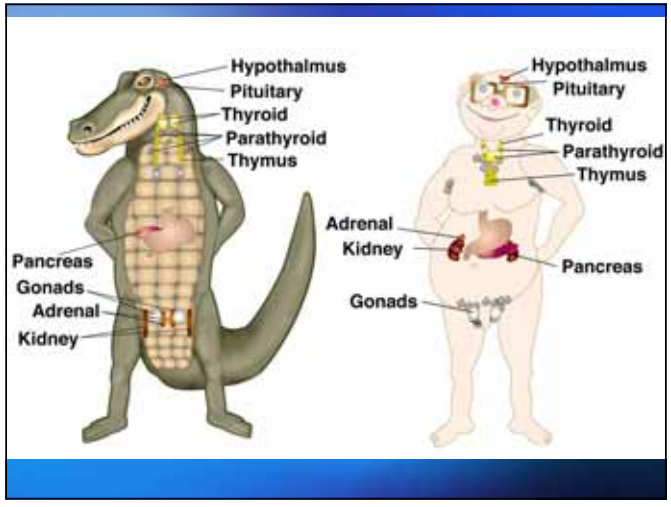
Fig. 2. Temporal trends of PCBs (diamonds and dotted line) and PBDEs (bars and full line) in blubber of male beluga whales from the SLE between 1988 and 2004 (adapted from Lebeuf *et al.*, 2004, 2007, and personal communication).



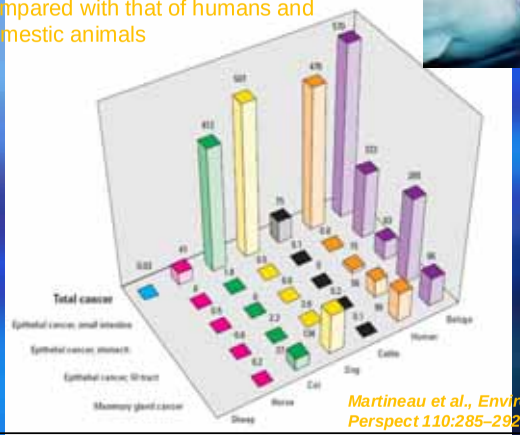
污染物的存在→ → → → → → → →效應

外在影響因素

對人體的影響雖不明確，但往往"始料未及"卻爲時已晚。



Adjusted annual rate (cases per 100,000) of cancer in the SLE beluga compared with that of humans and domestic animals



Martineau et al., Environ Health Perspect 110:285–292 (2002).

Polar Bears (Ursus maritimus) in greenland

Sonne et al., *Environ Health Perspect 112:1711–1716 (2004)*

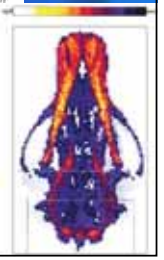
Table 1. Skull BMD [g/cm^2 ± SD (*n*)] for subadult and adult East Greenland polar bears from 1892 to 2002.

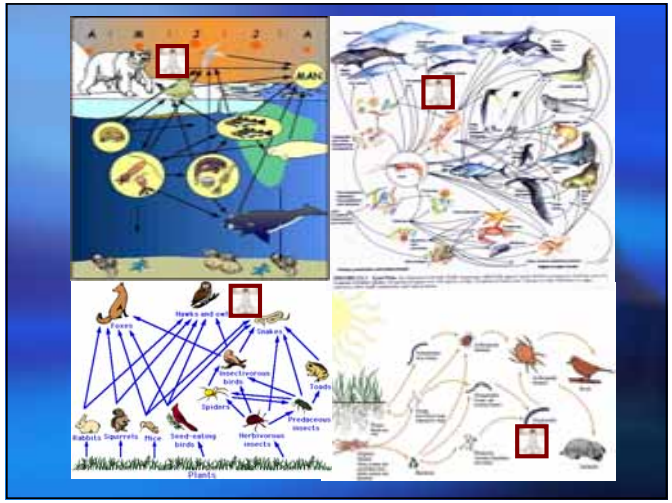
Period	Variable	Subadult females	Subadult males	Adult females	Adult males
1892–1932	BMD	1.67 ± 0.37 (7)	2.22 ± 0.19 (5)	1.99 ± 0.13 (9)	2.73 ± 0.21 (20)
	Age	2.6 ± 1.3 (7)	4.4 ± 1.3 (5)	12.7 ± 3.7 (9)	11.5 ± 4.5 (20)
1966–2002	BMD	1.55 ± 0.3* (17)	1.85 ± 0 .32* (35)	1.98 ± 0.13 (31)	2.49 ± 0.24**
	Age	2.8 ± 1 (17)	3.2 ± 1.1 (35)	12.1 ± 6.3 (31)	10.7 ± 5.5 (15)

Data are divided into two periods: 1892–1932 (supposed organochlorine and PBDE nonpolluted) and 1966–2002 (suppo organochlorine and PBDE polluted). BMD (g/cm^2) was obtained by DXA scanning of the entire skull, and age (years) obtained by counting the GLG of the lower I_2 tooth.

Table 3. Concentrations [mean ± SD (median), ng/g l.w.] of various contaminants in subcutaneous adip tissue of 58 East Greenland polar bears sampled during 1999–2001.

Compound	Subadults (*n* = 35)	Adult females (*n* = 14)	Adult males (*n* =
ΣPCB	6,597 ± 2,726 (6,089)	5,334 ± 2,150* (5,770)	8,637 ± 4,111* (8,2
ΣCHL	1,598 ± 884 (1,469)	1,379 ± 591 (1,353)	1,055 ± 517 (914)
ΣDDT	392 ± 209 (376)	358 ± 149 (366)	481 ± 331 (496)
ΣHCH	196 ± 68 (172)	195 ± 186 (151)	294 ± 210 (181)
Dieldrin	210 ± 100 (196)	174 ± 70 (154)	177 ± 81 (172)
HCB	99 ± 84 (70)	75 ± 82 (51)	51 ± 28 (48)
ΣPBDE	62 ± 33 (53)	53 ± 17 (53)	52 ± 16 (49)





Thanks for your attention

新興污染物檢測方法的開發與應用

Method Development and Application for Emerging Contaminants Analysis

丁望賢
國立中央大學化學系

摘要

新興污染物(Emerging Contaminants)定義為：現今政府相關單位監測計畫並未包含的化學物質，而且根據這些化學物質的性質，可能潛藏著對環境及健康的影響，雖然在今天影響並未展現，可是在未來或在下個世代可能會對環境及健康產生危害。目前「環境荷爾蒙」也被歸納為新興污染物的一部分。新興污染物並非表示最近才被釋放到環境中的化學物質，實際上，這些化學物質已在我們生活中大量使用，由於檢測技術的進步，以往無法測到化學物質，如今皆能在非常低的濃度下被檢測到。使用「Emerging」這一詞是為了引起科學團體和大眾的關注，了解到這些化學物質會透過不同的路徑釋放到環境中。有別於"傳統"的環境汙染物如有機氯農藥、重金屬、多氯聯苯、戴奧辛和多環芳香族碳氫化合物等，新興污染物包含許多不同的家用與工業用化學品，常見的如藥物、個人護理產品和家用化學品，人工香料、抗菌劑、界面活性劑與耐燃劑等。

新興污染物進入環境中的最大來源為未經處理的廢污水及廢污水處理廠的放流水。由於大部分的廢污水處理廠無法有效移除這些污染物，因此這類化學物質經放流水直接排放至自然水體中。進入自然水體的汙染物可藉由生物濃縮與累積效應進入生物體中，也可藉由食物鏈進入其他高階的生物體內，最後進入人體。某些進入自然水體的新興污染物，也可藉由飲用水的需求而進入被養養的動物體內或是人體內。這些新興污染物透過這一系列的循環，在環境中的濃度不斷累積，使得生物體內以及人體內的濃度不斷增加，最終將導致不可忽視的危害。

經多年的努力，本實驗室除了已建立檢測不同基質中壬基酚殘留物的相關檢測技術外，最近也完成檢測不同性質新興污染物的技術。檢測方法包含有樣品前處理與以現代化的氣相層析質譜儀(GC-MS)及液相層析質譜儀(LC-MS)為主的定性及定量技術。以下

列出本實驗室對臺灣環境中不同新興污染物所開發出的檢測技術及相關文獻，以供有興趣的專家學者參考，並於研討會中作回顧性之說明。

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戴奧辛生物篩檢法之評估及應用

Evaluation and application of Dioxin screening bioassay

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摘要

傳統之戴奧辛檢測大都藉由如高解析氣相層析質譜儀（HRGC-MS）等化學方法進行，其分析結果雖然相當準確，但是檢測成本高、耗時長、且檢測技術門檻高，如遇突發之污染狀況，恐無法在短時間內檢測大量樣品。為因應此種快速篩檢之需求，近年來發展出多項戴奧辛生物快速篩檢技術，除了可在短時間內檢測大量樣品之外，且檢測成本相對較低，目前已趨成熟並為各環保先進國家之頂尖實驗室採用。

