出國報告（出國類別：進修）

# 屠宰衛生檢查資訊回饋措施對上市豬隻病理病變之影響暨歐洲畜禽肉品中新興污染物質檢測方法之開發及研調分析

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# 摘要

肉品和肉類產品是人類飲食的主要來源。產食動物的健康應受到適當的監測和控制，以減少對食品供應鏈的風險。本研究包含以下策略：1.評估將病理報告回饋給來源養豬場後的結果；2.建立檢測方法並調查豬肉、小牛肉和嬰兒食品中環境污染物和動物用藥的流行情況。

在第一部份，收集並分析我國歷年的屠宰衛生檢查紀錄，並進一步分析屠宰衛生檢查紀錄對牧場端的影響。整體而言，大型養豬場的場數佔全國 7.8%，但生產頭數佔全國總屠宰頭數 45.2%。關於屠體中發現病理病變的百分比，在大型養豬場中，其變異係數（CV）為 45.4%，而小型養豬場為 75.9%。這表示大型養豬場的健康水平比中小型豬場更高。在研究的最後，將屠後檢查結果發送給來源養豬場後分析了病理性病變的的變化。從屠後檢查結果的統計資料指出，肝和肺的百分比每月逐漸降低 0.02%，而心臟與腎臟的病變百分比則逐漸增加。這反映出商業化的疫苗與動物用藥限制了肺部病變的發生率，但卻增加了潛在的心包膜炎病變，因此除了肉眼檢查，還需要一種先進的動物用藥的監測方法。即便如此，屠後檢查結果的回饋措施提高了政府資訊的透明度，生產者收到第一手的檢查資訊，並據以改善豬群的健康，從而使肉類產品更加安全。

在第二部分的研究中，開發了對全氟烷基物質（PFASs）、多氯聯苯（PCBs）、多環芳香烴（PAHs）、多溴二苯醚（PBDEs）、殺蟲劑及抗生素的高靈敏度檢測方法。定量限（LOQ）在 PFASs 中為 0.015-0.15 ng $g^{-1}$，在 PBDEs 中為 0.5 ng $g^{-1}$，符合歐盟第 2002/657/EC 號規定的標準。將開發的方法應用於幾種肉類產品的研究：豬肉、小牛肉和嬰兒食品。本研究結果顯示，豬肉、小牛肉和嬰兒食品中環境污染物的盛行率相當低，並且不會對人體健康構成威脅。

本研究所產出的結果包括對豬和牛肉中環境污染物和動物用藥檢測方法的清楚理解。此外，通過回饋郵件，使養豬者持續取得了從屠宰場端執行的檢查資訊，因此，他們採取了管理行動導致病理性病變的減少。本研究結果可以作為未來在整個食品鏈中保護肉品安全的參考方法。

# 目次

# 研究目的

目前歐盟雖全面在屠宰場由獸醫師實施官方控制，但執行的紀錄資訊僅保留在主管機關內。官方控制的紀錄包括動物來源資訊，以及屠後檢查的屠體病理病變紀錄。病理病變的紀錄與動物的健康品質有直接相關。本研究所在的義大利北部地區，主管當局收集了屠後檢查紀錄，並匯總陳報上級主管機關。在這種單向、向上的資訊流的架構下，農民沒有收到紀錄的副本。歐盟新的食品安全法規 Regulation 2017/625 設立了官方控制資訊管理系統（IMSOC），但此系統偏重於滿足主管機關的官方控制活動。一般來說，屠宰毛豬需要在牧場飼養 6 個月以上，牧場管理人扮演最主要控制豬隻健康的角色。本研究假設農民收到屠後檢查病理病變的報告之後，豬隻的屠後檢查病變結果不會有變化。本研究收集了臺灣各屠宰場的屠後檢查病理病變資訊，其中 2003 年至 2009 年有向牧場管理人提供屠後檢查資訊，然後從後續的屠後檢查紀錄中評估內臟病變的變化情形。

歐盟已禁止在食品鏈中使用全氟辛酸（PFOA）、全氟辛烷磺酸（PFOS)、多氯聯苯（PCBs），多環芳香烴（PAHs），多溴二苯醚（PBDEs），但在文獻中，魚樣品中仍然有微量殘留。為瞭解歐洲食品鏈中所存在的新興污染物質，在本研究中，針對多種殘餘物質開發了高敏感度的檢測和識別方法，然後將其應用於豬肉，小牛肉和動物源性成分的嬰兒食品樣本中，以探討本研究對家畜禽肉品的假設。

# 研究內容

## 一、歐盟肉品安全管理背景

歐洲各國之間的合作

西元 1952 年，6 個歐洲國家（比利時、法國、義大利、盧森堡、荷蘭及西德）簽訂了歐洲煤鋼共同體條約（European Coal and Steel Community, ECSC），該條約創設了一個煤礦與鋼鐵的共同市場並設立超國家機構以監督會員國之間的市場活動。1958 年，羅馬條約生效，設立了歐洲經濟共同體（European Economic Community, EEC）及歐洲原子能共同體（European Atomic Energy Community, EURATOM），深化了會員國之間的經濟合作。1987 年單一歐洲法（Single European Act）生效，促進了歐洲國家的整合並建立了內部市場（會員國的人力、貨物、服務及資金可以自由流動的市場），1993 年馬司垂克（Maastricht）條約生效，建立了歐盟（European Union）及歐元（Euro）。1999 年歐洲議會簽署了阿姆斯特丹（Amsterdam）條約，強化了歐盟的內部結構並強化民主原則。2003 年尼斯（Nice）條約生效，調整了歐盟各機構的組成及立法程序，為接下來的改革做準備。2009 年里斯本（Lisbon）條約生效，修訂了歐盟組織內的民主協商、精簡組織內部架構，並且強化以及提高組織本身之決策力。

有關歐盟肉品安全業務，為歐盟健康及食品安全總署（Directorate-General Health and Food Safety, DG SANTE）之職責，總署內之「健康及食品稽核分析局」（Health and Food Audits and Analysis, 前身為獸醫及食品辦公室，Food and Veterinary Office, FVO）為主要實施檢查、稽核之單位。歐盟健康及食品安全總署與歐洲食品安全局（European Food Safety Authority, EFSA）、歐洲藥物管理局（European Medicines Agency, EMA）、歐洲疾病管制中心（European Centre for Disease Prevention and Control, ECDC）、植物品種辦公室（Community Plant Variety Office, CPVO）及消費者、健康、農業與食物執行局（Consumers, Health, Agriculture and Food Executive Agency, CHAFEA）等機構緊密合作，規劃食品安全相關之法規命令、評估報告、動物防疫措施、教育訓練等以保障食品安全及歐洲消費者的健康。此外，各會員國的食品安全主管機關也與上述機構緊密合作，以確保適當地並持續地保障消費者的健康安全。

## 二、 歐盟肉品安全的立法

在歐洲食品安全管理中，最優先重要的其中之一就是屠宰過程的衛生管理。在第二次世界大戰之後，歐洲各國的屠宰衛生檢查主要在阻止豬旋毛蟲及牛結核病的散播。後續因為動物疾病預防及控制的技術的改良，前述的疾病在屠宰場內的發生率已經大幅減少，取而代之的是肉品食媒性病原（大腸桿菌、沙門氏菌、彎曲菌等）的監測管理。

歐盟有為數眾多的法規與肉品安全有關，其法規的管理密度居於第三位，僅次於車輛及化學品（如表 1）。

表 1. 歐盟與車輛、化學品及食品有關的法規數量

| 主管機關 | 行業別 | 法規數量 |
|---|---|---|
| 歐盟企業及工業總署（Directorate-General for Enterprise and Industry, EC, 2006） | 車輛（Automobiles） | 將近 100 部法規 |
| 歐盟內部市場，產業，創業和中小企業總署（Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs, EC, 2016） | 化學品（Chemical products） | 超過 70 部法規 |
| 歐盟健康及食品安全總署（Directorate-General Health And Food Safety, EU, 2013） | 食品與飼料（Food and feed） | 將近 70 部法規 |

從法規的密度可見，歐盟法規對消費者的保護是非常高的。不像其他消費性行業，消費者可以選擇不同的電視頻道、電信服務、手機製造商，或是不使用；而消費者必須食用食物而生存。食品業者掌握所有的資訊，比消費者握有較高的議價能力（Bargaining Power），因此，食品安全法規必須保護食品安全、也必須保護消費者的權益。

在 2002 年以前，歐盟尚無一部法規能管理所有食品的安全，而是散落於管理各種以食品名稱為主的法規中。隨著食品業者不斷研發新的食品與註冊創新的食品名稱，各種食品安全的隱憂

逐漸顯現。1996 年英國發生牛海綿狀腦病（Bovine spongiform encephalopathy, BSE），重創消費者信心。從 1997 年起，建立新的食品安全管理架構成為當務之急。1999 年比利時還發生了戴奧辛（dioxin）污染動物（雞、豬、牛）飼料事件，法規的訂定刻不容緩。歐盟隨後在 2000 年公布食品安全白皮書（White Paper on Food Safety），強調必須在食品供應鏈、食品科學、法規及食品安全管理等各面向重建消費者信心。新的食品法規以阻絕、預防、追溯性及透明化為原則。隨後於 2002 年公布食品法 Regulation 178/2002，定義了基礎原則，其中之一就是食品業者有追溯的義務。該法規同時也設立了歐洲食品安全局（EFSA），以科學基礎強化食品安全並鞏固消費者信心。

食品法 Regulation 178/2002 清楚定義了食品的追溯工作。食品業者有義務建立食品追溯系統，並且必須保存製造過程中的所有資訊。食品業者必須能辨識及記錄所製造的產品、原料的供應商及產品的買家，當主管機關提出需求時，食品業者必須儘速提送相關資訊。食品業者必須在營運前向主管機關登記，並取得登記編號，以獲得營運許可，並憑此登記編號與原料業者或下遊買家進行交易。

2004 年公布肉品安全有關的一系列法規 Regulation 852、853、854 及 882/2004。與動物來源有關的食品業者，必須記錄動物的來源。當動物被屠宰之後，必須記錄屠宰場編號。各會員國之間所使用的記錄動物方法可能有所不同（耳標、動物護照、條碼等），但所有這些方法必須帶有相同的資訊。屠宰衛生檢查的法源記載於 Regulation 854/2004，由官方獸醫師以肉眼檢查的方式進行屠前檢查、屠後檢查及動物福利等相關檢查。綜觀 2004 年公布的一系列法規，對食品業者賦予的義務如下，以更有效的保護食品安全：

1. 安全性：不安全的食品或飼料不可以進入市場。
2. 安全責任：食品業者有責任在製造、運輸、儲存或銷售時維護食品安全。
3. 追溯性：食品業者須迅速標記產品的原料商或是買家。
4. 透明化：當有理由懷疑某批產品有食品安全疑慮時，食品業者必須將相關資訊傳送給主管機關。
5. 應變計畫：當某產品出現食品安全問題時，食品業者必須立即將產品從市場下架。

6. 預防性原則：食品業者須識別並定期檢視製造過程中的重要管制點，並且須確認所使用的管制措施是適用的。
7. 合作性：食品業者必須與主管機關共同合作以降低食品安全風險。

以 Regulation 1760/2000 為例，為了向消費者傳達食品追溯資訊，在市場上的牛肉產品必須標示以下資訊：

1. 出生地點：牛隻出生地所在的國家。
2. 肥育地點：牛隻飼養或肥育的地點。
3. 屠宰地點及屠宰場編號：屠宰場所在地國家名稱及屠宰場編號。
4. 分切場地點及編號：分切場所在地國家名稱及分切場編號。

為了使消費者有更多的詳細資訊，食品業者可以在產品上標示批號、回溯號碼、來源牧場、牛隻品種等。所有標示的資訊是為了向消費者展示食品鏈資訊，鼓勵消費者在選購之前先瞭解產品。

回顧自 1996 年開始發生的全球重大食品安全事件，最終導致歐盟法規的改變，簡列如下（摘錄自世界各主要媒體新聞）：

1. 1996 年：英國發生牛海綿狀腦病。
2. 1999 年：比利時發生戴奧辛污染家禽、豬及牛飼料事件。
3. 2000 年：歐盟發表食品安全白皮書，以重建消費者信心。
4. 2002 年：歐盟發布食品安全法 Regulation 178/2002，設立歐洲食品安全局。
5. 2004 年：歐盟發布食品安全法規組合 Regulation 852、853、854 及 882/2004，使官方控制（official control）措施施加於動物產品之製造過程，使食品業者須遵守食品安全、動物防疫與動物福利等義務。
6. 2005 年：歐盟公佈微生物危害管控法規 Regulation 2073/2005 及旋毛蟲管控法規 Regulation 2075/2004。
7. 2006 年：印度發生 H5N1 禽流感感染。
8. 2008 年：愛爾蘭發生豬肉戴奧辛污染事件。

9. 2008 年：中國大陸發生牛奶摻混三聚氰胺事件。

10. 2009 年：墨西哥及美國發生新流感（又稱豬流感 H1N1）。

11. 2011 年：德國發生 O104 大腸桿菌感染事件。

12. 2013 年：歐洲發生馬肉摻混食品醜聞。

13. 2017 年：歐洲及超過 45 個國家發生雞蛋及禽肉芬普尼殘留事件。

因此，即使歐盟食品法自 2002 年發布以來，已超過 17 年，這 17 年來仍不時面臨許多新的挑戰。


圖 1. 食品安全事件與食品安全法規重疊年份比較圖（資料來源：本研究收集）

## 三、更聰明食品安全法規（Smarter rules for safer food）

上圖中歐盟重大食品安全事件，每件肉品安全或摻假事件大多涉及多種層級的複雜工作型態。為了制訂適當的法規以面對日新月異的肉品安全事件，歐盟將改善其食品安全管理架構，強化食品安全管理規定，並且以更精簡及易於執行的規定協助成員國順利執行。將近 70 部現有法規已重新組合並構建成為「更安全食品管理法」，具有八個編（title），該法規已公告為 Regulation