目前本團隊已建立三種戴奧辛生物篩檢法，包括 DR-CALUX®細胞檢測方法、美國 CAPE 公司之酵素免疫分析法（EIA）及 Procept®法。DR-CALUX®細胞檢測法是以老鼠肝癌細胞（mouse hepatoma H4IIE）穩定地轉植一段螢火蟲冷光報告基因，該基因與戴奧辛反應元素基因（DRE）連結在一起。一旦戴奧辛類化合物啟動細胞內之訊息傳遞機制，就會同時啟動報告基因而製造出冷光酵素。由於冷光酵素之產量與戴奧辛類化合物之間有劑量效應，藉由冷光之強弱與 TCDD 標準品產生之冷光強度比較即可推算出戴奧辛之含量。EIA 法是利用具有專一性的抗體與對戴奧辛類化合物結合進而達到定量之目的。EIA 試管壁上固著了定量之戴奧辛抗體，可與樣品中之戴奧辛類化合物結合，接著加入辣根過氧化酶(HorseRadish Peroxidase，HRP)與剩餘未鍵結的抗體連結，再用清水去除未連結的辣根過氧化酶。最後加入會與辣根過氧化酶結合呈色之呈色劑，最終之呈色會與辣根過氧化酶含量成正比，而與樣品戴奧辛含量成反比。Procept®法則是運用即時定量聚合酶連鎖反應技術（Real-time PCR）進行戴奧辛之定量。當樣品中存在戴奧辛類化合物時，會和試劑中之芳香烴受體蛋白質及具有 DRE 之 DNA 片段形成一複合體。然後利用特定抗體抓住此複合體後，洗去多餘試劑，接著以 Real-time PCR 量測 DNA 含量，即可間接推估戴奧辛類化合物之總毒性當量。

經實際比較測試，上述三種戴奧辛生物篩檢法之優缺點略如表。DR-CALUX®法之方法靈敏度高、適用於各類基質，且檢測結果相當穩定，但是技術建置費用及樣品分析費用相對較高。當搭配美國 CAPE 公司之矽膠—活性碳複合管柱執行淨化步驟時，在土壤及底泥樣品部分 DR-CALUX®測值及 HRGC/HRMS 測值比值範圍為 1.9～5.1（平均值為 2.4（n=21）），R^2=0.986；在植物及生物體樣品部分，比值範圍為 0.6～2.6（平均值為 1.4（n=23）），R^2=0.909；煙道氣樣品之比值範圍為 1.5～5.6（平均值為 2.6(n=18)），R^2=0.982）。EIA 法之技術建置費用及樣品分析費用最低且操作容易，但是方法靈敏度低，只適用於土壤、底泥檢測。應用於樣品檢測時，若已知樣品特定之修正因子，則 EIA 法之測值與 HRGC/MS 測值之相關性佳，比值介於 0.72~2.01 間（n=3）。至於 Procept®法，所需之建置及檢測費用、方法靈敏度、適用之基質範圍均介於前述二種方法之間，但實際進行飛灰、污泥檢測時，檢量線之穩定度不佳，而測值與 HRGC/MS 測值之比值介於 2.1~4.3 之間（n=3）。

關鍵字：戴奧辛生物篩檢法、DR-CALUX®細胞檢測方法、酵素免疫分析法（EIA）、Procept®法

表 戴奧辛生物篩測法比較表

項目 / 方法	DR-CALUX®法	EIA 法	Procept®法
認可	歐盟及美國環保署	美國環保署	美國環保署
技術建置費用	600 萬以上	200 萬以下	300 萬以下
分析時程	約 7 天	約 5-7 天	約 5 天
樣品分析費用	約 9000 元/樣品	約 4000 元/樣品	約 5000 元/樣品
分析量	約 120 件樣品/月	約 200 件樣品/月	約 200 件樣品/月
人員訓練時間	3 週以上	1 週	1 週
方法靈敏度	高	較低	中等
適用樣品基質	全部基質	土壤、底泥	土壤、底泥及飛灰

北台灣水庫中持久性有機污染物之傳輸、來源與累積特性研究

Transformation Processes, Possible Sources and Sink of PCDD/Fs in a Reservoir In Northern Taiwan

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Abstract

In this study, polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/F) deposition was sampled at Feitsui Reservoir in northern Taiwan via traditional cylindrical vessels (atmosphere) and sediment trap (water body) from January to December 2008. Fig. 1 shows that the atmospheric PCDD/F concentration and deposition flux measured in the vicinity area of the reservoir investigated ranged from 4.90 to 39.0 fg-I-TEQ/m^3 and 1.37 to 19.3 pg-I-TEQ/m^2-day, respectively. The highest atmospheric PCDD/F (39.0 fg-I-TEQ/m^3) and total suspended particle (128 μg/m^3) concentration is measured at February 2008 during the Asian dust storm event. In the meantime, the atmospheric PCDD/F deposition flux also increased from 4.14 to 9.89 pg-I-TEQ/m^2-day. In addition, the atmospheric PCDD/F deposition fluxes measured on winter are significantly higher than other seasons. The PCDD/F deposition flux measured in the water body at down stream of the reservoir investigated indicated that the PCDD/F deposition flux in the water body ranged from 10.3 to 29.8 pg-I-TEQ/m^2-day and 9.13 to 179 pg-I-TEQ/m^2-day measure at water depth of 20 and 70 m, respectively, and was significantly higher than the atmospheric PCDD/F deposition flux measured during same period. The temporal trend of the PCDD/F deposition flux in water body at 20 m water depth is quite similar with the atmospheric PCDD/F deposition flux. The significantly higher PCDD/F deposition flux in water body may be attributed to the

catchment erosion at down stream of the reservoir investigated. On September 2008, the PCDD/F deposition flux in the water body at 70 m water depth was dramatically higher than that measured at 20 m water depth during the intensive typhoon events. We consider that the dramatic increase of PCDD/F deposition flux at 70 m water depth was caused by the land slide occurred at up stream of the reservoir during the intensive typhoon period. Based on the PCDD/F deposition flux measured in atmosphere and water body (20 m water depth), the contribution of PCDD/F input sources of the reservoir investigated during different periods. Fig. 2 indicated that around 24 to 45% PCDD/F input flux in the reservoir investigated was contributed by the atmospheric deposition during normal periods. However, the contribution of atmospheric PCDD/F deposition increased to 57% during the Asian dust storm event. During the typhoon event, the contribution of atmospheric PCDD/F deposition decreased to 10% due to the significant catchment erosion in the reservoir investigated. The distribution of PCDD/F homologue observed in ambient air, atmospheric deposition, surface water, deposition in water body and sediment core samples. indicated that the highest distribution of PCDFs (80%) was observed at vapor-phase ambient air sample. The increasing trend of PCDDs was also observed from the sample of atmosphere, water body to sediment. The reasons contributing to the increasing trend of PCDDs are twofold. The first reason is that the PCDDs are mostly distributed in solid phase in ambient air. Therefore, the suspended particles collected in atmospheric deposition and water body would enhance the PCDD distribution. The second factor considered is the effect of catchment erosion in the reservoir investigated. The PCDD/F input sources in water body included atmospheric deposition and catchment erosion of the reservoir.

Acknowledgements

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關鍵字：戴奧辛(Dioxin)、大氣沈降(Atmospheric deposition)、長程傳輸(Long-range transport)、水中沈積物(Sediment trap)

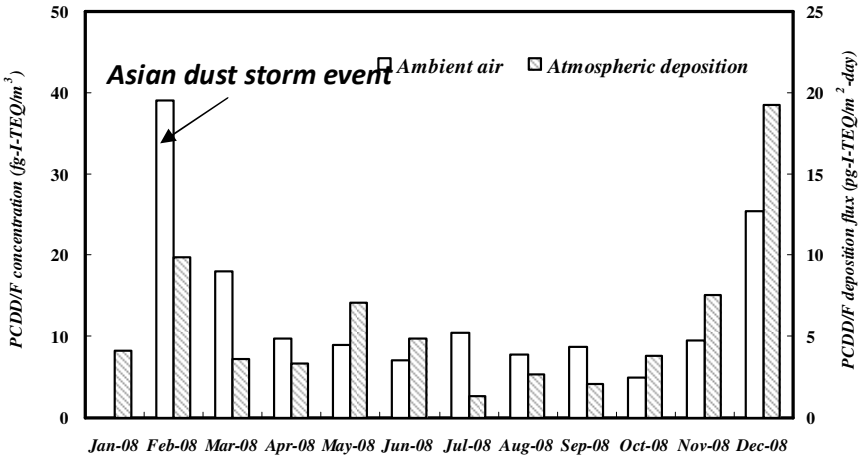

Figure 1 Atmospheric PCDD/F concentration and deposition flux measured in the vicinity area of the reservoir investigated.