2017/625 號法規。

表 2. 歐盟法規 Regulation 2017/625 摘要 （資料來源：作者自行整理）

| 編次 | 條號 | 摘要 |
|---|---|---|
| 1 | 1-3 | 本法規所涵括主題、範圍及定義 |
| 2 | 4-91 | 官方控制、邊境管理、財政來源及收費標準 |
| 3 | 92-101 | 科學實驗室資格標準 |
| 4 | 102-108 | 跨國行政協助、支援與合作 |
| 5 | 109-115 | 實施年度監測計畫 |
| 6 | 116-136 | 有關歐盟在第三國的管制權力、官方訓練活動、建立官方控制資訊管理系統（Information Management System for Official Controls，（IMSOC））等指引 |
| 7 | 137-140 | 違規處分，以及當有會員國管理系統崩潰（disruption）時的臨時管理措施 |
| 8 | 142-145 | 與其他相關法規的權責規劃 |

該法規有以下幾個特性：

1. 財源自籌性更佳（better self-finance）：自從 Regulation 882/2004 授權各國主管機關可以針對食品安全的官方控制設定適當的財源，各會員國可以自由訂定收費標準表。然而，各會員國仍然在設定檢查收費體系乙事承受極大壓力（Christodoulou et al., 2009）。新的法規定義了「強制性費用或規費」，主管機關「必須」為官方控制收取費用，這將比舊的法規 Regulation 852/2004 所定，主管機關「得」收取費用乙節，有更好的財源自籌性。
2. 建立 IMSOC 系統以支持官方控制業務：經由 IMSOC 系統，所有文件上的資料可以自動被查核，會員國之間的文件傳輸也會無紙化。儲存在伺服器中的檔案是唯一正本，而印出的紙本只是業務用的抄件。更進一步的說，系統將會收集到巨量的資訊，然後可以用來預測、預防、標定潛在的／新興的問題。
3. 持續化及常態化：各會員國必須研提並執行多年度監測管理計畫，以確保法規能有效的運作。
4. 官方控制崩潰（disruption）時的介入措施：當某一會員國控制不力，無法有效實施管控

措施，稱之為官方控制的崩潰，無疑地將引發新興風險。歐盟為確保食品安全管理的一致性，必須能做出反應，並採取行動將新興風險排除在食品供應鏈之外。回顧戴奧辛雞蛋事件以及芬普尼雞蛋事件，儘管歐盟向會員國投下高度的注意力以及督導措施，大量不安全的食品仍然在市場上存在了相當久的時間。新的法規賦予歐盟有介入的權力，能組織可調用的資源並打破領土限制，將食品風險排除在歐盟市場之外。

## 四、屠宰衛生檢查資訊現代化

屠宰衛生檢查在食品安全管理中最具有戰略性的地位。歐盟已經透過 Regulation 178/2002 法規 ，以及隨後公布的 Regulation 852、882/2004，將屠宰衛生檢查的組織架構予以一致化。義大利的研究指出，法規規定屠宰衛生檢查以視覺性（visual）的檢查為主，必要時可用觸診（palpation）或是嗅聞（smell）等感官性（sensory）檢查，然而，這會增加交叉污染的風險。2013 年，EFSA 發佈了一系列的指引文件應用於牧場與屠宰場，以使屠宰衛生檢查加速現代化，其中之一就是取消了常規的觸診及感官性檢查，改以在牧場端及屠宰場端加強執行生物風險的措施，並收集延伸性的資訊，以彌補取消屠後檢查的觸診及感官檢查的不足。在化學性污染物質的風險方面，應該要用更具整合性的採樣、檢測與介入措施以監測食品鏈中的化學物質。立陶宛的研究指出，在 10 年研究期間（2000-2009），豬隻屠後檢查有病理病變的比率平均值是 14.92%，從第 1 年起以 1.42%的趨勢逐年增加，所有豬隻外觀並沒有臨床症狀（Januškevičiene et al., 2010）。另一項研究指出，在家禽屠宰場中，檢出敗血症或傳染性華氏囊炎（Infectious bursal disease, IBD）的比例最低，因為農民在牧場裡就可以察覺該等疾病，反而是檢出腹水症（ascites）的比率高於牧場，因為農民很難在牧場內察覺到（Huneau-Salaün et al., 2014）。以上研究結果說明政府應重新組織屠前屠後檢查資訊，以滿足官方控制的需求。而屠後檢查資訊的回饋，也能對牧場管理者有助益。

## 五、食品鏈中的化學污染物質

1999 年 1 月，比利時發生了戴奧辛污染事件。一家動物飼料生產商使用受污染的動物脂肪

製作雞，豬和牛飼料。蛋雞攝入了受污染的飼料後，產蛋性能大幅下降，異常的場面引起了農場經理人的注意。經過深入調查，比利時當局於 1999 年 5 月 27 日向歐盟報告了警報資訊（Haron, 1999）。與微生物風險不同的是，戴奧辛並不能用加熱或冷凍的方式被去除。最好的方式是使用未受污染的原料製作產品。除此之外，持續性的監測食品污染物是從環境中移除污染物質的重要工作。另一方面，1995 年聯合國環境規劃署將持久性有機污染物（persistent organic pollutants, POPs）定義為「存在於環境中，可透過食物網而生物蓄積，並有可能對人類健康與環境造成不利影響的化學物質」。以水資源為例，因其在環境中長久存在的特性，水溶性的 POPs 逐漸地污染飲用水。動物攝取了污染的飲用水，並在體內累積了環境污染物質，最終使污染物質通過食品供應鏈從農場到達餐桌，影響了人類的健康。2004 年，斯德哥爾摩公約生效，共同簽署的國家同意禁止或限制 POPs 的使用。同年歐盟還公布了控制 POPs 的法規 Regulation 850/2004，值得注意的是，全氟烷基物質（perfluoroalkyl substances, PFASs），多氯聯苯（polychlorinated biphenyls, PCBs），多環芳香烴（polycyclic aromatic hydrocarbons, PAHs），多溴二苯醚（polybrominated diphenyl ethers, PBDEs）和殺蟲劑（農藥）都是水溶性的，並能夠進入動物或人類飲用水供應體系，對人類的健康增加風險。

1. 全氟烷基物質（PFASs）

   PFASs 是各種全氟化烷基化合物的統稱，個別化合物的碳原子數目及官能基（羧基或硫酸根）可定義化學名。1947 年，明尼蘇達礦業製造公司（3M）合成了全氟辛酸（perfluorooctanoic acid, PFOA），隨後在 1949 年合成了全氟辛烷磺酸（perfluorooctane sulfonic acid, PFOS）。PFASs 被廣泛應用於防水紡織品、不粘鍋、煎鍋、容器和各種包裝材料。在生產此類產品時，PFASs 可能會擴散到空氣中，然後污染周圍的土壤和水。Gebbink 等人於 1997 年在人血清中檢測到 PFOS。Adinehzadeh 等人在 1998 年指出 PFOA 可能對大鼠具有肝毒性。PFOA 和 PFOS 被公認為是內分泌干擾物質，會對生殖系統產生不良影響，對兒童、孕婦或老年人等弱勢人群的免疫系統產生抑制作用。當動物攝入受污染的飲用水後，PFASs 最終會聚集到食品供應鏈中。2014 年美國環境保護署（USEPA）將 PFOA 和 PFOS 列入了新興風險物質（Emerging Risk substance）清單。此外，EFSA 提

出了 PFOA（每天 1,500 ng / 每公斤體重）和 PFOS（每天 150 ng / 每公斤體重）的成人每日容許攝取量。在 EFSA 提出的科學資料中，共有 25 種不同的 PFAS 化合物。 EFSA 還提到 PFASs 最常在魚類、飲用水和肉類產品被報告。另一項歐洲範圍的調查指出，有 11 條河流不斷排放 PFOA 和 PFOS 到河水中，並最終將河水注入到歐洲海洋（Lindim et al., 2016）。2002 年美國自願性停止生產 PFOA 和 PFOS。2006 年歐盟宣布禁止使用 PFOA 和 PFOS。2019 年歐盟發布了 Regulation 2019/1021，該法規廢除了 Regulation 850/2004，以對持久性有機污染物施加更多限制。在該法規中歐盟鼓勵成員國監控環境中的 PFAS、收集結果並流通資訊，以便評估斯德哥爾摩公約 2004 年起執行後的結果。

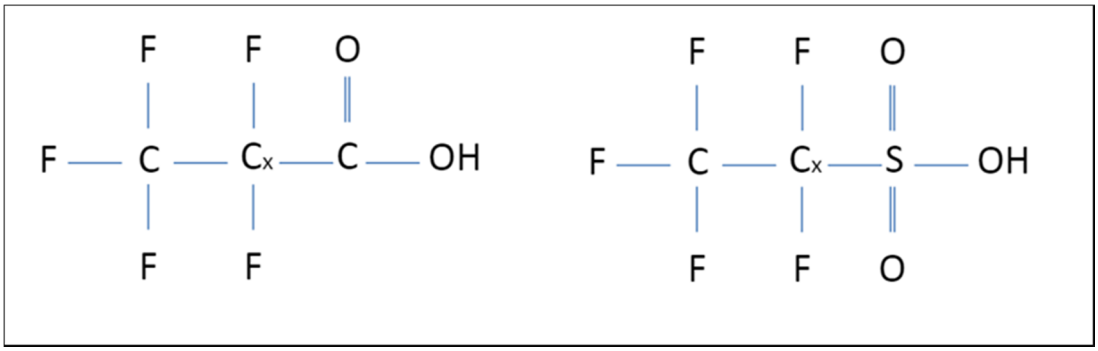


圖 2. 全氟化烷基酸（左圖），及全氟化烷基磺酸（右圖）。資料來源：EFSA。

2. 多氯聯苯（polychlorinated biphenyls, PCBs），多環芳香烴（polycyclic aromatic hydrocarbons, PAHs），多溴二苯醚（polybrominated diphenyl ethers, PBDEs）

除了 PFASs 之外，多氯聯苯（PCBs）、多環芳香烴（PAHs）及多溴二苯醚（PBDEs）也是水體、沈積物及野生生物中常見的污染物質（Erickson 1997；Safe 2003）。這三類化合物具有相似的親脂性及抗分解的理化特性（Xua et al., 2013）。由於這三類化合物的高度生物蓄積特性對人類及動物產生多樣化的毒理效應，因此評估污染的發生情形是重要的任務（Van den Berg et al., 2006; Robertson and Hansen, 2001）。PCBs 是合成的聯苯物質，依照氯原子在苯環上的位置與數量，依序在名稱後編給 BZ 編號，以識別同類物（Ballschmiter & Zell, 1980），以此定義了 PCB 1 至 PCB 209。自然界不存在天然形成的 PCB。PCB 沒有氣味，在常溫下 PCB 呈淺色液體至黑色臘狀固體。由於具有不可燃燒及絕佳的電絕緣性質，因此 PCB 被應用於冷卻劑、潤滑劑、電力設備、電力穩壓器等。然

而，由於在製造或處理過程中的意外洩漏、設備或容器的破損，或含有 PCB 的產品被非法掩埋，PCB 被釋放至環境之中。PCB 可以在環境中停留很長時間，並且不會自然分解（Haddaoui et al., 2016）。PCB 也可分佈在糧食作物中（Liu et al., 2019）。因此，動物或人攝入被 PCB 污染的飼料或食物後將開始在體內累積。從 1977 年開始，美國停止生產 PCB。PCB 已被證實可對大鼠產生中毒性肝病，也發現工人暴露在 PCB 污染的工廠中與肝癌的發生有關（Bosetti et al., 2003; Mallin et al., 2004; Ruder et al., 2014）。在動物模型中，PCB 可干擾甲狀腺素濃度（Gaum et al., 2016）。

PBDEs 由二苯醚結構組成，依照溴分子在苯環上的位置與數量，給予編號標示。每個 PBDE 的標示也適用 BZ 編號規則（從 PBDE 1 到 PBDE 209）。PBDE 的主要用途是在紡織品、電子設備和電腦零件中添加作為阻燃劑。不斷大量廢棄的電腦和電子產品被丟棄在環境中，因此，地下水流經廢棄物，使 PBDEs 流進入地下水層。PBDEs 也會引起內分泌和甲狀腺素干擾（Linares et al., 2015）。PAH 是多重芳香烴的組合物，通常不溶於水（Choi et al., 2010），然而，似乎更習於藏身在土壤之中，被微生物分解後成為可溶性代謝產物（Johnsen et al., 2005），PAH 主要引起人類肺癌和膀胱癌的風險（Mastrangelo et al., 1996）。