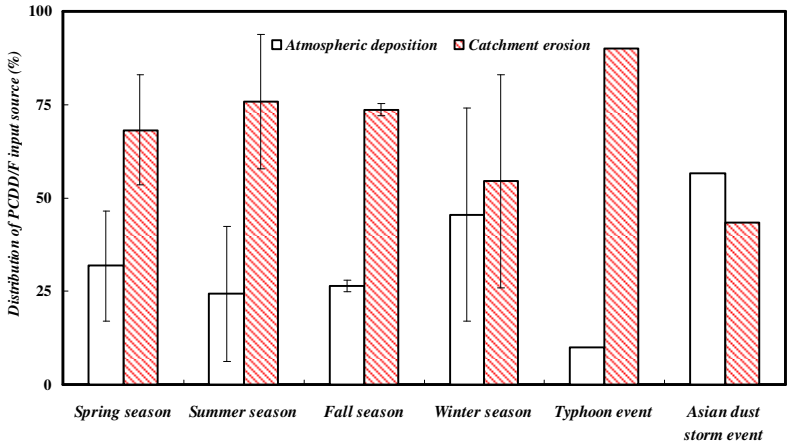

Figure 2 Contribution of PCDD/F input sources of the reservoir investigated during different periods.

壁報論文

十溴聯苯醚之替代方析方法開發

Development of Alternative Method for Analysis of Decabromodiphenyl Ether (BDE-209)

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摘要

十溴聯苯醚(decabromodiphenyl ether, BDE-209)因為具有耐高溫、結構穩定不易破壞等特性，因此常作為阻燃劑(flame retardant)使用。隨著 BDE-209 的大量使用，河川底泥、灰塵等環境介質已能偵測出 BDE-209 的存在，顯示其環境污染問題已相當嚴重。傳統的 BDE-209 分析方法以索氏萃取法(sohxlet extraction)搭配氣相層析質譜儀(GC/MS)為主，但索氏萃取法有耗時、耗溶劑及樣本回收率不佳等缺點，在實驗室強調速度與效率的今天，並不符合經濟效益。此外 BDE-209 具有不易揮發及高溫下易分解等特性，在氣相層析樣本氣化過程中可能會產生熱分解，也增加了定性與定量的難度。本研究使用微波萃取法及搭配紫外光偵測器之液相層析儀，期能開發出適用於 BDE-209 之分析方法。經測試後選定以丙酮為萃取溶劑，利用微波加熱升溫至 120℃後，持續加熱 10 分鐘，樣本冷卻後過濾定容，即可注入液相層析儀進行分析，較低濃度之樣本則吹氮濃縮後再次分析。液相層析儀之分析條件包括以甲醇為流動相，流速 1.0 ml/min、樣本注射體積 10 μl、樣本分析時間 12 分鐘、紫外光偵測器設定為 230 nm。在此條件下，標準品之 BDE-209 約在 8.7 分鐘檢測出，0.6 - 60 g/mL 範圍內檢量線之線性可達 0.999 以上。其他測試結果顯示本方法之偵測下限為 5400 ng/g，定量下限為 6000 ng/g、樣本回收率為 80%(±20%)，準確度為 95 %，配合吹氮濃縮之方法偵測極限為 300 ng/g。以汽車灰塵進行測試，其檢測濃度約為 23000 ng/g，與文獻資料濃度範圍相當。以此分析方法與文獻資料比對，研判應用微波萃取配合吹氮濃縮法配合 HPLC/UV 分析之檢測方法，應足以測量環境中污染程度較高之 BDE-209 含量。

關鍵字：十溴聯苯醚、微波萃取法、液相層析儀

室內環境灰塵中十溴二苯醚含量之檢測分析

Measurement of Decabromodiphenyl Ether (deca-BDE) in Indoor Environment

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摘要

十溴二苯醚(decabromodiphenyl ether, deca-BDE)為使用廣泛的溴系阻燃劑，長期大量使用的結果已造成環境污染。Deca-BDE 之檢測主要以索氏萃取法和氣相層析質譜儀進行，此方法之樣本前處理程序耗時，且氣相層析質譜儀在氣化樣本的過程中，可能會導致 deca-BDE 裂解，進而影響量化結果。本研究以微波萃取裝置及高效液相層析儀進行 deca-BDE 的分析，並針對室內環境灰塵中的含量進行檢測。本研究以汽車、辦公室及住家室內收集之灰塵為研究對象，灰塵樣本先經烘乾過篩後，以丙酮進行微波萃取，冷卻過濾後即以 HPLC 進行分析，樣本濃度若低於檢量線範圍時則以吹氮濃縮樣本，過濾後再行分析。初步分析汽車內灰塵樣本中 deca-BDE 含量範圍為 12000~38000 ng/g，另分析辦公室內灰塵樣本中 deca-BDE 含量為 3800 ng/g，與文獻中相同類型的樣本含量相當。本研究使用之檢測方法，儀器檢量線範圍在 0.4~100 ng/ L 之間，樣本平均回收率為 85%，加上樣本前處理簡便快速，後續將繼續進行其他環境中 deca-BDE 之檢測分析。

關鍵字：十溴二苯醚、室內環境污染、微波萃取、高效液相層析儀

性轉換是造成台灣鮭魚(*Oncorhynchus masou formosanus*)瀕危的原因?

Sex Reversal Causes the Extinction of Formosa Landlocked Salmon (*Oncorhynchus masou formosanus*)?

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摘要

陸封台灣鮭魚(*Oncorhynchus masou formosanus*)目前僅棲息於台灣中部大甲溪上游支流的七家灣溪流域，是名列紅單(red list)的瀕危物種。保育瀕危族群必須瞭解族群結構，其中雌雄性比(sex ratio)會關鍵性決定小族群的有效族群數目(effective population size)，而性別決定(sex determination)機制又決定族群的性別比例(sex ratio)。鮭科魚類除非在繁殖季節才顯現第二性徵，否則不易以外形辨性別。台灣鮭魚雄魚幼形成熟(neoteny)，所以即使在繁殖季節亦難分辨雌雄。我們以 HPLC 高效液相層析儀螢光法測量 18 個非生物的棲地樣本(養殖池、種魚池、溪水、農地排出水、溪流底泥、飼料、蔬菜、溪畔林、土壤)和台灣鮭魚體內壬基苯酚和辛基苯酚的濃度。我們也成功開發出準確、簡單、快速、經濟、非侵入性的基因型雄性性別分子標記(male-specific sex marker)，準確的斷定已知性別的櫻鮭家族表型。調查歷年台灣鮭魚野外族群的雌雄性比，我們發現台灣鮭魚野外族群性比顯著失衡，且有兩尾台灣鮭魚的基因型和表型不吻合（發生性轉換 sex reversal）。我們同時也注意到台灣鮭魚體內和其周遭棲地環境都充斥高濃度的壬基苯酚。本研究試圖探討造成此兩現象的可能肇因，並提出環境污染物質導致生物發生性轉換時，伴隨的阿里效應(Allee effect)也會嚴重威脅到瀕危族群之生存。

關鍵字：台灣、鮭魚、環境荷爾蒙、瀕危、性別

從個體、組織及分子層次探討多溴聯苯醚對魚類的影響

Assessing the Effects of PBDEs on Fish at the Organism, Tissue and Molecular Levels

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摘要

多溴聯苯醚（PBDEs）是新興的持久性有機污染物，主要用途為添加於商業製品中做為阻燃劑之用，不僅應用廣泛且結構穩定不易被分解，因此在環境中大量分佈。另外，PBDEs 為親脂性，可藉由各種途徑進入生物體內蓄積，具有成生物累積及生物放大效應。PBDE-47 為生物體中最常見且含量最高的多溴聯苯醚異構物。動物及體外研究顯示，PBDEs 可能具有類戴奧辛毒性，並可能影響性別分化、甲狀腺功能及神經系統。然而，PBDEs 對魚類影響之資料仍然相當缺乏。本實驗利用斑馬魚作為模式生物，同時從個體、組織及分子的層次研究 PBDE 對魚類的影響。實驗中從孵化後第 20 天到第 90 天以添加不同濃度 PBDE-47 的飼料餵食 (control, solvent control, 10, 100, 1000 ng/g)，並在選擇飼料中濃度時參考野外研究文獻，以求暴露劑量能接近環境濃度。

在個體層次方面，我們觀察幼魚的外型發育和記錄體重體長，並利用動物軌跡影像分析系統分析幼魚的運動行為參數，以作為神經毒性的指標。實驗結果顯示，斑馬魚的生存、生長及外型發育並未受到 PBDE-47 影響，但運動行為（包括活動距離及活動時間）則顯著降低。本結果顯示行為毒理研究可有效偵測 PBDE 潛在之慢性神經毒性。

在組織層次方面，在孵化後 90 天實驗結束時，進行全魚組織切片，觀察各組實驗魚的雌雄比及性腺發育狀況，探討 PBDE-47 是否具有干擾性別分化或性腺發育的能力。實驗結果顯示，各組的雌雄比例均無顯著差異，也沒有雙性個體的產生；並且各組樣本之精卵巢中，成熟卵細胞與卵巢總細胞數比，以及成熟精細胞與精巢總發育面積比，各組間均無顯著差異。顯示持續暴露在此環境劑量的 PBDE-47 並不會造成斑馬魚性別分化異常或性腺發育的改變。