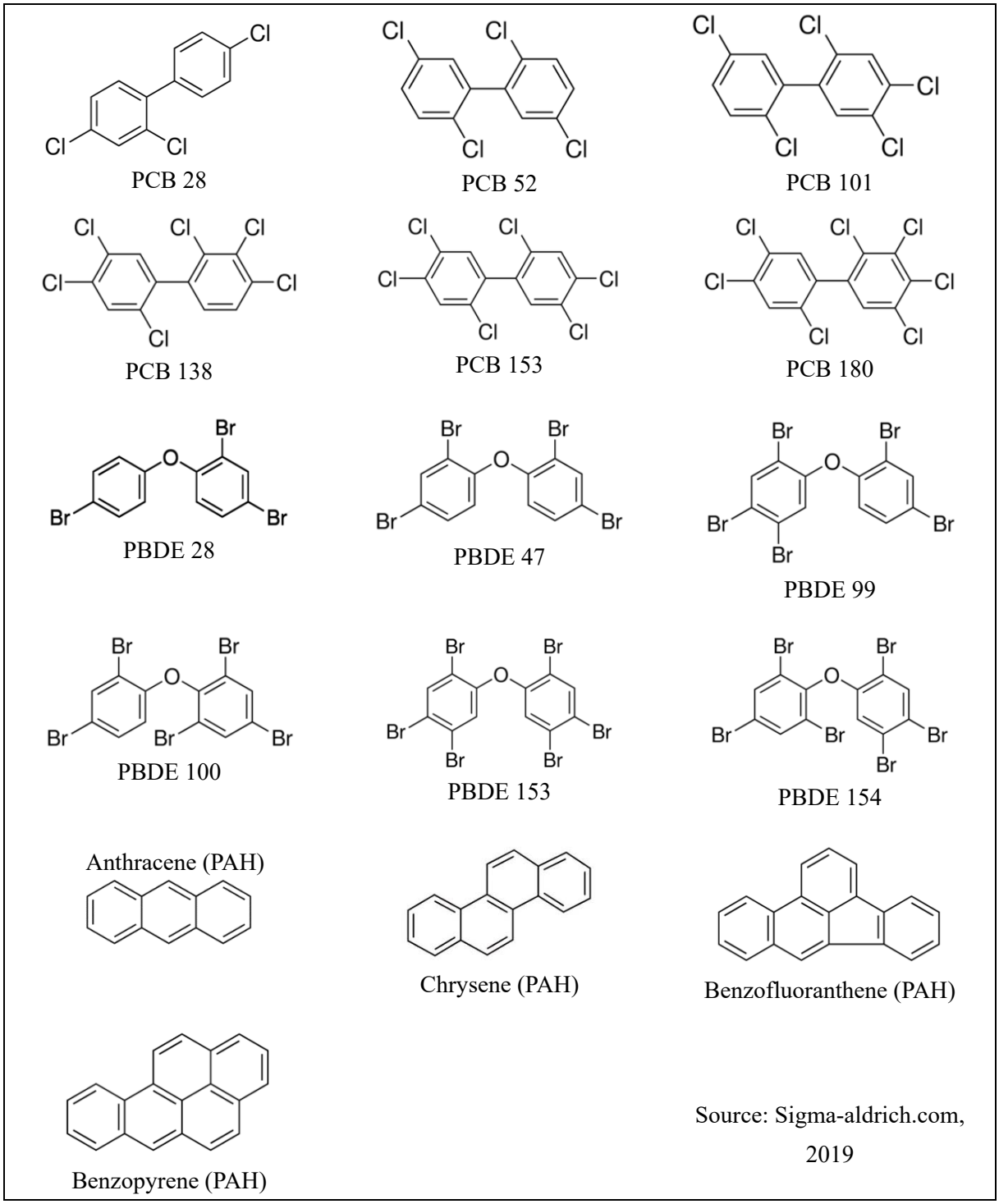


圖 3: 有關 PCBs、PAHs 及 PBDEs 的名稱與分子結構。

3. 殺蟲劑

殺蟲劑是殺死害蟲或真菌微生物的農業用化學物質，它們被施用在作物、蔬菜或水果的生長田地上。灌溉水和風可以使殺蟲劑分佈在田地，或排水溝渠，進而擴大了污染範圍。家畜禽攝入被污染的飲水與作物後，殺蟲劑能分佈在組織中（Vijay & Vikas, 2011）。DDT

（dichlorodiphenyltrichloroethane）是知名的殺蟲劑，能蓄積在屠宰山羊與綿羊的組織中（Nath et al., 1998）。過去的研究已指出 DDT 有造成影響兒童神經組織發育的風險（Eskenazi et al., 2006）。

4. 食品中的動物用藥殘留

為了促進動物的成長、預防疾病、治療疾病和提高飼料效率等因素，農場管理人可能會在飼料中添加動物用藥。動物攝入飼料後，藥物或其代謝產物的原始形式可能會分佈並積聚在動物的組織或器官中。農場管理人必須遵守「停藥期」規定，以便讓動物有時間將攝入的藥物完全代謝。未能妥善遵守停藥期或非法使用動物用藥將導致食品中藥物殘留。以芬普尼（fipronil）及三亞螨（amitraz）為例，屬於抗寄生蟲藥，通常作為治療犬或貓的跳蚤、虱子或壁蝨感染，然而，芬普尼非法用於產蛋雞將會在雞蛋中殘留（Maclachlan, 2008），然後雞蛋將流入市場供人食用。在食品供應鏈中，動物體內的藥物殘留無法用肉眼檢查，屠宰後也無法經由製造程序加以排除。最終，它進入了人類的食物，對人類健康也構成了風險。

含有藥物殘留的食品對人體健康有害。長期攝入殘留藥品的食品的人將逐漸積累潛在的風險。弱勢群體例如兒童、病情嚴重的患者、對特定藥物過敏的人，攝入後可能會發生疾病，甚至死亡。可危害人類健康的情形如下：

A. 藥物超敏反應（hypersensitivity）：攝入β-內醯胺（β-lactam, 青黴素家族）藥物可導致大多數過敏綜合症候群。

B. 致癌作用：Sulphamethazine、Oxytetracycline、Furazolidone 類。

C. 致畸胎作用：Aminoglycosides、Polymyxins、Tetracycline、Vancomycin、Fipronil。

D. 腎毒性：Aminoglycosides、Methicillin、Cephalosporins、Polymyxins、

E. 骨髓毒性：抑制了造血能力，Vancomycin、Chloramphenicol。

F. 中毒反應（Clenbuterol、Amitraz）：Clenbuterol 具有β-致效劑的作用，用於治療呼吸道疾病。然而，它被誤用作為增加瘦肉的生長促進劑。患者食用後出現心跳過快和心房顫動（Sporano et al., 1998）。Amitraz 的過量使用會導致中樞神經系統和呼吸系統的抑

制。

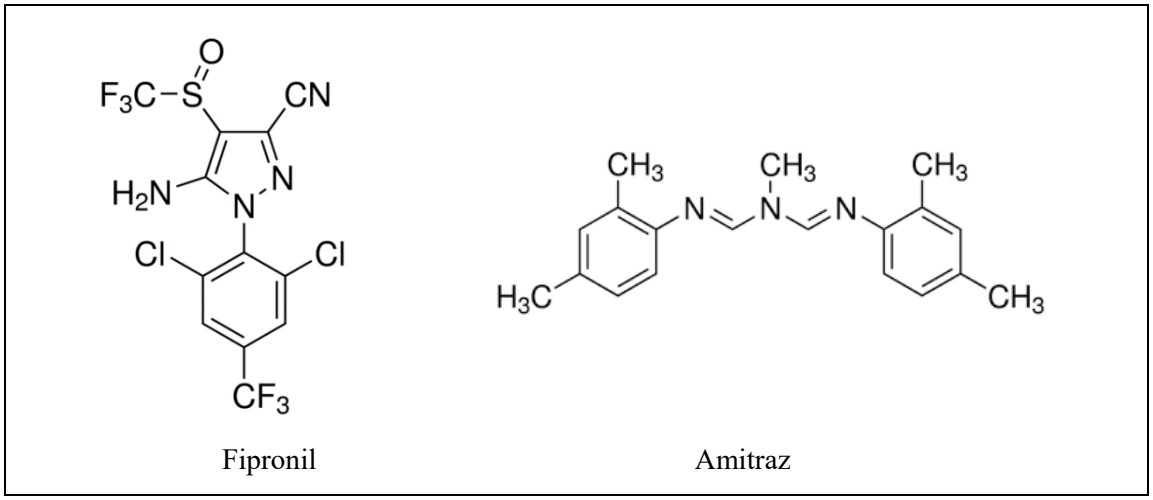


圖 4. 抗寄生蟲藥 Fipronil 與 Amitraz 的分子結構圖。來源： Sigma-aldrich.com, 2019

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## 研究主題一：從屠宰場向豬隻牧場回饋屠後檢查資訊可能會影響上市豬隻的健康水準


### 摘要

根據歐盟法規 Regulation 853/2004，食品鏈資訊是往下一階段（單向）傳遞的，在屠宰場內，屠宰衛生檢查獸醫師應逐頭檢查，在切除不適合食用的病變之後，還須記錄檢查結果。因此，隨著時間的推進，可以用來評估每個牧場的豬群健康水準。本研究汲取臺灣 2001 年至 2017 年各豬隻屠宰場屠後檢查資料，其中在 2003 年至 2009 年期間，各牧場都會收到每月屠後檢查報表。以 2011 年為例，微型、小型養豬場場數佔全國出豬總場數 53.6%，但屠宰頭數僅佔全國屠宰頭數 9.8%；相反地，大型養豬場佔總出豬牧場數 9.7%，但卻生產了 49.8%的屠宰毛豬。再分組收集每組牧場豬群的病變紀錄比率，據以計算各組的變異係數（coefficient of variation, CV）值。微型、小型牧場的 CV 值變化較大（78.5%、59.3%），而中型及大型牧場的 CV 值變化較小（分別為 50.2%、42.5%）。最後，以月平均值作圖，肺臟病變比率從 2005 年起逐年下降，肝臟病變從 2007 年起有反轉向下的趨勢，而心臟與腎臟病變的比率仍逐年上升。本研究結果說明了將屠後檢查結果回饋給來源牧場後可以降低肝臟與肺臟的病變比率。


### 簡介

屠宰衛生檢查是現代化食品安全及公共衛生管理最重要的業務之一。屠宰衛生檢查獸醫師在屠宰場內進行屠前檢查、屠後檢查，以及設施設備、屠宰作業等離線檢查，涵蓋了動物防疫、屠宰衛生、動物福利及公共衛生等多面向的施政目標（EFSA, 2011）。除了反映公共衛生的管理之外，屠後檢查病理病變記錄通常能反映出一個牧場潛在的問題。丹麥的主管機關與屠宰場公會及牧場協會協議架設電腦資訊系統（Willeberg et cl., 1984），以協助辨識高發生率的屠體病理病變，並提供給專業獸醫人員參用。屠後檢查資訊的另一個潛在的延伸應用是用來並評估動物福利（Ellerbroek et al., 2011），根據丹麥豬場使用的有損動物福利的指標性病變，最高發生率的病

變是膿瘍及咬尾（Cleveland-Nielsen et al., 2004）。雖然上市屠宰豬隻的病變比率不能代表全國性豬群（含有種豬、哺乳豬及肥育豬等非屠宰豬隻），但仍可視為恆定的指標且不會隨著時間而影響（EFSA, 2011; Harley et al., 2012）。儘管屠後病理病變的資訊可以有益於牧場改善生產管理，但歐盟法規 Regulation 854/2004 並未規劃將屠後檢查病變資訊傳遞給來源牧場及牧場特約獸醫師參考（Harley et al., 2012）。我國曾經建立自願性的資訊回饋體系以收集屠後檢查病理病變資訊並寄送至豬隻來源牧場。本研究將探討回饋資訊對於屠後病理病變的影響，特別注重在隨著時間推移的變化，並強化屠宰衛生檢查資訊的使用。

## 1.1 材料與方法

### 1.1.1 資料來源

本研究資料汲取自 2001 年至 2017 年我國 69 家豬隻屠宰場屠後檢查病理病變資料。屠宰衛生檢查獸醫師依據規定須逐頭檢查豬隻屠體與內臟，當屠體或內臟檢出病變時，獸醫師必須切除此病變並做成書面紀錄，在 2004 年至 2005 年期間，本局實施了一系列在職訓練與溝通工作，將屠宰衛生檢查判定標準達到全國性一致化。在 2003 年至 2012 年期間，有自願性的收集豬隻來源牧場資料，並在 2003 年至 2009 年期間每月向各牧場寄送豬隻健康回饋報表供農民參考。該報表可用來審視牧場疾病防疫管控策略及飼養管理計畫的成效。

### 1.1.2 資料分析方法

資料分析工作劃分為三個工作項目：

#### 工作項目 1：牧場的維度（dimension）

各牧場的維度具有很大的異質性，以 2005 年生產屠宰豬隻頭數計算，維度從 1 頭至 98,094 頭。家庭式管理的牧場大多是微型、小型牧場，而大型牧場需要雇用更多的專業工作人員來管理牧場以獲取更好的經濟效率。由於豬隻拍賣市場常以 50 頭豬隻為計算單位，本研究將牧場規模以下列年生產頭數分組計算：1-100（微型牧場）、101-600（小型牧場）、601-2,500（中型牧場）以及 2,500（大型牧場）以上。

### 工作項目 2：以地理位置評估豬隻牧場數、生產頭數以及屠宰頭數

以 2011 年為例，有 6,129 個牧場生產了 7,062,407 頭毛豬並且在 19 個縣市完成屠宰衛生檢查。

### 工作項目 3：以各臟器檢出的病理病變計算病變比率

經檢視屠後檢查資料庫，最常被檢出病變的臟器為肝臟、腎臟、心臟及肺臟。在此將每月收集的病變計數訂為 $P_{organ,month}$ ，其計算式：

$$P_{organ,month} = \frac{Number\ of\ pathological\ records\ of\ the\ organ}{Numbers\ of\ pigs\ slaughtered} \times 100\%$$