在分子層次方面，我們利用即時定量 PCR（QPCR）技術，分別於孵化後 38 天（斑馬魚性腺分化尚不明顯）及孵化後 90 天（斑馬魚性腺分化完成）兩時期檢測基因表現，探討 PBDE-47 對於斑馬魚發育時期中的基因表現量之影響。於孵化後 38 天分析的目標基因包括性別分化相關基因（卵黃原蛋白 *vtg*、抗穆勒氏管荷爾蒙 *anti-mullerian hormone*、*cyp11b*）、戴奧辛毒性反應基因（芳香烴受體核轉移蛋白 *arnt*、芳香烴受體 *ahr2*、細胞色素 P450 *cyp1a1*）及甲狀腺相關基因（促甲狀腺激素 *TSH*、第一型脫碘酵素 D1、甲狀腺素運送蛋白 TTR）等九個基因；於孵化後 90 天進行戴奧辛毒性反應基因及甲狀腺相關基因等六個基因。結果顯示，在孵化後 38 天，PBDE-47 對於基因表現的影響並非隨著濃度增加而抑制或刺激表現，以 *cyp11b*、*ahr2* 來說，其基因表現量在低濃度處理組與高濃度處理組間有顯著差異，而 *D1* 於低濃度處理組中有顯著差異，高濃度組則無影響。在孵化後 90 天，觀察到 *ahr2* 與 *TSH* 在高濃度處理組與控制組有顯著差異，且 *ahr2* 在低濃度與高濃度處理組之間也有顯著差異。我們的結果顯示，PBDE-47 可能會影響性別分化、甲狀腺相關及戴奧辛指標基因的表現。根據實驗結果，我們認為 PBDE-47 的類戴奧辛毒性遠比 TCDD、PCB 來得低，但可能透過干擾 *cyp11b2*、*TSH*、*D1* 基因表現干擾魚類正常生理作用。

綜言之，長期低劑量的 PBDE 暴露會對魚類行為及基因表現產生影響。本研究也顯示，利用斑馬魚作為模式物種可以同時在不同生物組織階層測試環境荷爾蒙的潛在毒性。

關鍵字：多溴聯苯醚(PBDEs)、斑馬魚(zebrafish)、動物行為(animal behavior)、基因表現(gene expression)

三丁基錫對台灣九孔之毒性研究及台灣九孔對三丁基錫之累積

The Toxicity and Accumulation of Tribulyltin Chloride on Taiwan Abalone, *Haliotis diversicolor supertexta*

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摘要

台灣沿海地區之養殖九孔於近十年間發生大量死亡情形，而絕多數研究僅以生物性致病因子進行探討，有鑑於此，本研究針對台灣重要經濟水產生物-九孔，以源自船底塗料且廣泛污染於海洋環境之有機錫物種-三丁基錫，進行急毒性測試與九孔對三丁基錫累積之研究。

第一部分之研究，以空白實驗組、溶劑(EtOH)控制組與濃度分別為 5 μg L^{-1} 、10 μg L^{-1}、20 μg L^{-1}、40 μg L^{-1} 之三丁基錫海水，對九孔進行 7 天之急毒性測試，經 GC-PFPD 分析求得九孔對三丁基錫($Sn(C_4H_9)_3^+$)之半致死濃度(LC50)為 48-h LC_{50}=35.42 μgL^{-1}、72-h LC_{50}=20.78 μgL^{-1}、96-h LC_{50}=16.28 μgL^{-1} 與 120-h LC_{50}=13.78 μgL^{-1}，並於實際平均濃度為 40.16 ± 1.04 μgL^{-1} 之氯化三丁基錫海水，測試第 24 小時之後，發現九孔體表周邊所有感覺突觸嚴重遭受三丁基錫侵蝕而斷裂，且體表出現瓦解現象。

第二部分以九孔暴露於實際平均濃度為 2.66 ± 1.3 μgL^{-1} 之氯化三丁基錫海水，進行 7 天累積實驗。結果顯示，雌性與雄性九孔之肌肉組織對三丁基錫之累積速率無顯著差異；攝食與不攝食情況下，九孔肌肉組織對三丁基錫累積速率分別為 128.57 ng (Sn) /g dry-wt 與 158.50 ng (Sn) /g dry-wt，生物累積係數(Bioaccumulation factor, BAF)分別為 338.35 與 417.10，顯示九孔於短期暴露之實驗，對三丁基錫之累積主要來自於水體環境。此外，本實驗於 2008 年 5 月由台東地區沿海養殖環境購回之九孔對 MBT、DBT 與 TBT 之平均累積含量分別為 186.79、173.33 與 30.84 ng (Sn) /g dry-wt，其中，丁基錫(BTs)主要累積於內臟組織，TBT 主要累積於肌肉組織($P<0.05$)，並於所有九孔個體中檢出微量三酚基錫(TPT)，檢出率為 100%。

關鍵字：九孔(*Haliotis diversicolor supertexta*)、三丁基錫(tri-bulyltin)、急毒性測試(acute toxicity test)、生物累積因子(Bioaccumulation factor, BAF)

多溴聯苯醚在室內環境中之分布及來源調查研究

Distribution and Sources of Polybrominated Diphenyl Ethers in Indoor Environment

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摘要

人們經常使用及接觸的消費性用品，如電腦、電器及家具（含發泡材料），隨著防火觀念的提升，多添加有阻燃劑。國內外相關研究顯示，目前最被廣泛使用在各類消費性產品之阻燃劑為多溴聯苯醚（PolyBrominated Diphenyl Ethers，PBDEs），此類物質與其他持久性有機污染物不同之處，在於一般室內空氣之濃度比室外空氣高出甚多，推測原因應是室內使用電子、電機產品及家具等造成之結果，逸散至環境中。現代人們之生活型態，大部分活動時間都在室內環境，來自室內環境空氣中 PBDEs 的曝露機會增加。對室內環境中 PBDEs 分布及來源之了解，有助於降低來自一般日用品的 PBDEs 暴露機會。

本研究調查對象分室內環境空氣及塑膠軟墊、塑膠玩具及用具等兩類別。使用之檢測方法，室內環境空氣部分，採樣方法參考環保署公告之「空氣中戴奧辛及呋喃採樣方法」（NIEA A809.10B），使用高流量採樣器採樣，吸附劑採用石英纖維濾紙和聚氨甲酸酯吸附材料。石英纖維濾紙收集粒徑大於 1 μm 以上的粉塵（內含固相 PBDEs），聚氨甲酸酯吸附材料吸附氣相多溴聯苯醚。採樣前先以正己烷溶劑以索氏萃取清洗聚氨甲酸酯 24 小時。分析方法為採樣後將石英纖維濾紙和聚氨甲酸酯，以索式法萃取後，將萃液進行硫酸酸洗及管柱淨化，使用氣相層析儀/質譜儀分析 27 種 PBDEs 同源物；塑膠軟墊、玩具、及用具部分，參考國際電工技術委員會（International Electrotecthnical Commission，IEC）之方法草案，先以液態氮脆化材料後，使用研磨機磨碎，過篩至 1 mm 以下，以索式法萃取，將萃液進行硫酸酸洗及管柱淨化，使用氣相層析儀/質譜儀分析 27 種 PBDEs

同源物。

室內環境空氣分別來自影城、幼稚園、航空站、辦公大樓及百貨公司等 5 處室內場所，PBDEs 濃度分布介於 46.2~261 pg/m^3，平均值為 117 pg/m^3，濃度較高之室內環境來自幼兒活動環境中，主要為幼稚園及百貨公司內幼童嬉戲處，兩處有一共通點，即使用大量之塑膠軟墊、塑膠玩具及用具等。有鑑於一般塑膠成品中，多有使用 PBDEs 阻燃劑，因此對幼稚園內之塑膠軟墊、塑膠玩具及用具等進行分析，濃度範圍為 802~5468 ng/g，平均值為 2360 ng/g，可能為室內環境空氣 PBDEs 來源之一。

關鍵字：多溴聯苯醚、室內環境、塑膠、玩具、氣相層析儀/質譜儀

雙酚 A 對斑馬魚之累積影響及累積平衡
The Study on Accumulation and Equilibrium of Bisphenol A to zebra danio

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摘要

隨著石化工業的快速發展，以致環境中有愈來愈多具有內分泌干擾作用的物質，如壬基酚(NP)、雙酚A、鄰二甲苯類、多氯聯苯等化合物。2001年鄭茂先、吳德生等學者在環境雌激素雙酚A(BPA)之生殖毒理研究，結果證實該物質對人類及動物的健康和繁殖確有極為不利的影響，此外，2008年9月3日由美國國立衛生研究院「全國毒物計畫」（NTP）發表的報告，顯示酚甲烷可能會改變腦部的發展和行為，同時增加兒童、嬰兒和胚胎發生前列腺癌的可能，因而受到大眾的普遍關注。雙酚A是已獲證實的內分泌干擾物，其具有類似雌激素的作用，因而又稱為類雌激素，如進入生物體內，將有可能干擾正常的內分泌系統。雙酚A影響成人健康程度，尚有待進一步的研究與討論，依當前研究結果顯示，對於嬰兒及胎兒之影響，很有可能超過成人。

魚類的繁殖過程是在雌激素調節下進行的，因此，環境中的類雌激素對魚類生長，具明顯的影響能力。斑馬魚是一種常見的熱帶淡水魚，具有體型小、世代週期短、卵膜透明使胚胎發育過程易於觀察、繁殖率高且飼養管理價廉等特點。另斑馬魚之受精方式為體外受精，幼魚孵化120天後即能進行繁殖(即性腺已達成熟)，這些生物特性使斑馬魚廣泛被應用在毒理學之研究上，因此，本研究選擇斑馬魚作為模式(試驗)生物。

依據前置試驗結果，斑馬魚成魚之24小時LC_{50}位於7.0-10.0 mg/L間(8.5±1.5 mg/L)，而在正式試驗中，發現暴露於不同BPA試驗濃度(100μg/L及500μg/L)環境中之魚體卵囊，皆有水腫(edema)之現象。而累積試驗結果顯示，成魚內臟累積能力遠大於其他組織，500 μg/L成魚試驗組之內臟及其他組織的最高累積濃度分為119.61μg/g及8.42μg/g(前者約為後者的14.21倍)，100 μg/L成魚試驗組為131.89μg/g及44.72μg/g(前者約為後者的2.95倍)。因此，從以上結果可以推知，以魚體發育程度為基礎作比較，成魚的內臟比起幼魚對BPA的累積能力強許多。在經過4天清水馴養後，原500 μg/L及100 μg/L之BPA試驗組，成魚魚體內之殘留累積濃度最低分別為28.41μg/g及26.80μg/g，減少74.5%與75.1%；500 μg/L幼魚試驗組則為80.08μg/g，減少58.4%。

環境細菌對烷基苯酚類環境荷爾蒙的分解
The Degradation of Alkylphenol Environmental Hormones by Bacteria in the Environment

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摘要

烷基苯酚聚氧乙基醇(alkylphenol polyethoxlates, $APEO_n$)為一廣泛使用於工業、農業與家用活動之非離子性界面活性劑，主要包括壬基苯酚聚氧乙基醇(nonylphenol polyethoxylates, $NPEO_n$)及辛基苯酚聚氧乙基醇(octylphenol polyethoxylates, $OPEO_n$, in average n=9.5)，其一旦被排放至自然環境中，經常會生成更不易分解且具環境荷爾蒙效力的代謝產物如烷基苯酚和帶 1-3 單位之烷基苯酚聚乙氧基醇，對於人體健康與環境生態具危害性。因辛基苯酚(octylphenol, OP)誘導魚類產生卵黃前質的能力為雌激素之 4×10^{-4} 倍，在與雌激素受體結合能力上，此化合物為烷基苯酚聚氧乙基醇代謝物中之最高者。因此本研究以辛基苯酚為篩選目標，由宜蘭縣圓山之已知長期施灑農藥與肥料之稻田表土、含有辛基苯酚之土壤縮模(microcosm)及辛基苯酚藥罐中，篩選出 18 株能以辛基苯酚為唯一生長碳源之菌株，經 BioLog 碳源利用指紋法、脂肪酸圖譜及 16S rDNA 序列分析等三種鑑定方法發現，在所篩選的菌種中，屬革蘭氏陰性菌之 *Pseudomonas* 屬佔 11 株，其餘菌株包括 *Alcaligenes defragrans*、*Bordetella brochiseptica*、*Zoogloea ramigera*、*Inquilinus limosus* 及 *Methylobacterium radiotolerans*。在碳源利用上，依碳源利用情形大致可將分解微生物分為三類:第一類細菌為可分解 $OPEO_n$ 及其代謝產物 $OPEO_2$、$OPEO_1$、OP、octylcatechol (OC)之菌株，多屬於 *Pseudomonas* 屬；第二類細菌為可分解 $OPEO_1$、OP、OC，但無法分解 $OPEO_n$ 及 $OPEO_2$，除了 *Pseudomonas* 屬的菌株，還包括 *Zoogloea ramigera* 及 *Bordetella brochiseptica*；第三類細菌為可分解 OP、OC，但無法分解 $OPEO_n$、$OPEO_2$ 及 $OPEO_1$，主要為 *Pseudomonas putida*、*Alcaligenes defragrans*、*Bordetella brochiseptica* 及 *Inquilinus limosus*。目前世界各國包括台灣，在其環境中如河流、排放水及其底泥中都偵測到各種不同濃度的烷基苯酚類代謝物如 OP、OPEOn (n=1~3)及一系列的羧酸化產物，本研究所篩選之環境微生物對於 OP 及其相關化合物具各種不同的代謝能力，以期未來能運用於環境中烷基苯酚類環境荷爾蒙的防制上。

線上衍生化技術結合氣相層析質譜儀檢測環境水樣及個人護理產品中三氯沙之含量

Determination of Triclosan in Water Sample and Personal Care Products by On-line Derivatizaion coupled with GC/MS

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摘要

三氯沙 Triclosan (TCS，5-chloro-2-(2,4-dichloro-phenoxy)-phenol)為一種抗菌化學品，廣泛使用於個人衛生護理產品中，如洗髮精、香皂、除嗅劑、乳霜、漱口水、牙膏等，在這些產品中三氯沙一般的添加範圍為 0.1~0.3%(w/w)，台灣環保署規定三氯沙添加劑量不得超過 0.3%。由於三氯沙有對格蘭式陽性菌、黴菌及酵母菌具有抑菌活性，在規定的濃度限制下對人類不具急毒性，也不具致癌性，對眼睛及皮膚並無刺激性，因此被業者大量使用。然而，三氯沙的結構相似於其它多氯鄰羥基二苯醚類化合物 (Polychlorinated Phenoxyphenols)，而這類化合物目前已發現可以環化形成具有毒性的多氯戴奧辛，最近研究結果顯示，三氯沙可藉由加熱、陽光或高能量紫外光源，在水中進行光解產生水解及脫氯作用，形成氯酚和鄰苯二酚等產物，甚至可以轉換成戴奧辛成分之一的 2,8-DCDD。

本篇研究主要是以氣相層析質譜儀的大體積進樣裝置搭配矽烷化衍生試劑進行線上衍生化技術，步驟簡單且迅速地分析待測物，並利用固相萃取技術作為水樣的前處理方法。待測物在去離子水中之回收率為 104%以上，相對標偏差值(RSD)則都在 7%以下。對台灣不同環境樣品進行檢測，待測物的回收率大多可在 50%以上，相對標準偏差在 16%以下，在水樣中三氯沙的濃度範圍為 1~1333 ng/L，甲基三氯沙並無偵測出，而本方法之定量偵測極限(LOQ)對三氯沙及甲基三氯沙分別為 0.9ng/L 與 1.0ng/L。本實驗並針對個人護理產品(如牙膏、洗面乳)進行檢測，三氯沙的濃度範圍為 0.07% ~ 0.28%，均在政府的規範內。

以微波輔助頂空固相微萃取法搭配氣相層析質譜儀檢測水樣中的多環麝香化合物

Determination of Synthetic Polycyclic Musk in Aqueous Samples by Microwave-Assisted HS-SPME & GC/MS

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摘要

人工合成麝香化合物(synthetic musk fragrances)是一種廣泛被使用在清潔劑、香水、洗髮精和其他許多個人護理產品的化學物質。依其物理與化學性質，可被歸類為具疏水性與半揮發性的新興環境汙染物。具有這些性質的汙染物在進入環境後可透過食物鏈造成生物累積與放大效應。目前文獻指出，在空氣、淡水、海水和底泥中分別都能檢測出多環類麝香化合物的殘留。由於多環類麝香化合物具有高脂溶性，在水生動物的體內也能見到這類化合物的蹤跡，甚至在人類的脂肪組織或母乳中也存在著多環類麝香化合物殘留。

本研究以發展微波輔助固態微萃取法(Microwave-Assisted Headspace Solid-Phase Microextraction，簡稱 MA-HS-SPME)當作樣品的前處理步驟，從環境水樣萃取六個常見的多環類麝香化合物(synthetic polycyclic musks)，再結合氣相層析質譜儀做為檢測工具。最佳化的萃取條件是取 20 mL 水樣置於 40 mL 萃取瓶(水樣體積與頂空體積為 1:1)，添加 4 g NaCl，使用 65 μm PDMS/DVB 的纖維在 180 W 下加熱並萃取 4 分鐘。定量極限(Limit of Quantification，簡稱 LOQ)低於 0.2 ng/L。在環境水樣中，HHCB 與 AHTN 為主要被檢出的多環類麝香化合物，以標準添加法定量結果介於 1.2 至 37.3 ng/L 之間，回歸係數則高於 0.981。本研究所發展方法，具有高靈敏度與穩定的檢測結果。可以用來檢測環境水樣中多環麝香化合物的殘留與流布。

環境樣品中之甲基汞檢測技術研究
Study on Methyl Mercury Analytical Technique in Environmental Samples

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摘要

汞是一種具有高毒性的重金屬，於環境中流布可能造成生物體內毒性累積及環境污染，而其毒性又以甲基汞為最，已被我國列為「持久性有機污染物國家實施計畫」中須建立檢測技術之化合物之一。環顧國內尚缺環境基質(如生物體及底泥等)中有機汞測定之前處理方法。本研究除開發「生物體鹼性消化法」及「土壤底泥溶劑萃取(Solvent Extraction, SE)」前處理之甲基汞-冷蒸氣原子螢光法檢測技術(CVAFS)外」，並對常見之環境基質中甲基汞做污染調查之初探。其中，生物體鹼性消化- CVAFS 檢測技術之 MDL 可達 3.0 ng/g；土壤底泥 SE-CVAFS 檢測技術之 MDL 可達 0.08 ng/g，相關係數 R 值都可達 0.999 以上；以參考標準物質驗證方法，生物樣品精密度為 8.84 %，準確度為 86.96 ~ 104.8 %；土壤底泥精密度為 10.2% ，準確度為 85.2 ~ 105.2%。