## 1.2 研究結果

### 工作項目 1：牧場的維度（dimension）

在 2005 年，有 56.2%的養豬場為微型、小型養豬場，所生產豬隻佔屠宰頭數的 11%，有 36%為中型養豬場，佔屠宰頭數的 43.8%；大型養豬場數佔 7.8%卻生產了 45.2%的屠宰毛豬。CV 值反映出了各分組牧場豬群健康水準的變異性。微型、小型養豬場具有高度的變異性（75.9%、59.4%），而中、大型養豬場有較低的變異性（分別是 54.6%及 45.4%）（圖 1.A）。到 2011 年，微型、小型養豬場佔的比率下降了（-2.6%），生產的屠宰豬隻頭數比率也比 2005 年更少（-1.2%），中型養豬場微幅增加（+0.7%），但生產的屠宰豬隻比率減少了（-3.4%）。大型養豬場的比率增加 1.9%，所佔屠宰豬隻的比率也提高至 49.8%（+4.6%）。2011 年小型養豬場病理病變比率的 CV 值仍然很高（78.5%、59.3%），而中型與大型養豬場的 CV 值都有下降（分別是-4.4%、-2.9%）（圖 1.B）。

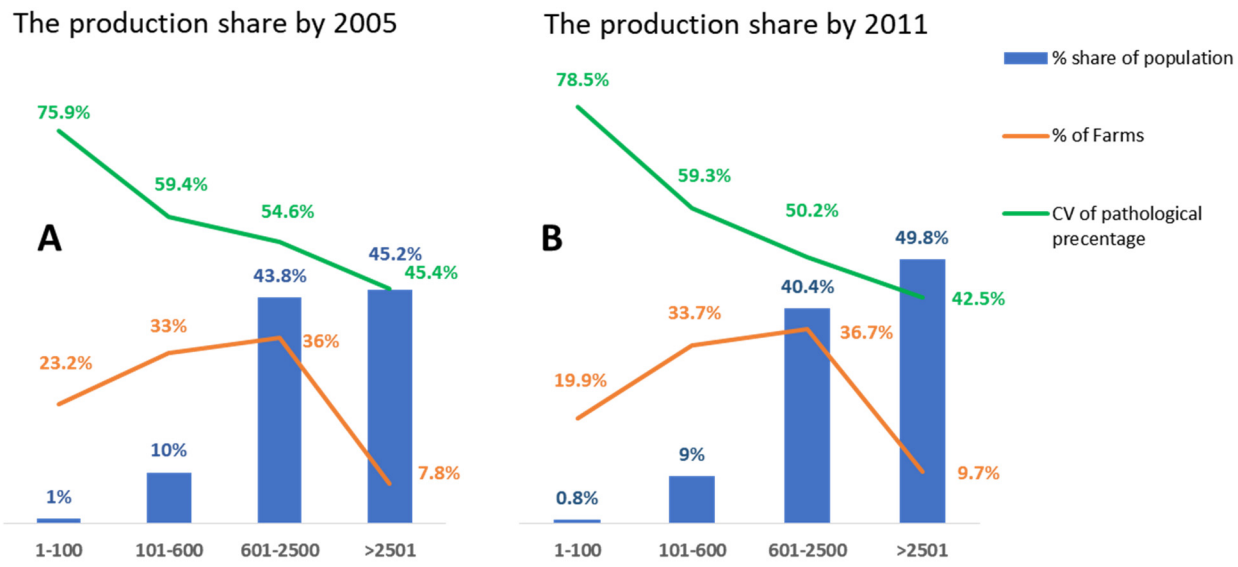


圖 1. 各規模養豬場比率、病理病變的 CV 值以及生產屠宰豬隻的比率圖。(A)2005 年及(B)2011 年。

## 工作項目 2：以地理位置評估豬隻牧場數、生產頭數以及屠宰頭數

臺灣的中部與南部縣市分布有密度最高的養豬場及生產豬隻頭數（圖 2. A 及 B）。由於有大量在地豬肉消費需求，豬隻被運送到消費人口較密集的北部、中部及南部縣市屠宰（圖 2. C）。

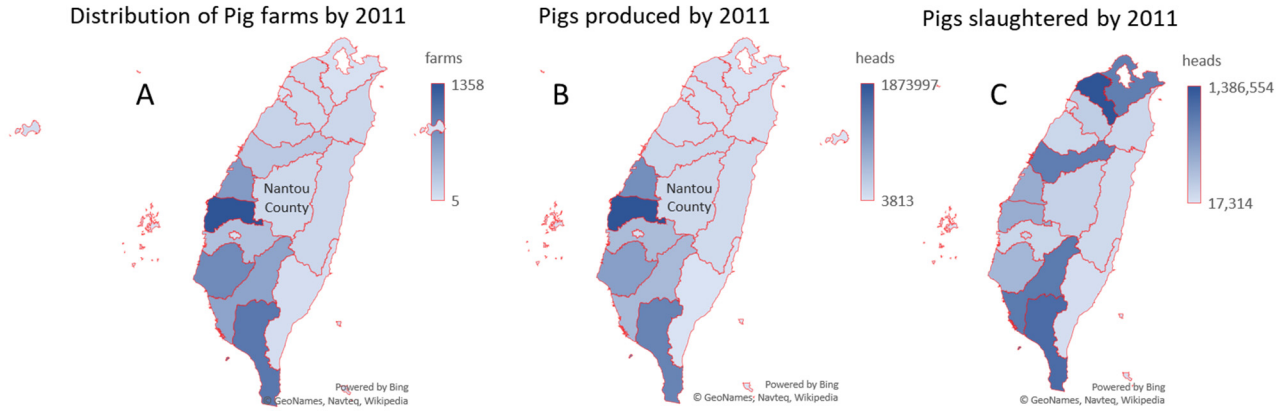


圖 2. 豬隻牧場、生產頭數以及屠宰頭數分布在各縣市的密度圖。

## 工作項目 3：以各臟器檢出的病理病變計算病變比率

以年度平均值作圖，肺臟病理病變的比率似乎有逐年減少的趨勢，而腎臟與心臟的病變比率逐年增加，肝臟的病變比率範圍較大，在 5.38%至 12%之間（圖 3）。最常見的病理病變分別是肝臟的蛔蟲斑、囊腎／水腎、心包膜炎以及肺炎／胸膜肺炎。

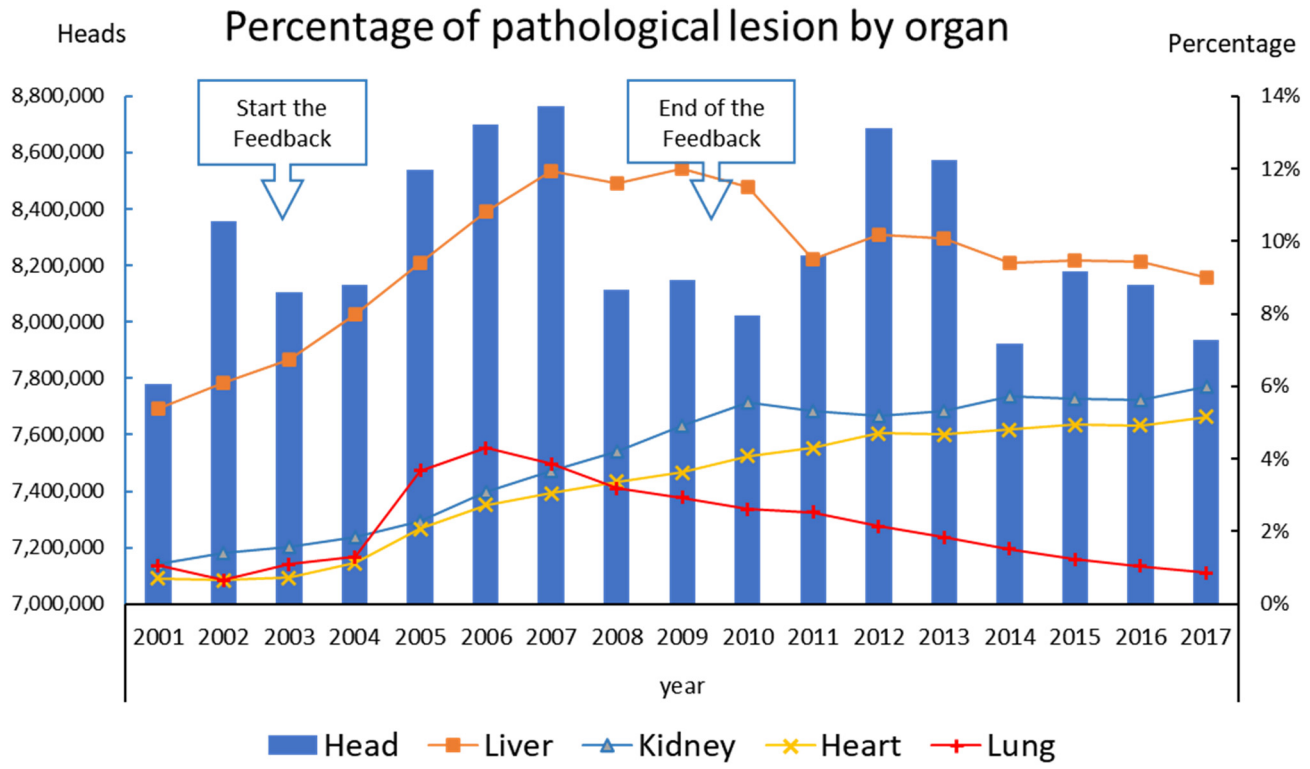


圖 3. 各臟器病理病變比率年度平均值曲線圖。

若以各月份平均值作圖，可發現肝臟病變有季節性的循環現象；更進一步地說，五月及六月是相對高峰期，而一月及二月是相對低點（圖 4）。

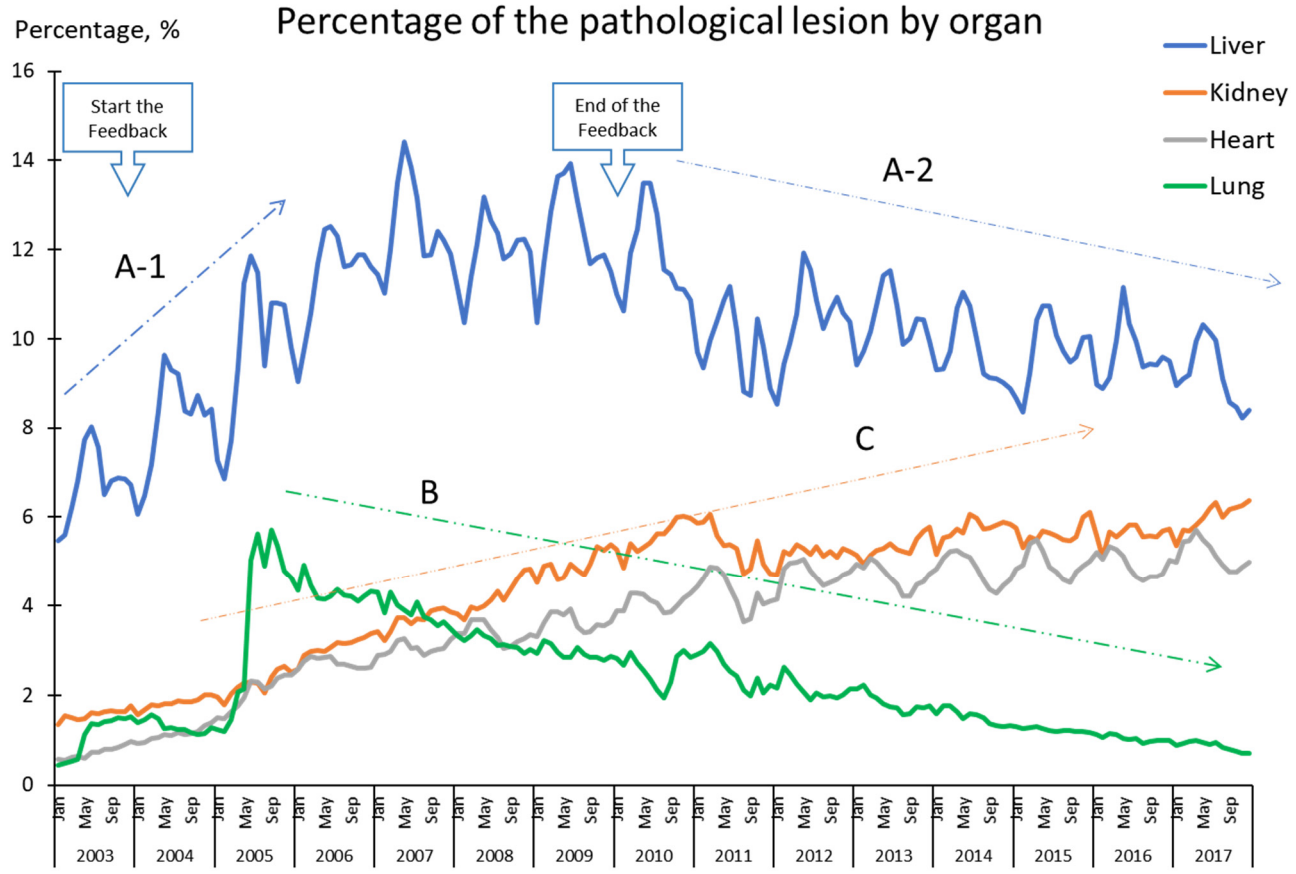


圖 4. 各臟器病理病變比率月份平均值曲線圖。

如圖 4 所示，肝臟病變的比率從 2003 年起逐漸增加（如 A-1 段），並且在每年 5 月或 6 月份達高峰，在 1 月或 2 月到達相對低點。2009 年起停止寄發豬隻健康資訊回饋報表之後，肝臟病變的比率有逐年降低的趨勢（A-2 段）。長達 7 年期間的資訊回饋報表停止寄發之後，從 2009 年起一直到研究結束年度（2017 年）肝臟與肺臟的病變比率還持續降低。