關鍵字：持久性有機污染物、甲基汞

戴奧辛類污染物大氣連續採樣器驗證及差異性研究計畫
Evaluation of Dioxin in Ambient Air using High-Volume Sampler

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摘要

污染源排放之戴奧辛進入食物鏈的初級機制是透過大氣沉降而流布於環境中，故大氣中戴奧辛的濃度分布與其傳輸行為是評估戴奧辛危害風險時的重要依據。行政院環保署環境檢驗所自民國 88 年 3 月起，即展開環境空氣中戴奧辛檢測方法研究工作。也由歷年計畫結果發現，台灣地區空氣中戴奧辛之濃度分布已普遍下降，顯見本署污染防治政策成效良好，惟此現象亦凸顯出目前採樣方法猶待突破之瓶頸。現行公告方法之採氣流量規範為 225 L/min，若以目前環境空氣中普遍濃度範圍而言，一個樣品需要超過三天以上的採樣時間，方能達到待測物可以被偵測檢出的濃度。對於人力、經費等成本及經濟效益均是很大的負擔，尤其是以商業為導向之檢測機構。目前日本已公告之空氣中類戴奧辛污染物採樣方法[1]中採氣流量可達 700 L/min，且已有多家公司均已開發出符合該規範之高流量採樣器。有鑑此新型採樣器可以有效縮短採樣時間，降低整體環境戴奧辛監測之成本，並可藉以監測至更低濃度之待測物，殊為國內空氣中戴奧辛類污染物採樣之重要發展方向。本計畫與中央大學張木彬教授及中央研究院環境變遷研究中心紀凱獻博士合作，進行戴奧辛類污染物大氣自動連續採樣器驗證，了解該採樣器之適用性、比較其與國內現行方法之差異、建立採樣之技術並作為未來方法公告修訂之參考依據。

計畫結果顯示高流量環境空氣採樣器（HV series）在七天採樣時間內，運用不同採氣流量進行採樣時之相對誤差為 0.56% ，顯示該採樣器在長時間不同流量的採樣條件下，仍有相當穩定的表現。而高流量環境空氣採樣器及 PS-1 採樣器之採樣結果差異。則是在 ±5%左右。然而高流量環境空氣採樣器之採氣流量如果增加到 700 L/min，在相同的

採樣時間內，其採樣結果與 PS-1 採樣器的差異會些微增加，但仍在 10%以內。綜上所述，本研究初步結果顯示高流量環境空氣採樣器的穩定性良好，且與 PS-1 之採樣誤差相當接近。所以 HV series 在環境空氣中戴奧辛類污染物採樣分析之適用性亦應屬可行。如此將可大幅縮減採樣所需之時間和人力，並且針對環境空氣中更低濃度污染物之區域進行有效之監測，所測得之數據亦更有可信度。

關鍵字：戴奧辛（Dioxin）、空氣採樣（Air Sampling）、高流量環境空氣採樣器（High Volume Sampler）

活化的 aryl hydrocarbon receptor 及 glucocorticoid receptor 的交互作用
Crosstalk between Activated Forms of the Aryl Hydrocarbon Receptor and Glucocorticoid Receptor

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Abstract

Pyrene, benzo[*a*]pyrene (BaP), and indeno[1,2,3-cd]pyrene (IND) are polycyclic aromatic hydrocarbons (PAHs) with four to six annealed phenyl rings. Dexamethasone (Dex) is a synthetic agonist of glucocorticoids. This is the first report showing that BaP and IND distinctly enhanced Dex-induced transactivation of the glucocorticoid receptor (GR). The aryl hydrocarbon receptor (AhR) ligands, BaP and IND, did not directly activate the GR, and Dex, did not activate the AhR. Whenever BaP and IND were added to Dex-treated cultures, they were present with Dex for longer periods, and higher enhancement of Dex-induced transactivation of the GR was found, which indicates that the freshly activated AhR is essential for synergistic interactions with the activated GR. The degree of enhancement of the Dex-induced transactivation of the GR by PAHs, BaP ≈ IND > pyrene, paralleled the potency of PAHs in activating the AhR. This synergistic interaction was more distinct in an ovarian granulosa cell line (HO23) than in HepG2, 293T, and HeLa cell lines. In addition, Dex suppressed BaP- and IND-induced transactivation of the AhR and decreased BaP-induced cytochrome P450 (CYP) 1A1 expression. Dex also counteracted the BaP-induced decrease in cell viability. Increases in both stress and pollution have accompanied the development of modern society and cities. Furthermore, ovarian granulose cells are essential for reproduction. This report shows the significance of *in vitro* endocrine-related results, which provide a clue for molecular studies of an interactive mechanism between the AhR and GR, and should be confirmed by future *in vivo* studies.

建立檢測環境類雌激素汙染之轉基因斑馬魚
Establish Living Transgenic Zebrafish to Detect Environmental Estrogens

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Abstract

The environmental estrogens are environmental pollutants that can trigger estrogen-like response for human or other animals. Several different kind of environmental estrogens such as the pesticide DDT, biphenol A and PCBs have been extensively reported to reduce the sperm mobility, induce the sex reversal or mammary cancer. Therefore, how to detect the potential environmental estrogens pollution has become an important and public issue today. The current methods to detect the environmental estrogens are largely relayed on electrochemical technology, blood vitellogenin measurement or cell-based reporter assay. However, these methods are suffered from the drawback of either high cost or lower sensitivity. The specific aim for this study is to build up an simple *in vivo* assay method to monitor environmental estrogens in a non-invasive manner. To achieve this goal, we created one stable transgenic zebrafish of Tg(vtg-4.1K:EGFP) that harboring EGFP reporter gene under the control of 4.1kb *vitellogenin* (*vtg*) promoter. Normally, the *vtg* gene is exclusively transcribed in female liver during reproductive season. However, the *vtg* expression can be ectopically induced in male liver or even in embryonic stage after exposure to environmental estrogens. As we expected, the synthetic estrogen of EE2 are able to induce EGFP expression in the liver of transgenic embryos when exposure to EE2 higher than 100 ng/L. To enhance the sensitivity, currently we sought to use adult transgenic male to evaluate the dose- and time-dependent induction of EGFP expression by EE2 and other environmental estrogens.

Induction of Reproductive Toxicity in Male Rats by Motorcycle Exhaust

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Abstract

Motorcycle exhaust (ME) is a major source of air mobile pollutants in urban areas where motorcycles are a popular means of transportation. ME poses a potential health hazard because the gas phase of ME contains carcinogens benzenze and 1,3-butadiene and the particulate phase contains carcinogenic polycylic aromatic hydrocarbons benzo(a)pyrene and benz(a)anthracene. The major objectives of the present study were to investigate the male reproductive toxicity of ME and the underlying mechanisms of toxicity. Male rats were exposed to 1:10 diluted ME by inhalation 1 hour each in the morning and afternoon, Monday through Friday, for 4 weeks. ME inhalation produced marked decreases of sperm content in the testis and the cauda epididymis. ME caused histopathological changes including testicular spermatocytic necrosis and seminiferous tubule atrophy and cauda epididymal formation fo clusers of pyknotic and necrotic sperm cells. ME induced testicular proinflammatory cytokine interleukin (IL)-6 protein level and mRNA expression.

Cotreatment with vitamin E at 50 mg/kg orally for 4 weeks reversed the ME-mediated decrease of spermatid number and induction of IL-6 protein and mRNA in the testis.

The present findings show that ME causes male reproductive toxicity and induces testicular IL-6 in rats by mechanisms involving induction of oxidative stress and inhibition of steroidogenesis.