在 2005 年期間，本局將肺臟病變的處置方式均一化。不論屠宰業者是否自發性的將肺臟病變廢棄，屠宰衛生檢查獸醫師必須切除肺臟病變並且記錄。此均一化的處置實施後，肺臟的病變紀錄從 2005 年起急遽升高，因此肺臟的廢棄病變資料也經由資訊回饋報表即時寄送給來源牧場。6 個月之後，肺臟的病變比率開始穩定的下降（如 B 段）。腎臟及心臟的病理病變比率在資訊回饋期間及停止資訊回饋之後仍然保持逐漸增加的趨勢（C 段）。

從病理病變比率的變化可發現有季節性循環的現象。除了肝臟病變之外，腎臟病變比率在 1 月及 2 月達相對最低點，而在 11 月及 12 月達到相對高點（表 1）。

表 1. 2003 年至 2017 年期間各臟器的病理病變最高最低月份

| 臟器名稱 | 月份別 | |
|---|---|---|
| | 相對最高 | 相對最低 |
| 肝 | 1 月、2 月 | 5 月、6 月 |
| 腎 | 1 月、2 月 | 11 月、12 月 |
| 心 | 1 月、2 月、8 月 | 3 月、5 月、6 月、12 月 |
| 肺 | 8 月、9 月、10 月、11 月、12 月 | 2 月、3 月、4 月 |

在 2003 年至 2009 年期間，本局向來源牧場寄發豬隻健康回饋報表，因養豬牧場出豬期程具有動態性，因此每月寄發報表的收件牧場數為浮動數值，以 2003 年為例，寄發數在 3,073 至 3,842 場之間，平均值大約是每月 3,567 場（表 2）。

表 2. 每月寄發豬隻健康回饋報表的收件牧場數

| Year 2003 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| 3,799 | 3,073 | 3,691 | 3,579 | 3,842 | 3,413 | 3,222 | 3,698 | 3,707 | 3,704 | 3,512 | 3,563 |

Average: 3,567

儘管豬隻健康資訊回饋報表自 2009 年 12 月後停止寄送，全國屠宰豬隻肝臟及肺臟的病理病變比率仍然以每月下降 0.02%的趨勢持續進行，而腎臟及心臟的病理病變並沒有下降的趨勢（分別是月增率 0.008%及 0.013%），並且出現季節性循環的現象（圖 5）。

以上初步分析的結果反映出四種主要臟器病理病變的描述性統計結果可以做為主要評估牧場豬隻健康水準變化的參數。

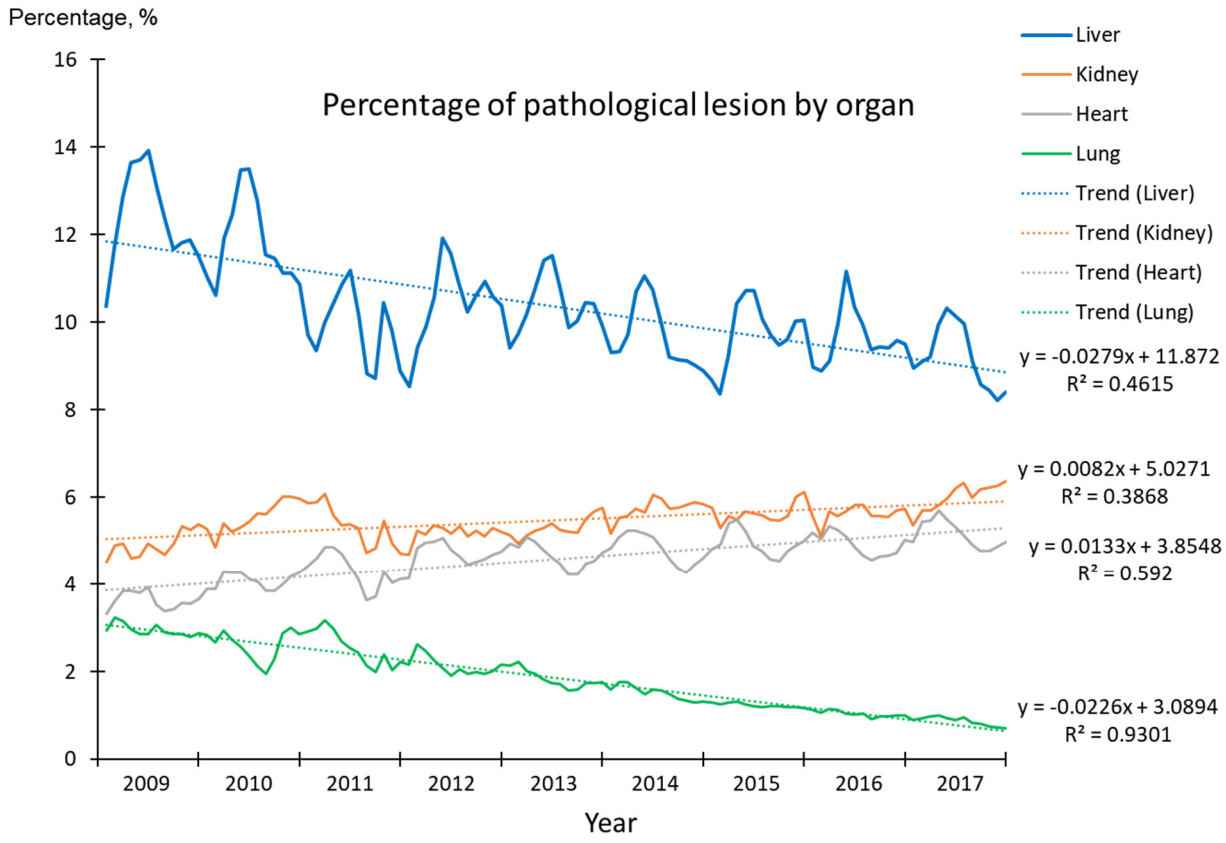


圖 5. 在 2009 年 12 月停止寄發豬隻健康回饋資訊報表後，屠宰豬隻各臟器病理病變比率趨勢圖。

## 1.3 討論

### 工作項目 1：牧場的維度（dimension）

2011 年全國中型及大型養豬場的場數佔全國總場數的 46.4%，且生產了佔全國 90.2%的屠宰毛豬。根據歐盟統計局（EUROSTAT）在 2014 年公布的統計資料略以，歐盟國家（28 個會員國）2011 年在養頭數 400 頭以上養豬場佔總場數 1.7%，生產了佔總數 77.9%的成豬，但是，各會員國之間存在很大的差異。在比利時、捷克、丹麥、愛沙尼亞、愛爾蘭、西班牙、義大利、賽浦路斯、荷蘭、瑞典及英國等 12 個會員國，400 頭以上的養豬場佔全國總場數的 90%，而波蘭、羅馬尼亞只佔總場數的 33%。飼養規模小於 10 頭的養豬場在羅馬尼亞（佔 62.8%）、克羅埃西亞（45.3%）、斯洛維尼亞（佔 31.4%）、立陶宛（佔 28.8%）及保加利亞（佔 25.8%）等國家最多。以歐盟層級而言，雖然小型牧場只生產了佔總數 3.8%的屠宰豬隻，但是佔全部牧場

總數的 73.3%。目前缺乏歐盟內大型或小型牧場屠宰豬隻病理病變的 CV 值，即便如此，來自塞爾維亞的研究資料指出，在小型養豬場的豬隻屠宰後具有病理病變的盛行率很高（Čobanović et al., 2019）。歐盟法規 Regulation 852/2004 並未規定須將屠宰後病理病變資料提供給來源牧場做參考，也許新的食品安全法規 Regulation 2017/625 所設立的 IMSOC 系統，會是將強制性收集屠後檢查結果及來源牧場資訊並做連結以加強預防食品安全風險的法源依據。

## 工作項目 2：以地理位置評估豬隻牧場數、生產頭數以及屠宰頭數

我國的國土面積為 36,197 平方公里，與歐盟會員國相比，面積與荷蘭（37,824 平方公里）相當，我國與其他國家並無陸路相連，所有的屠宰豬隻都是國內生產的。養豬場高度集中於中部與南部縣市，同時也是主要的農業生產縣市，而豬隻的屠宰活動分別集中在北、中、南部地區，因為那裡具有大量的居民，大多數消費者偏好購買 24 小時內屠宰的豬肉。與歐盟國家相比，豬隻的生產活動是跨國境的，2013 年丹麥、德國、西班牙、法國、荷蘭及波蘭生產了 3 分之 2 的豬隻，而德國與波蘭是進口最多屠宰用豬隻的國家。

## 工作項目 3：以各臟器檢出的病理病變計算病變比率

豬隻屠後檢查的病理病變比率是用來評估豬群健康水準的有力參數，丹麥衛生計畫（Danish health scheme）是最早實踐收集屠宰衛生檢查資料並用來改善來源豬場的健康情形（Willeberg et al., 1984）。豬隻病理病變的每月報表及趨勢變化情形，反映出豬場端的流行病學、風險因子及針對疾病控制的生產管理策略（Sanchez-Vazquez et al., 2011, and Correia-Gomes et al., 2017）。在英國，肝臟乳白斑病變在 3、4 月發生最少，在 9、10 月發生最多；心包炎在 12 月、1 月份發生最少，在 5、6、7 月發生最多（Correia-Gomeset al., 2017）。豬蛔蟲是肝臟發生乳白斑的主要原因，並且導致肝臟在屠後檢查時被判定廢棄。儘管我國養豬場在 2003 年起開始收到豬隻健康資訊回饋報表，肝臟病理病變的比率仍持續上升直到 2006 年，隨後才發生反轉並逐漸下降，季節性起伏的現象仍然存在，但是整體而言以每月 0.02%的速率下降。

下降的趨勢反映出養豬農民採取了正確行動以減少豬蛔蟲症的發生。一項針對屠體病理病變與屠體重量的研究指出，豬隻平均每日增重為 441.1 g，但是患有肺部疾病的豬隻卻明顯減少，如嚴重肺炎（-39.4 g）及粘黏性胸膜肺炎（-32.8 g）、地區性肺炎（-11.9 g）、輕度胸膜炎（-10.4 g）、

中度胸膜炎（-9.8 g）（Schuh et al., 2000）。除此之外，肺臟病變的趨勢線也反映了我國的養豬農民在 2005 年起從豬隻健康資訊回饋報表中收到了高盛行率（5.7%）的資訊後，隨即採取正確的行動以減少肺臟病變的盛行率，到了 2017 年，肺臟病變的盛行率只有 0.87%。對比於立陶宛的 10 年期研究，在無法回饋檢查資訊給養豬牧場的情形下，屠宰場內檢出的病理病變以每年 1.42%的趨勢逐漸增加。（Januškevičienė et al., 2010）。因此，回饋屠後病理病變的資訊至養豬牧場以促使病理病變在牧場端就地減少，無疑地是未來的趨勢。

腎臟的病理病變主要是水腎或是多發性囊腎，此類病變大多是遺傳性的（Wells et al., 1980 and Wijeratne et al., 1980），而且不會造成食品安全風險。

心臟的病理病變主要是纖維素性心包膜炎，主要的病理機轉是來自於 *Pasteurella multocida* 以及黴漿菌 Mycoplasmas （*Mycoplasma hyopneumoniae*, *M. hyosynoviae* and *M. hyorhinis*）（Pors et al., 2011 and Buttenschøn et al., 1997）。至於為何發生肺臟病變減少、心臟病變卻增加，推測是以商業化的疫苗免疫或抗生素的處置限制了肺臟病變的盛行率，但卻增加了心臟的潛在感染（Buttenschøn et al., 1997 and Paladino et al., 2017）。因此，除了以肉眼執行屠宰衛生檢查之外，以先進的方法監測肉品中的動物用藥殘留，甚至包括其他有害物質物質的污染是必須的。

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## 研究主題二：歐洲家畜禽肉品中全氟烷基物質（PFASs）、多氯聯苯（PCBs），多環芳香烴（PAHs），多溴二苯醚（PBDEs）、殺蟲劑、動物用藥品檢測方法之開發與應用


### 摘要

人類的飲食中包括大量的肉品及肉類產品，但由於存在殘留物質，因此，大量被消費的肉品須要針對殘留物質做監控。歐盟委員會尚未對某些環境污染物（例如全氟烷基物質（PFASs）和多溴二苯醚（PBDEs）規定最大限量；歐洲食品安全局（EFSA）科學小組進一步建議，應收集更多食品中 PFASs 的發生數據，以提高未來暴露量計算的準確性。因此，本研究以液相層析-串聯高解析度質譜儀（LC-HRMS）和氣相層析-串聯質譜儀（GC-MS/MS）對來自 8 個歐盟國家豬肉中的 PFASs 和 PBDEs 微量污染物的分佈進行了研究。除僅在 1 個奧地利豬肉中檢測到全氟辛酸 PFOA（其濃度為 0.531 ng $g^{-1}$）外，未檢出 PFASs。在 77 個樣本中的 3 個中檢測到 PBDEs：1 個德國的豬肉樣本存在濃度為 0.53-0.77 ng $g^{-1}$ 的 PBDEs 同類物；其餘 2 個分別來自荷蘭（PBDE 153, 0.53 ng $g^{-1}$）和義大利（PBDE 100, 0.62 ng $g^{-1}$）。研究結果闡明豬肉樣品中的 PFOA 與 PBDEs 濃度偏低，並不會對人類造成風險。正如歐洲委員會所建議，須要進行持續性的的研究以不斷監測在食品中的存在。

為了保護嬰兒，食品安全監控勢在必行。本研究也調查嬰兒食品中新興風險物質的存在，其中一些污染物為內分泌干擾物，並在 112 種不同類別的嬰兒食品（肉品、魚、蔬菜、水果及奶酪）及 108 件小牛肉樣品中分析了持久性有機污染物（POPs），全氟烷基物質（PFASs），殺蟲劑和抗生素。研究結果顯示，未發現任何殘留。建議持續監測並收集數據，供歐洲食品安全局和歐洲藥品管理局評估並發布敏感族群的暴露量與成人每日攝取量之用。