以雌激素及雙酚 A 探討對澤蛙(*Rana limmocharis*)卵黃蛋白前質(Vitellogenin)之影響

Effects of Natural Estrogen and Bisphenol-A on Induction of Vitellogenin in *Rana limmocharis*

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摘要

多數的水體環境中常會發現化學物質的存在，近這幾年來，除了一般傳統的指標調查之外，許多的專家學者與環保人士皆相當關注仿雌激素或環境荷爾蒙物質所造成的影響。本研究選用澤蛙(*R.limmocharis*)作為指標生物，在曝露於雌激素(17β-estradiol)及雙酚 A(Bisphenol-A)七天及十四天之後，量測其體內卵黃蛋白前質(vitellogenin, VTG)含量的變化情形。VTG 則以 BioRad 之 Experion Pro260 自動電泳分析儀量測澤蛙卵黃蛋白前質含量。本研究結果顯示：(1)澤蛙相當適合作為兩棲類族群檢測環境中雌性激素活性之指標生物。(2)實驗組中，在曝露雌激素十四天後，其 VTG 誘導率為最高者，約佔總蛋白質比例之 0.2%，相當於對照組別 VTG 含量的 3 倍。(3)本實驗數據顯示，雌激素對澤蛙 VTG 的誘導效力大於雙酚 A。(4)從活體實驗中發現 VTG 誘導率與本實驗所注射之雌激素及雙酚 A 濃度具正相關性。因此，從上述訊息得知，雌激素與雙酚 A 的確會使澤蛙體內 VTG 產生誘導，而澤蛙作為指標生物確具可行性。

關鍵字：澤蛙、卵黃蛋白前質、仿雌激素、Experion Pro260 自動電泳分析儀

十溴聯苯醚經哺乳途徑對子代發育及早期神經行為的影響
The Effects of Decabrominated Diphenyl Ether on the Offspring Development and Early Neurobehavioral Through Breast Milk

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摘要

研究背景：十溴聯苯醚(Decabrominated Diphenyl Ether；PBDE 209)是一種阻燃劑，使用量佔全球含溴阻燃劑之第二位，主要添加於耐衝擊聚苯乙烯中作為電子設備之外殼，如電視機、顯示器...。由於其為添加型阻燃劑，因此不會與聚合物結合而易於釋放於環境。文獻指出，在受污染的人類母乳中存有多溴聯苯醚(polybrominated diphenyl ether; PBDEs)，其中也包括 PBDE 209。研究顯示動物出生後直接暴露於 PBDEs，在成年後會出現神經毒性。母乳是新生兒理想的營養來源，但母乳也是人體毒物排除途徑之一，目前並不清楚由哺乳途徑暴露於 PBDE 209 對子代發育及神經行為之影響。本研究利用動物實驗評估後代經由哺乳途徑暴露於 PBDE 209 對發育及早期神經行為的影響。

研究方法：ICR 母鼠分娩後隨機分成六個劑量組，每個劑量組有六隻母鼠。暴露方式是以餵食針每天強迫餵食 5、10、100、500 或 1500 mg/kg 的 PBDE 209，控制組動物則餵食玉米油，直到離乳，此期間仔鼠均以母奶作為唯一的食物來源。哺乳期間觀察仔鼠的發育，包括體重變化、耳羽打開、長毛、長牙、耳道打開、開眼的時間等發育指標，並且進行早期神經行為測試，包括懸崖迴避試驗（第 3-7 天、平面翻正試驗（第 5-9days）、負趨地性試驗（6, 8, 10 天）、空中翻正反射試驗（12-15 天）以及前肢懸掛（14-16 天）等早期神經行為實驗；另於離乳當天測量每隻幼鼠肛門至生殖口的距離(Ano-genital distance; AGD)。

實驗結果：在發育指標方面，耳羽打開、長毛、長牙、耳道打開、開眼的時間，暴露組與對照組並無顯著差異，但在體重的變化上，1500 mg/kg 的暴露組顯著重於其他各組(100 mg/kg 暴露組除外)。離乳當天測量每隻仔鼠之 AGD，發現母仔鼠在 100 mg/kg 及 1500

mg/kg 暴露組的 AGI(AGD/體重)顯著小於控制組(p<0.05)，公仔鼠則是 100 mg/kg 暴露組的 AGI 小於控制組(p<0.05)。早期神經行為的各項實驗參數以每窩的成功率(每窩在一定時間內完成動作的個體數/每窩總隻數)作為結果，經統計後各參數的成功率在各組間均無顯著性差異。但是，本研究觀察到懸崖迴避以及平面翻正實驗在劑量-反應上呈現 U-shaped 趨勢，但無統計差異。

討論：本研究初步顯示十溴聯苯醚可能透過哺乳途徑進入子代區室，影響子代的生長及 AGI，疑似環境荷爾蒙，需進行進一步的確認。在神經行為方面，懸崖迴避以及平面翻正實驗是測試個體神經反射功能，其在劑量-反應上呈現 U-shaped 趨勢，值得進一步探討 PBDE 209 對神經的影響機制。另外，其他動物實驗研究報告指出出生時直接暴露 PBDEs，成年期會有過動現象，因此，本研究未來將進一步探討其對後代成年期之神經毒性。

關鍵字：多溴聯苯醚(polybrominated diphenyl ether)、十溴聯苯醚(decabrominated diphenyl ether)、早期神經行為(early neurobehavioral)、發育(development)

畸形蛙體內卵黃前質素(vitellogenin)、雄性素(testosterone)及雌性素(17β-estradiol)之測定

Measurement of Vitellogenin, Testosterone and 17β-estradiol Concentration in Malformed Frogs

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摘要

本研究團隊於 2008 年 7~9 月於高屏溪與東港溪上游一帶共捕獲 47 隻畸形蛙。為了探討捕獲之兩生類的致畸因子，本研究初步測定捕獲畸形蛙及正常蛙肝臟中卵黃前質素(vitellogenin)、雄性素(testosterone)及雌性素(17β-estradiol)之含量。卵黃前質素濃度分析結果顯示，高屏溪畸形公蛙及母蛙之卵黃前質素濃度分別為，1.46±0.61μg/mg 及 2.15±0.87μg/mg；東港溪畸形公蛙及母蛙分別為 2.23±1.47μg/mg 及 3.00±1.73μg/mg；樣區中捕獲之正常公蛙及母蛙卵黃前質素濃度分別為 1.09±0.56μg/mg 及 1.47±0.85μg/mg。雄性素濃度分析顯示，於高屏溪畸形公蛙(146.17±61.2pg/mg)、東港溪畸形公蛙(186.37±96.8pg/mg)及正常公蛙(218.74±112.1pg/mg)三者間並無顯著差異。雌性素濃度分析顯示，高屏溪畸形公蛙(0.02±0.06ng/mg)、東港溪畸形公蛙(0.02±0.01ng/mg)及正常公蛙(0.02±0.02ng/mg)間亦無明顯差異；高屏溪畸形母蛙(6.45±2.63ng/mg)及東港溪畸形母蛙(9.00±5.18pg/mg)則較高於正常母蛙(3.91±2.77ng/mg)。經分析結果顯示，高屏溪與東港溪上游一帶畸形蛙肝臟中卵黃前質素均有偏高的狀態，再加上畸形雌蛙的雌性素濃度高於正常雌蛙，此結果似乎意味著畸形蛙可能比起正常蛙有接觸較多的類雌性素之物質(estrogen-like)。

關鍵字：畸形蛙、卵黃前質素(vitellogenin)、雄性素(testosterone)、雌性素(17β-estradiol)

福壽螺與梯形福壽螺的成長與攝食之比較

Ecological Comparisons of Growth and Feeding between *Pomacea canaliculata* and *Pomacea scalaris*

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摘要

福壽螺(*Pomacea canaliculata*)從1980年引進台灣之後，其分佈遍及臺灣全島並延伸至離島，但梯形福壽螺(*Pomacea scalaris*)僅分佈於高雄及屏東一帶，除了地理分佈上的差異，目前對於梯形福壽螺的相關探討很少，因此，本論文進行福壽螺及梯形福壽螺的成長與攝食之比較。結果顯示梯形福壽螺受精卵所需的孵化時間(10.4±1.3天)較梯形福壽螺(12.2±2.3天)短，且福壽螺剛孵化的幼螺體型較小，而福壽螺的成長率較高，約兩星期後兩物種成長出現明顯差異，孵化後75日之成長方程式，福壽螺為 $y = 0.29x + 1.09$ ($p < 0.001$)，梯形福壽螺為 $y = 0.16x + 1.68$ ($p < 0.001$)；攝食消化構造的比較發現，福壽螺的顎片、齒舌、中間齒、與胃均明顯大於梯形福壽螺，但腸道長度兩物種無顯著差異；食物通過福壽螺消化道的時間(1.4±0.7天)較梯形福壽螺(2.9±0.7天)短，且福壽螺之每日近似消化率(AD，approximate digestibility) (44.41±24.02%)較高；在胃內含物與排遺物的分析發現，兩物種食性差異不大，唯福壽螺的攝食量較多，而梯形福壽螺食物中含有較多的石頭，碳氮穩定同位素分析發現，兩物種的δ^{13}C值與δ^{15}N值差異不大，表示兩物種食物來源與營養階層相近，此結果和胃內含物與排遺物分析相呼應；但在穩定同位素分析亦發現，屏東九如及萬巒五溝水地點間的δ^{13}C值與δ^{15}N值均有顯著差異，表示兩地點的攝食環境有差別。雖然福壽螺與梯形福壽螺均為外來種，但福壽螺在成長與攝食上的優勢，可能是造成其擴張能力較梯形福壽螺強的重要原因之一。

關鍵字：福壽螺(*Pomacea canaliculata*)、梯形福壽螺(*Pomacea scalaris*)、成長(growth)、攝食(feeding)

出生前暴露 PBDE-47 對子代成鼠自發性行為、學習與記憶影響

Effects of Prenatal Exposure of PBDE-47 on Spontaneous Behavior, Learning and Memory in Adult Mice Offspring