## 2.1 材料與方法

作為內標（internal standard）的 $^{13}$C-labeled PFOS (MPFOS) 、 $^{13}$C-labeled Perfluorononanoic acid (MPFNA)以及 17 種 PFASs 標準品購自 Fluka 公司，作為內標的 FBDE（3-fluoro-2,2',4,4',6-pentabromodiphenyl ether）以及 PBDEs 同類物（PBDE 28; PBDE 33; PBDE 47; PBDE 99; PBDE 100; PBDE 153 and PBDE 154）標準品、PCBs 同類物（PCB 28; −52; −101; −138; −153 and −180; 內標為 PCB 209）購自 AccuStandard 公司。固相萃取管柱（solid-phase extraction cartridge, SPE）購自 Waters 公司；QuEChERS 試劑（QuE Citrate、QuE-Z SEP 等）購自 Supelco 公司，有機氯殺蟲劑 Organochlorine pesticides (OCs) (aldrin; α-hexachlorocyclohexane (α-HCH); β-hexa-chlorocyclohexane (β-BHC); hexachlorobenzene (HCB); dichlorodiphenyldichloroethylene (DDE); dichlorodiphenyltrichloroethane (DDT); dichlorodi- phenyldichloroethane (DDD); endosulphan I; endo-sulphan II; endosulphan sulphate; endrin; heptachlor; heptachlor epoxide; lindane and trans chlordane) 購自 Restek 公司。有機磷殺蟲劑 Organophosphorus pesticides (OP): chlorpyriphos diazinon, disulphoton, ethoprophos, mevinphos and phorate, and 4-nonylphenol (IS for OCs and OPs) 購自 Sigma-Aldrich.。多環芳香烴 PAHs: chrysene、benz(a)anthracene、benzo(b)fluoranthene 及 benzo- (a)pyrene 購自 Restek 公司。所有溶劑均為 HPLC 等級。動物用藥: amoxycillin, ampicillin,benzylpenicillin, cefquinome, ceftiofur, cefalexin,ciprofloxacin, chloramphenicol, chlortetracycline,cloxacillin, danofloxacin, dicloxacillin, dimetridazole,doxycycline, enrofloxacin, florfenicol, florfenicolamine, flumequine, furaltadone, furazolidone,lincomycin, lomefloxacin, marbofloxacin, nalidixic acid, nitrofurazone, oxolinic acid, oxytetracycline,ronidazole, spiramycin, sulphadiazine, sulphathiazole,sulfadimethoxine, sulphadimidine, sulfamerazine,tetracycline, thiamphenicol, tiamulin,tilmicosine, tinidazole, trimethoprim, tylosin 以及 enrofloxacin-d5（作為內標） 購自 Merck 公司。40 種殺蟲劑（名稱詳如表 4）標準品購自 Sigma-Aldrich 公司。

### 2.1.1 樣品採集

豬肉樣品由供應商中取得，豬隻屠宰前體重介於 130 至 160 公斤之間，共有來自 8 個國家（奧地利、丹麥、法國、德國、荷蘭、義大利、波蘭及西班牙）的 77 件豬肉樣品，樣品取得期間

介於 2016 年 12 月至 2017 年 5 月之間。小牛肉則是從米蘭當地的超市供應鏈中採集的，來自 3 個國家共 108 個樣品，牛隻年齡不超過 8 個月，屠宰日期介於 2018 年 5 月至 2019 年 5 月之間。所有樣品均冰存在-20℃冰箱中，在分析之前置於室溫下解凍。

嬰兒食品從當地市場中收集，共 112 個，其中 45 個是肉品類（小牛肉，豬，馬，羊肉，兔子，雞肉，火雞），13 個魚類（鮭魚，鯛魚，鱒魚，鱸魚，鱈魚），47 個水果與蔬菜類，7 個是乳酪類，另外從塞爾維亞的超市收集了 11 種不同基質的樣品，以擴大國際範圍。樣品詳細資料如表 1。

表 1. 所收集嬰兒食品樣本分類表

| Meat | Fish | Fruit/vegetables | Cheese |
|---|---|---|---|
| Veal | Plait | Apple | Cheese (bovine milk) |
| Swine | Hake | Plum | |
| Horse | Plait and potatoes* | Pear | |
| Lamb | Trout and vegetables* | Pear and blueberry | |
| Rabbit | Bream and vegetables* | Apple and blueberry | |
| Chicken | Bream and potatoes* | Apple and banana | |
| Turkey | Bass and vegetables* | Apple and peach | |
| Veal and ham | Cod and potatoes* | Apple and apricot | |
| Chicken and carrots* | Cod and vegetables* | Banana and kiwi | |
| Chicken with green beans and zucchini* | Salmon and vegetables* | Mixed fruit | |
| Veal and vegetables* | | Carrot and apple | |
| Veal and carrots* | | Legumes | |
| Veal and potatoes* | | Zucchini | |
| Veal, broccoli and carrots* | | Broccoli | |
| Veal, potatoes and mushrooms* | | Carrots, potatoes and zucchini | |
| Turkey, corn and potatoes* | | Sweet potato and carrots | |
| | | Tomato and vegetables | |
| | | Peas and spinach | |
| | | Mixed vegetables | |
| Total n = 45 | n = 13 | n = 47 | n = 7 |

*for mixed categories, meat and fish represented the major component as declared in the label.

### 2.1.2 PFASs 萃取

將 1 g 均質化樣品置入 15mL 離心管，加入 Acetonitrile 溶劑，Vortex 混合 1 分鐘後，置於超音

波水浴槽中常溫震盪 30 分鐘。從水浴槽中取出，在 4℃以 4,612× g 離心 10 分鐘，將上清液置於梨型瓶並裝上真空旋轉乾燥儀以 35℃水浴進行乾燥。乾燥後的梨型瓶加入去離子水 10mL 重新溶解。在此同時，將固相萃取（SPE）WAX 管柱進行活化（依序以 3 mL 5% $NH_3$/methanol、3 mL methanol 及 3 mL 去離子水流經管柱）。將樣品溶液注入 SPE 管柱並緩慢流出，將流出液廢棄。將 SPE 管柱依序以 3 mL of 25mM acetate buffer pH 4.5 及 2 mL methanol 沖洗。最後以 3 mL 5% $NH_3$/methanol 沖提管柱。將沖提液置於梨型瓶並裝上真空旋轉乾燥儀以 35℃水浴進行乾燥。乾燥後的樣品以 100 μL methanol:ammonium formate 20 mM (10:90 v/v)定容，並裝入螺旋蓋樣品瓶 Screw Vial，置於 LC-HRMS 上機分析。

### 2.1.3 抗生素萃取

將 1 g 均質化樣品置入 15mL 離心管，加入 5 mL McIlvaine buffer, 100 μL 20%TCA，以 Vortex 混合均勻後置於超音波水浴槽中常溫震盪 30 分鐘。從水浴槽中取出，在 4℃以 4,612× g 離心 10 分鐘，將上清液取出，並加入 3 mL hexane，以 Vortex 混合均勻後用相同條件離心，廢棄上層 hexane 溶劑，再加入新的 3 mL hexane，以相同條件重複混合、離心並廢棄上層 hexane 溶劑。在此同時，將固相萃取（SPE）HLB 管柱進行活化（依序以 3 mL methanol 及 3 mL 去離子水流經管柱）。將樣品溶液注入 SPE 管柱並緩慢流出，將流出液廢棄。將 SPE 管柱依序以 6 mL methanol:water (5:95 v/v) 沖洗，最後以 5 mL methanol 沖提管柱。將沖提液置於梨型瓶並裝上真空旋轉乾燥儀以 35℃水浴進行乾燥。乾燥後的樣品以 200 μL methanol:water (10:90 v/v) in 0.1% formic acid 定容，並裝入螺旋蓋樣品瓶 Screw Vial，置於 LC-HRMS 上機分析。

### 2.1.4 POPs、殺蟲劑萃取

本研究採用 QuEChERS（quick, easy, cheap, effective, rugged and safe）萃取法。將 1 g 均質化樣品置入 50 mL 離心管中。加入 QuE Citrate 萃取劑，加入 10 mL Acetonitrile 並劇烈搖晃避免結塊。以 Vortex 混合 1 分鐘，在 4℃以 4,612× g 離心 10 分鐘。抽取上清液，加入 QuE-Z SEP 淨化劑，以 Vortex 混合 1 分鐘，在 4℃以 4,612× g 離心 10 分鐘。將上清液分成兩份置於梨型瓶並裝上真空旋轉乾燥儀以 35℃水浴進行乾燥。乾燥後的樣品以分別以 1 mL hexane 及 100 μL methanol:water (10:90 v/v) in 0.1% formic acid 定容，並裝入螺旋蓋樣品瓶 Screw Vial，分別送至

GC-MS/MS 及 LC-HRMS 上機分析。

### 2.1.5 液相層析-串聯高解析度質譜儀（LC-HRMS）分析

本研究使用 Thermo 公司 Surveyor 液相層析儀，使用 C18 層析管柱，分別使用 ammonium formate 20 mM 及 Methanol 作為移動相（mobile phase）A、B。檢液注入量為 20 μL。偵測儀器採用 Thermo 公司 Q-Exactive Plus 質譜儀，具有一個離子阱（orbitrap）。詳細分析條件請參見已發表文章（附錄 1）。儀器產出之資料檔以 Xcalibur 軟體判讀。分析方法的驗證資料請見表 2。

表 2. 17 種 PFASs 化合物驗證資料表，其中電噴灑離子化 electrospray ionization（ESI）均設為 negative。

| Compound* | Name | Formula | Exact mass [m/z] | Transition [m/z] | LOD (pg $g^{-1}$) | LOQ (pg $g^{-1}$) | Recovery (%) | intra-day CV (%) (n=5) | inter-day CV (%) (n=7) |
|---|---|---|---|---|---|---|---|---|---|
| PFBA | Perfluorobutyric acid | $C_4HF_7O_2$ | 212.9792 | 168.98836 | 10 | 30 | 99 | 6 | 20 |
| PFPeA | Perfluoropentanoic acid | $C_5HF_9O_2$ | 262.97601 | 218.98560 | 10 | 30 | 104 | 15 | 14 |
| PFBS | Perfluorobutane sulfonate acid | $C_4F_9HO_3S$ | 298.94299 | 98.95434 | 5 | 15 | 119 | 19 | 20 |
| PFHxA | Perfluorohexanoic acid | $C_6HF_{11}O_2$ | 312.97281 | 268.98288 | 10 | 30 | 112 | 11 | 15 |
| PFHpA | Perfluoroheptanoic acid | $C_7HF_{13}O_2$ | 362.96962 | 318.97949 | 5 | 15 | 109 | 7 | 10 |
| PFHxS | Perfluorohexane sulfonic acid | $C_6F_{13}HO_3S$ | 398.9366 | 98.95437 | 5 | 15 | 101 | 19 | 20 |
| PFOA | Perfluorooctanoic acid | $C_8HF_{15}O_2$ | 412.96643 | 368.97681 | 8 | 24 | 114 | 8 | 11 |
| PFNA | Perfluorononanoic acid | $C_9HF_{17}O_2$ | 462.96323 | 418.97385 | 20 | 60 | 110 | 8 | 11 |
| PFOS | Perfluorooctane sulfonic acid | $C_8F_{17}HO_3S$ | 498.93022 | 79.95598 | 10 | 30 | 84 | 13 | 17 |
| PFDA | Perfluorodecanoic acid | $C_{10}HF_{19}O_2$ | 512.96004 | 468.97064 | 28 | 84 | 87 | 5 | 9 |
| PFUdA | Perfluoroundecanoic acid | $C_{11}HF_{21}O_2$ | 562.95684 | 518.96729 | 30 | 90 | 87 | 13 | 20 |
| PFDS | Perfluorodecane sulfonic acid | $C_{10}F_{21}HO_3S$ | 598.92383 | 79.95593 | 50 | 150 | 81 | 10 | 15 |
| PFDoA | Perfluorododecanoic acid | $C_{12}HF_{23}O_2$ | 612.95365 | 568.96436 | 5 | 15 | 80 | 12 | 20 |
| PFTrDA | Perfluorotridecanoic acid | $C_{13}HF_{25}O_2$ | 662.95046 | 618.96094 | 30 | 90 | 80 | 8 | 16 |
| PFTeDA | Perfluorotetradecanoic acid | $C_{14}HF_{27}O_2$ | 712.94726 | 668.95795 | 50 | 150 | 83 | 10 | 15 |
| PFHxDA | Perfluorohexadecanoic acid | $C_{16}HF_{31}O_2$ | 812.94088 | 768.95093 | 50 | 150 | 80 | 9 | 13 |
| PFODA | Perfluorooctadecanoic acid | $C_{18}HF_{35}O_2$ | 912.93449 | 868.94507 | 50 | 150 | 80 | 16 | 20 |