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摘要

多溴聯苯醚類（PBDEs）為一種阻燃劑，普遍存在於環境和人體組織中。2,2',4,4'-tetrabtromodiphenyl ether（PBDE–47）是 PBDEs 的同源物，研究指出臍帶血中 PBDE-47 與母親血液中的 PBDEs 呈現高度相關，此表示 PBDE 會透過胎盤進入胎兒的循環系統。本研究利用動物模式探討暴露 PBDE-47 對仔鼠成年後之學習與記憶能力的影響。CD-1 母鼠在懷孕第六天（GD6）起分四組進行 PBDE-47 每天一次餵食暴露，劑量各為 0.1 mg/kg、0.5 mg/kg、2.5mg/kg 直到 GD17。在出生後第 60 天，從各組母鼠每一胎中，隨機選擇 3 隻公鼠、3 隻母鼠進行開放空間（open field）的自發行為評估；於出生後 66-70 天進行測試學習與記憶能力的莫氏水迷宮（Morris Water Maze, MWM）實驗。自發行為評估中母鼠的站立次數暴露組顯著較控制組低（$p<0.05$），隨劑量上升站立次數有降低的趨勢；0.1、0.5 mg/kg-b.w.-day 公鼠總活動量較控制組低（$p<0.05$），各劑量間有 U 型效應（U-shaped），在 MWM 的各項參數則無統計上的顯著差異。從本研究的結果中推測，胎盤暴露 PBDE-47 會對子代成鼠的自發行為產生影響，且對母鼠的效應可能較公鼠大。

關鍵字：PBDE-47(2,2',4,4'-tetrabtromodiphenyl ether)、自發行為(spontaneous behavior)、學習與記憶(learning and memory)

Effects of Prenatal Exposure of PBDE-47 on Spontaneous Behavior, Learning and Memory in Adult Mice Offspring

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Abstract

Brominated flame retardants are a novel group of global environmental contaminants. Within this group the polybrominated diphenyl ethers (PBDEs) constitute one class of many that are found in electrical appliances, building materials, and textiles. 2,2',4,4'-tetrabtromodiphenyl ether (PBDE-47) is one of congeners of PBDEs and found with high level in human cord blood. The purpose was to evaluate learning and memory in adult male mice prenatally exposed to PBDE-47. Female mice were orally exposed to 0, 0.1, 0.5, or 2.5 mg of PBDE-47/kg of body weight from gestation day 6 (GD6) to GD17. Three male and female offspring were subjected by random sapling per litter to observe the performance of locomotion, resting, rearing and total activity for spontaneous behavior on postnatal day 60 (PND60) , as well as the latency of Morris water maze (MWM) for learning and memory on PND 66-70. The behavior tests showed that the dose-response related on female offspring mice and the U-shaped related in male offspring mice. No significant changes were observed in the latency of MWM. The results indicated that mice prenatally exposed to PBDE-47 might alter spontaneous behavior in female adult offspring rather than the male mice.

Keywords: 2,2',4,4'-tetrabtromodiphenyl ether (PBDE–47), spontaneous behavior, learning and memory

戴奧辛排放係數之調查研究-以固定污染源用一般或事業廢棄物為燃料為例

Investigation of Dioxin Emission Factor – A Case Study of Stationary Pollution Sources Using General or Industrial Waste as Fuel

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摘要

戴奧辛的來源最主要來自燃燒，國內管制對象包括大型廢棄物焚化爐、中小型廢棄物焚化爐、煉鋼業電弧爐、鋼鐵業燒結工廠、鋼鐵業集塵灰高溫冶煉設施、以及固定汙染源，後者廣泛包括各種工業製程，如行政院環境保護署發布之「自中華民國 96 年 1 月 1 日，火化場、輪胎裂解製程、電力業汽電共生業燃煤鍋爐、觸媒再生製程、造紙黑液鍋爐、鋁二次冶煉、銅二次冶等固定污染源，應每 2 年定期檢測戴奧辛排放 1 次」。桃園縣為全國第一工業大縣，各類工業製程相較其他縣市為高，本研究因此針對桃園縣列管之戴奧辛固定污染源用一般廢棄物或事業廢棄物為燃料之事業單位，分析排放管道戴奧辛之檢測濃度與其原料量或污染防制設備系統的操作條件間的相關性，探討影響排放係數的因子，建立本土化之戴奧辛排放係數資料庫。

於 96~98 年調查期間，新增污染源以銅二級冶煉之製程的戴奧辛排放係數為最高，其中 A 科技廠及 B 化工廠排放係數偏高(345.7~27350.3 ng-TEQ/T 及 50.8~8268.0 ng-TEQ/T)，排放係數偏高原因分別為進料量及污染防治設備不足或老舊所致。C 染整廠之排放係數介於 496~5009 ng-TEQ/Ton，排放係數高的原因為使用木屑為輔助燃料，木屑原物料中含有包覆著皮革的大型木製傢俱，皮革原料大部分為塑膠，燃燒後會產生高濃度之戴奧辛。D 紙廠排放係數介於 16.5~451 ng-TEQ/Ton，顯示進料篩選、焚化操作和污防控制皆運轉良好。

關鍵字：戴奧辛、排放係數、銅二級冶煉

戴奧辛數據之不確定度探討-以空氣及土壤為例

Uncertainty in Dioxin Data–A Case Study of Ambient Air and Soil

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摘要

戴奧辛主要來自不完全燃燒，或是來自儲存在自然環境中，皆會釋放到周界空氣，透過呼吸途徑被人體吸入體內，沉降到土壤環境，透過食物鏈途徑被人體攝入體內，危害人體健康，環境與健康之研究議題，因此日益受到重視。戴奧辛數據之不確定度，受到許多參數影響，如從樣品採集時之溫度、溼度、風向、風速等氣象條件，以及汙染源的變動，到實驗室分析時之前處理、儀器跳動、偵測極限等參數影響。量測不確定度評估，可以推估實驗室分析之不確定度；相對之下，評估現場採樣之不確定度，困難許多。應用統計技術，找出異常值，剔除不確定度高之檢測數值，確定使用有效數據，合理解讀環境現象，非常重要。

本研究設計數學統計方法，利用測值之相對標準差(RSD)與不確定度成正比之關係，探討戴奧辛數據的不確定度，推估出合理的 RSD 範圍，以找出異常值。以 90~95 年間新竹垃圾焚化爐鄰近區域周界空氣和土壤戴奧辛數據，驗證提出之構想。首先計算所有採樣點之 17 種同源物的 RSD，皆超過 200%，異常偏高，表示有異常數據。其次，使用主成分分析，發現 D 點測值的分布，明顯不同於其他採樣點，表示異常。接著，再計算 D 點以外所有採樣點之 17 種同源物的 RSD，降到低於 80% (正常範圍 60%~80%)，確認 D 點測值的確異常。土壤戴奧辛數據之正常 RSD 範圍介於 70%~90%，比周界空氣高，可能因土壤樣品取樣的均勻性較空氣樣品差。最後將用圖示法，以客觀表示判定之依據。

關鍵字：戴奧辛、不確定度、主成分分析、相對標準差

懷孕期間暴露 BDE-47 對成熟子代公鼠發育及生殖功能影響

Effects of Prenatal Exposure to Tetrabromined Diphenyl Ether (BDE-47) on Developmental and Reproductive Toxicity in Mice Offspring

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摘要

多溴聯苯醚類(polybrominated diphenyl ethers, PBDEs)是一種溴系阻燃劑，在環境與生物組織均可檢測出 PBDEs。而四溴聯苯醚(tetrabromined diphenyl ether, BDE-47)，是存在人體血液或母乳中最常見的 PBDEs 同源物，已成為新的環境污染議題。本研究探討透過胎盤暴露 BDE-47 的子代小鼠，是否對仔鼠的成長發育及雄性生殖功能方面有影響。懷孕母鼠於懷孕期第 6 天至第 17 天每日以胃管餵食方式進行暴露，劑量分別為 0 (corn oil)、100 μg BDE-47 /kg 、500 μg BDE-47 /kg、2500 μg BDE-47 /kg。於出生後進行生長發育評估，並於第 85 天進行生殖功能之分析。研究結果發現親代暴露 500 μg BDE-47 /kg 造成其子代雄性比例上的減少，並達顯著差異 ($p < 0.05$)。生長發育方面，500 μg BDE-47 /kg 使得子代生長發育指標，有遲緩的狀況。雄性子代尿道至肛門口距離 (anogential distances, AGD)，以體重校正後之數值經統計分析顯示，500 μg BDE-47 /kg 使得 AGD 縮短，並且達到顯著差異 ($p < 0.05$)。而暴露 BDE-47 的子代小鼠，其精子型態異常情形達到顯著差異($p < 0.05$)。精子功能方面，粒線體功能(Mitochondrial Membrane Potential, MMP)100 μg BDE-47 /kg 相較於控制組有顯著的差異($p < 0.05$)；反應性氧化物(Reactive Oxygen Species, ROS) 500 μg BDE-47 /kg 則達統計上的顯著差異($p < 0.05$)。將於事後利用迴歸分析來進行兩者之間相關性探討；睪丸毒性方面則沒有顯著的危害。以及本研究結果將釐清胎兒在子宮內暴露不同劑量之 BDE-47，如何影響雄性子代的發育與生殖功能，提供 PBDEs 類的環境荷爾蒙對人類生殖健康潛在危害之重要資訊。

關鍵字：多溴聯苯醚類，四溴聯苯醚，胎盤暴露，發育，生殖