*= reported in alphabetic order

表 3. 39 種抗生素的分子量及片段等資料

| Compound name (39 compounds) | Formula | Exact mass [m/z] | Transition [m/z] | ESI mode +/- |
|---|---|---|---|---|
| Amoxicillin | $C_{16}H_{19}N_3O_5S$ | 366.11182 | 114.00109 | + |
| Ampicillin | $C_{16}H_{19}N_3O_4S$ | 350.11690 | 106.06545 | + |
| Benzylpenicillin | $C_{16}H_{18}N_2O_4S$ | 335.10600 | 176.06030 | + |
| Cefalexin | $C_{16}H_{17}N_3O_4S$ | 348.10125 | 158.02704 | + |
| Cefquinome | $C_{23}H_{24}N_6O_5S_2$ | 529.13224 | 134.09634 | + |
| Ceftiofur | $C_{19}H_{17}N_5O_7S_3$ | 524.03629 | 126.01212 | + |
| Chloramphenicol | $C_{11}H_{12}Cl_2N_2O_5$ | 321.00505 | 257.03409 | + |
| Chlortetracycline | $C_{22}H_{23}ClN_2O_8$ | 479.12157 | 444.08377 | - |
| Ciprofloxacin | $C_{17}H_{18}FN_3O_3$ | 332.14050 | 288.15005 | + |
| Danofloxacin | $C_{19}H_{20}FN_3O_3$ | 358.15615 | 314.16579 | + |
| Dimetridazole | $C_5H_7N_3O_2$ | 142.06110 | 112.06335 | + |
| Doxycycline | $C_{22}H_{24}N_2O_8$ | 445.16054 | 410.12305 | + |
| Enrofloxacin | $C_{19}H_{22}FN_3O_3$ | 360.17180 | 316.18188 | + |
| Florfenicol | $C_{12}H_{14}Cl_2FNO_4S$ | 355.99319 | 185.02769 | + |
| Florfenicol amine | $C_{10}H_{14}FNO_3S$ | 248.07512 | 130.06515 | - |
| Flumequine | $C_{14}H_{12}FNO_3$ | 262.0874 | 244.07686 | + |
| Furaltadone | $C_{13}H_{16}N_4O_6$ | 325.11426 | 100.07608 | + |
| Furazolidone | $C_8H_7N_3O_5$ | 226.04585 | 95.03703 | + |
| Lincomycin | $C_{18}H_{34}N_2O_6S$ | 407.22103 | 126.12775 | + |
| Lomefloxacin | $C_{17}H_{19}F_2N_3O_3$ | 352.14672 | 265.11438 | + |
| Marbofloxacin | $C_{17}H_{19}FN_4O_4$ | 363.14631 | 320.10410 | + |
| Nalidixic acid | $C_{12}H_{12}N_2O_3$ | 233.09207 | 205.06041 | + |
| Nitrofurazone | $C_6H_6N_4O_4$ | 199.04618 | 152.96921 | + |
| Oxolinic acid | $C_{13}H_{11}NO_5$ | 262.07100 | 244.06044 | + |
| Oxytetracycline | $C_{22}H_{24}N_2O_9$ | 461.15546 | 426.11816 | + |
| Ronidazole | $C_6H_8N_4O_4$ | 201.06183 | 140.04529 | + |
| Spyramicin | $C_{43}H_{74}N_2O_{14}$ | 422.26428 | 174.11231 | + |
| Sulfadiazine | $C_{10}H_{10}N_4O_2S$ | 251.05972 | 156.01120 | + |
| Sulfadimethoxine | $C_{12}H_{14}N_4O_4S$ | 311.08085 | 156.07666 | + |
| Sulfadimidine | $C_{12}H_{14}N_4O_2S$ | 279.09102 | 149.02325 | + |
| Sulfamerazine | $C_{11}H_{12}N_4O_2S$ | 265.07537 | 156.01135 | + |
| Sulfathiazole | $C_9H_9N_3O_2S_2$ | 256.02089 | 156.01120 | + |
| Tetracycline | $C_{22}H_{24}N_2O_8$ | 445.16054 | 410.12305 | + |
| Thiamphenicol | $C_{12}H_{15}Cl_2NO_5S$ | 353.99752 | 185.02805 | + |
| Tiamulin | $C_{28}H_{47}NO_4S$ | 494.32986 | 192.10501 | - |
| Tilmicosine | $C_{46}H_{80}N_2O_{13}$ | 435.2903 | 174.11232 | + |
| Tinidazole | $C_8H_{13}N_3O_4S$ | 248.06995 | 121.03193 | + |
| Trimethoprim | $C_{14}H_{18}N_4O_3$ | 291.14517 | 245.10294 | + |
| Tylosin | $C_{46}H_{77}NO_{17}$ | 916.52643 | 174.11229 | + |

表 4. 40 種殺蟲劑的分子量及片段等資料

| Compound name (40 compounds) | Formula | Exact mass [m/z] | Transition [m/z] | ESI mode +/- |
|---|---|---|---|---|
| Atrazin | $C_8H_{14}ClN_5$ | 216.10105 | 174.05385 | + |
| Azinphos-ethyl | $C_{12}H_{16}N_3O_3PS_2$ | 346.04435 | 114.96143 | + |
| Azinphos-methyl | $C_{10}H_{12}N_3O_3PS_2$ | 318.01305 | 142.99245 | + |
| Azoxystrobin | $C_{22}H_{17}N_3O_5$ | 404.1241 | 372.09729 | + |
| Benalaxyl | $C_{20}H_{23}NO_3$ | 326.17507 | 148.11185 | + |
| Bitertanol | $C_{20}H_{23}N_3O_2$ | 338.1863 | 70.04069 | + |
| bupirimate | $C_{13}H_{24}N_4O_3S$ | 317.16419 | 108.01172 | + |
| Buprofezin | $C_{16}H_{23}N_3OS$ | 306.16346 | 201.10551 | + |
| Cadusafos | $C_{10}H_{23}O_2PS_2$ | 271.09498 | 158.96980 | + |
| Chlorfenvinphos | $C_{12}H_{14}Cl_3O_4P$ | 358.97681 | 155.04663 | + |
| Cyproconazol | $C_{15}H_{18}ClN_3O$ | 292.12112 | 70.04073 | + |
| Cyprodinil | $C_{14}H_{15}N_3$ | 226.13387 | 108.08103 | + |
| Diazinon | $C_{12}H_{21}N_2O_3PS$ | 305.10833 | 169.07928 | + |
| Ethoprophos | $C_8H_{19}O_2PS_2$ | 243.06368 | 130.93852 | + |
| Ethoxyquin | $C_{14}H_{19}NO$ | 218.15394 | 190.12244 | + |
| Fenamiphos | $C_{13}H_{22}NO_3PS$ | 304.11308 | 217.00816 | + |
| Fenarimol | $C_{17}H_{12}Cl_2N_2O$ | 331.03994 | 81.04534 | + |
| Fludioxonil | $C_{12}H_6F_2N_2O_2$ | 266.07356 | 227.04482 | + |
| Flusilazole | $C_{16}H_{15}F_2N_3Si$ | 316.10761 | 165.06987 | + |
| Furalaxyl | $C_{17}H_{19}NO_4$ | 302.13868 | 95.01640 | + |
| Kresoxim-methyl | $C_{18}H_{19}NO_4$ | 314.13868 | 222.09219 | + |
| Malathion | $C_{10}H_{19}O_6PS_2$ | 331.04334 | 99.00809 | + |
| Metalaxyl | $C_{15}H_{21}NO_4$ | 280.15433 | 220.13306 | + |
| Methidathion | $C_6H_{11}N_2O_4PS_3$ | 302.96913 | 145.00656 | + |
| Oxadixyl | $C_{14}H_{18}N_2O_4$ | 279.13393 | 219.11262 | + |
| Paraoxon-methyl | $C_8H_{10}NO_6P$ | 248.03185 | 234.02864 | + |
| Phosalone | $C_{12}H_{15}ClNO_4PS_2$ | 367.99414 | 182.00029 | + |
| Piperonyl butoxide | $C_{19}H_30O_5$ | 356.24315 | 177.09122 | + |
| Pirimicarb | $C_{11}H_{18}N_4O_2$ | 239.15025 | 72.04513 | + |
| Pirimiphos-ethyl | $C_{13}H_{24}N_3O_3PS$ | 334.13488 | 198.1058 | + |
| Pirimiphos-methyl | $C_{11}H_{20}N_3O_3PS$ | 306.10358 | 108.05595 | + |
| Profenophos | $C_{11}H_{15}BrClO_3PS$ | 372.94242 | 344.91083 | + |
| Propachlor | $C_{11}H_{14}ClNO$ | 212.08367 | 170.03662 | + |
| Propargite | $C_{19}H_{26}O_4S$ | 368.18901 | 231.17419 | + |
| Pyrazophos | $C_{14}H_{20}N_3O_5PS$ | 374.0934 | 194.55950 | + |
| Quinalphos | $C_{12}H_{15}N_2O_3PS$ | 299.06138 | 147.05527 | + |
| Simazine | $C_7H_{12}ClN_5$ | 202.0854 | 132.03226 | + |
| Tetrachlorvinphos | $C_{10}H_9Cl_4O_4P$ | 364.90653 | 127.01553 | + |
| Tetraconazole | $C_{13}H_{11}Cl_2F_4N_3O$ | 372.02881 | 91.05791 | + |
| Triazophos | $C_{12}H_{16}N_3O_3PS$ | 314.07228 | 162.06616 | + |

### 2.1.6 氣相層析-串聯質譜儀（GC-MS/MS）分析

本研究使用 Thermo 公司 GC Trace 1310 chromatograph 氣相層析儀串聯 TSQ8000 三重四級杆（Triple quadrupole mass spectrometry, QqQ）電子撞擊模式（electron impact mode）質譜儀，使用氦氣作為載流氣體（carrier gas）。詳細分析條件請參見本人已發表期刊文章（附錄 1 及附錄 2）。分析後之資料以 Xcalibur 軟體判讀。38 種 POPs 同類物的分析資料請見表 5。

表 5. 38 種 POPs 同類物分析資料表

| Compound name (38 compounds) | Formula | Retention time (min) | Precursor ion [m/z] | Transition ion [m/z] | Collision energy (V) |
|---|---|---|---|---|---|
| α HCH | $C_6H_6Cl_6$ | 17.83 | 180.9 | 145 | 10 |
| β BHC | $C_6H_6Cl_6$ | 19.35 | 180.9 | 145 | 10 |
| Aldrin | $C_{12}H_8Cl_6$ | 23.83 | 260.9 | 191 | 30 |
| Anthracene | $C_{14}H_{10}$ | 37.77 | 226.1 | 224.1 | 10 |
| Benzofluoranthene | $C_{18}H_{10}$ | 42.02 | 252.1 | 250.1 | 30 |
| Benzopyrene | $C_{20}H_{12}$ | 42.02 | 252.1 | 250.16 | 30 |
| Chlorpyrifos | $C_9H_{11}Cl_3NO_3PS$ | 24.33 | 278 | 109.1 | 20 |
| Chrysene | $C_{18}H_{12}$ | 37.76 | 228.1 | 226.2 | 30 |
| o,p'-DDT | $C_{14}H_9Cl_5$ | 33.06 | 235 | 165.1 | 20 |
| p,p'-DDD | $C_{14}H_{10}Cl_4$ | 32.53 | 235 | 165.1 | 20 |
| p,p'-DDE | $C_{14}H_8Cl_4$ | 33.06 | 246 | 176.1 | 30 |
| p,p'-DDT | $C_{14}H_9Cl_5$ | 34.22 | 235 | 165.1 | 20 |
| Diazinon | $C_{12}H_{21}N_2O_3PS$ | 19.00 | 304.1 | 179.2 | 10 |
| Disulfoton | $C_8H_{19}O_2PS_3$ | 19.64 | 142 | 81 | 10 |
| Endosulfan I | $C_9H_6Cl_6O_3S$ | 28.53 | 372.8 | 265.9 | 20 |
| Endosulfan II | $C_9H_6Cl_6O_3S$ | 28.54 | 240.9 | 205.9 | 10 |
| Endrin | $C_{12}H_8Cl_6O$ | 31.31 | 262.9 | 193 | 30 |
| Ethoprophos | $C_8H_{19}O_2PS_2$ | 15.83 | 158 | 97 | 20 |
| Heptachlor | $C_{10}H_5Cl_7$ | 22.21 | 271.8 | 236.9 | 10 |
| Heptachlor epoxide | $C_{10}H_5Cl_7O$ | 26.39 | 352.9 | 262.9 | 10 |
| Hexachlorobenzene | $C_6Cl_6$ | 18.13 | 283.8 | 248.9 | 20 |
| Lindane | $C_6H_6Cl_6$ | 21.03 | 219 | 183 | 10 |
| Mevinphos | $C_7H_{13}O_6P$ | 12.33 | 127 | 109 | 10 |
| PBDE 28 | $C_{12}H_7Br_3O$ | 32.39 | 246 | 139 | 30 |
| PBDE 33 | $C_{12}H_7Br_3O$ | 31.98 | 247.9 | 139 | 30 |
| PBDE 47 | $C_{12}H_6Br_4O$ | 38.33 | 483.7 | 325.9 | 20 |
| PBDE 99 | $C_{12}H_5Br_5O$ | 40.90 | 563.6 | 403.8 | 20 |
| PBDE 100 | $C_{12}H_5Br_5O$ | 41.60 | 563.6 | 403.8 | 10 |

| | | | | | |
|---|---|---|---|---|---|
| PBDE 153 | $C_{12}H_4Br_6O$ | 43.13 | 483.7 | 376.8 | 30 |
| PBDE 154 | $C_{12}H_4Br_6O$ | 44.20 | 483.7 | 323.8 | 30 |
| PCB 28 | $C_{12}H_7Cl_3$ | 22.09 | 256 | 186 | 20 |
| PCB 52 | $C_{12}H_6Cl_4$ | 23.54 | 291.8 | 222 | 25 |
| PCB 101 | $C_{12}H_5Cl_5$ | 28.32 | 325.8 | 255.9 | 25 |
| PCB 138 | $C_{12}H_4Cl_6$ | 33.23 | 359.8 | 289.9 | 25 |
| PCB 153 | $C_{12}H_4Cl_6$ | 34.82 | 359.8 | 289.9 | 25 |
| PCB 180 | $C_{12}H_3Cl_7$ | 38.01 | 393.8 | 323.8 | 25 |
| Phorate | $C_7H_{17}O_2PS_3$ | 17.07 | 121.1 | 65 | 10 |
| Trans chlordane | $C_{10}H_6Cl_8$ | 28.29 | 372.8 | 265.9 | 20 |

以 Xcalibur 軟體判讀 Chrysene、PBDE 28、及 PCB 28 的畫面如圖 1。

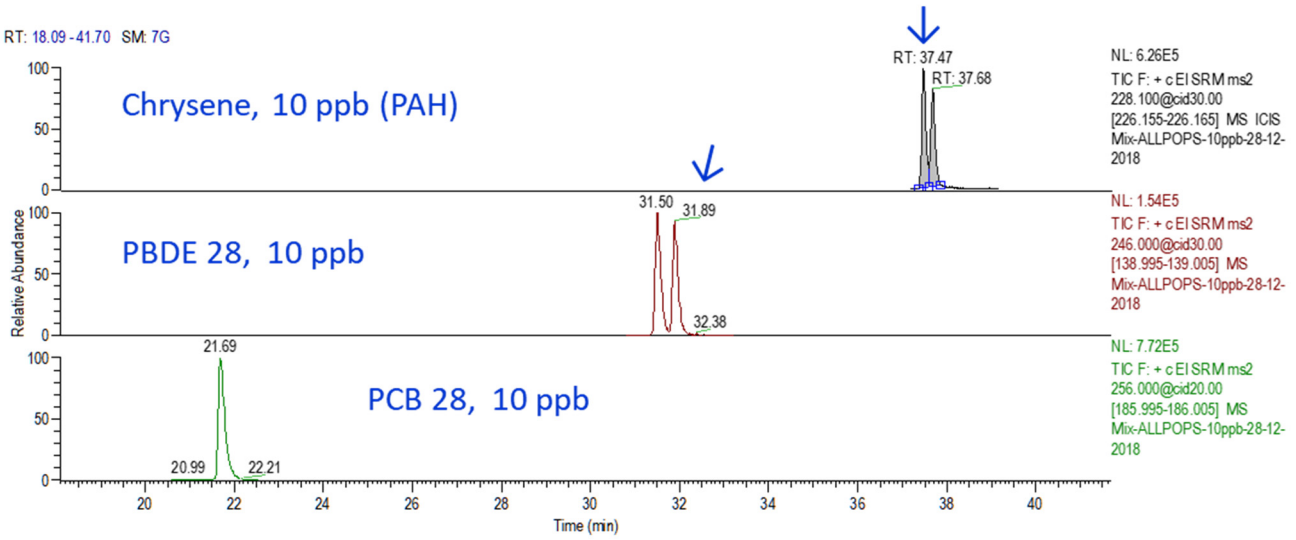


圖 1. Chromatograph of standard reference of Chrysene, PBDE 28, and PCB 28.

### 2.1.7 分析效能與方法驗證

驗證方法係依據歐盟執委會 SANTE/2015 規定辦理。針對 PFASs，本分析方法顯示出高特異性，在各分析物所屬的滯留時間（retention time）點並無出現其他干擾性訊號，且具有高的信噪比（Signal-to-noise ratio, S/N ratio），即使濃度降低到 pg $g^{-1}$ 層級也具有良好的信噪比。每種分析物的信號均符合相對滯留時間 2.5%範圍內，信噪比大於 3，符合歐盟執委會建議的公差範圍內（European Commission, 2002）。各分析物平均回收率（recovery）在 80%至 117%之間，顯示了本分析方法的效率。經審視每個分析物的信噪比，17 個 PFASs 分析物的偵測極限（limit of

detection, LOD）介於 5 pg $g^{-1}$ 之間，定量極限（limit of quantification, LOQ）介於 15pg $g^{-1}$ 至 150 pg $g^{-1}$ 之間。

所製作各分析物的檢量線（calibration curve）在工作範圍內呈現良好的線性關係，$R^2$ 值均大於 0.99。重複性（repeatability）與再現性（reproductivity）的精確度（Thompson et al., 2010）是以 one-way analysis of variance ANOVA 計算的，分別低於 19%及 21%，符合歐盟法規的要求。

依據上列的分析結果，本研究所選用的分析方法，靈敏度很高。

有關 POPs、抗生素及殺蟲劑的分析效能及驗證資料，可參閱本研究團隊（國立米蘭大學）之前已發表過的文章（Chiesa et al., 2017;2018a;2018b;2018c;2018d;2018e）

## 2.2 分析結果與討論

在 77 個豬肉樣品之中，僅有 1 個奧地利的樣品測出 PFOA 殘留（濃度 0.531 ng $g^{-1}$），1 個德國樣品測出多種 PBDEs 殘留，分別介於 0.53 至 0.77 ng $g^{-1}$ 之間，荷蘭與義大利各有 1 個樣品分別測出 PBDE 153（0.53 ng $g^{-1}$）及 PBDE 100（0.62 ng $g^{-1}$）殘留（表 6）。

表 6. 來自不同生產國家的豬肉樣品中的化學殘留物的定量結果.

| Production area | N° of Sample | Analyte detected | | Concentration (ng $g^{-1}$ fresh weight) |
|---|---|---|---|---|
| Austria | 7 | PFOA | (n=1) | 0.53 |
| Denmark | 8 | n.d. | | - |
| France | 8 | n.d. | | - |
| Germany | 10 | PBDE 28 | | 0.57 |
| | | PBDE 33 | | 0.73 |
| | | PBDE 47 | | 0.60 |
| | | PBDE 99 | (n=1) | 0.74 |
| | | PBDE 100 | | 0.77 |
| | | PBDE 153 | | 0.70 |
| | | PBDE 154 | | 0.53 |
| Netherland | 8 | PBDE 153 | (n=1) | 0.53 |
| Italy | 20 | PBDE 100 | (n=1) | 0.62 |
| Poland | 8 | n.d. | | - |
| Spain | 8 | n.d. | | - |

n.d.=Not detected

在 117 件嬰兒食品及 108 件小牛肉樣品中未發現 PFASs、POPs 抗生素及殺蟲劑的殘留。

根據本研究所獲得的結果，可以進行一些考慮。歐盟尚未規定食品中 PBDE 的最大限量，因為尚未對人體內 PBDE 的風險特徵進行定義，儘管最近的研究已經評估了關於人體組織（例如血液、人乳）中 PBDE 濃度與健康影響（免疫、生殖、發育、遺傳毒性和致癌作用）（ATSDR, 2011）。比較前人在文獻中的結果，研究發現比來自西班牙的豬肉中 PBDE 的含量比其他文獻記載的地區還低，如西班牙（109 ng $g^{-1}$）（Bocio et al., 2003），加泰隆尼亞（32.3 ng $g^{-1}$）（Perellò et al., 2009），瑞典（63.6 ng $kg^{-1}$）（Domingo et al., 2004）和中國大陸（8.074 ng $g^{-1}$）（Gong et al., 2014）。由於其具有親脂性，Törnkvist 等人（2011 年）表示，對 PBDE 攝入總量的最大貢獻者是魚類（39%）和乳製品（31%），其次是肉類（17%）。Vouriner 等（2012 年）研究了波羅的海三個地區的大西洋鮭魚中 PBDE 的生物放大作用，他們證明了 PBDE 的蓄積性取決於年齡和脂肪含量。人類可以以多種方式暴露到 PBDE，主要接觸途徑是從被污染的食物、環境（空氣，土壤）以及皮膚與受污染產品的接觸。多項研究表明，與嬰兒或學步兒童相比，嬰幼兒的 PBDE 暴露量更高，這是因為它們的體重較小，並且經常與地板灰塵接觸（ATSDR, 2011）。在文獻中，關於 PBDE 飲食攝入的信息很少。PFOA 由於其化學結構，在環境中非常穩定，並具有抗生物降解和水解的能力（ATSDR, 2009）。與 PBDEs 不同，在活生物體中，PFASs 與血液、肝臟和雞蛋中的白蛋白結合，但不會在脂肪組織中積聚。由於它們的水解特性，它們在水環境中的含量更高，與其他食品相比，在魚類中的含量也更高。

儘管本研究發現其他樣品中的 PFOS 似乎與魚類樣品中的主要化合物無關（Chiesa et al., 2018; Squadrone et al., 2015; Guerranti et al., 2013），但仍發現了豬肉中的低濃度殘留（15 pg $g^{-1}$; 0.74 ng $g^{-1}$）（Guerranti et al., 2013; Noorlander et al., 2011）。

EFSA 食品鏈污染物科學小組（Scientific Panel on Contaminants in the Food Chain）建議，應收集更多食品中 PFASs 的存在數據，以提高未來暴露量計算的準確性（EFSA, 2008）。隨後，歐洲委員會發布了關於監測成員國食品中 PFASs 的委員會建議書 2010/161 / EU（EFSA, 2012）。本研究結果有助於人們了解 PFASs 在豬肉中的存在。根據這些結果，對人類沒有風險，但是，正如歐盟 EFSA 所建議的那樣，須要進行進一步的研究以持續監測其在食品中的存在。

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# 研究發現與結論

藉由屠宰衛生檢查資料的研析，本研究發現豬隻肝臟的病理病變百分率隨著季節循環而起伏，這一點與英國的研究（Correia-Gomeset al., 2017）相符。將屠後檢查資訊回饋給養豬場長達 7 年之後，肝臟與肺臟的病變比率都下降了。本研究結果對於歐盟未來的官方控制措施有很好的參考作用。歐盟的 IMSOC 系統已經將屠前檢查及屠後檢查於資料庫的格式、紀錄、傳輸及交換定下了統一規範，牧場與所生產豬隻的資訊也一併被收錄在資料庫，農民已經依照法律規定登記在資料庫中，若能開辦一致性的屠後資訊回饋作業，將能協助農民更有效的進行動物健康及動物防疫管理。

歐盟從 2006 年禁用 PFOA 及 PFOS，本研究已經開發了高靈敏度的多重殘留分析方法來檢測環境污染物、抗生素及殺蟲劑。本研究在所有的豬肉樣品中發現了微量的 PFOA 及 PBDEs，不會對人體健康造成風險，可說明歐洲管制措施已有成效，肉品安全性也相對提高。

在資源有限的情形下，本研究運用兩種儀器（LC-HRMS 及 GC-MS/MS）開發了多重殘留分析方法，並運用了兩種萃取技術（QuEChERS 及固相萃取技術），從 112 件嬰兒食品與 108 個小牛肉樣品中分析了 134 種分析物（17 種 PFASs、 38 種 POPs、 39 種抗生素、及 40 種殺蟲劑）。本研究沒有發現抗生素、殺蟲劑及環境污染物，這說明了歐盟對於抗生素、殺蟲劑及環境污染物質的控制活動是有效的。

人類的健康主要建立在絕佳的食品安全上，而絕佳的食品安全建立在絕佳的動物健康上，因此，維護動物的健康就是保護人類的健康。總結來說，本研究已經證明屠後檢查資訊回饋到養豬場可以減少屠宰豬隻的病變百分比。資訊回饋可以提高動物公共衛生管理措施的透明度，因為這可以填補動物生產者的資訊缺口，最終使動物族群處於低度病變的狀態。其次，本研究還探討了歐盟當前的食品污染物質安全問題，而且開發了分析方法來識別並定量動物源性食品中的已知污染物質。可想而知的是，歐盟的食品安全仍然受到各種產業創新、環境污染問題及氣候變遷的挑戰，本研究工作所組織的科學內容毫無疑問的針對維護歐盟動物健康及動物源性食品的化學殘留物的安全性至關重要。

# 相關研習活動

一、 2016 年 10 月 13 日起多次赴米蘭市 Mercato Ittico 魚市場（有公務水產品獸醫師駐場檢查）協助執行採樣工作，實地汲取義大利官方獸醫師在水產肉品安全領域的職責、肉品追溯資訊管理與執行情形。倫巴第衛生局在此派駐有 1 位公務獸醫師主任及 4 名獸醫師，主要針對水產品採樣進行肉眼檢查，並配合年度監控計畫採樣送實驗室檢驗。肉眼檢查主要是依法檢查活體存活情形、解剖檢查寄生蟲（檢出蟲體須整批退運，不得流入市場）、及產品標籤的合規性等。

二、 2017 年 5 月 19 日赴帕維亞（Pavia）省內 Melca 屠宰場（有公務屠檢獸醫師駐場檢查）及牛隻牧場（畜養 Frisona 種乳牛及 Chianina、Piemonte 等 3 種原生種肉牛）參訪，並與屠檢獸醫師主任就小型規模屠宰場之屠宰衛生檢查、肉品追溯資訊管理與執行情形交換意見。

三、 動物疾病診斷中心

筆者在 2017 年 7 月 7 日赴倫巴第及艾米利亞羅馬涅大區動物疾病預防及診斷研究所洛迪診斷中心（Sezione di Lodi, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna）短期研習，該診斷中心隸屬區域性（regional）公共衛生體系，為公務獸醫服務體系之一環，除監控轄區內動物健康、動物用藥處方及肉品安全情形之外，該中心亦對洛迪省及鄰近省市內的政府機關、屠宰場、畜牧業、乳、蛋、魚、肉品有關行業及公、私領域獸醫單位提供病理學、病毒學、血清學、微生物學、分子生物學及寄生蟲學等科學診斷或諮詢服務，在該轄區內的動物健康、抗體力價監測、用藥處方管理及肉品安全的官方控制業務扮演重要角色。下一年度起該轄區內之經濟動物獸醫師須全數將處方箋即時經由電腦網路申報至中央資料庫，以利該中心即時督導查核獸醫師處方合規情形。該中心主任亦針對在牛隻屠宰場內針對屠檢病變收集大量數據以分析歸納屠宰動物來源及可能病因，進而事先預防或阻斷病變重複發生情形之研究模式慷慨提供經驗與建議。研習期間洛迪市政府因市內有市民溜狗之後未清理狗便之情形，爰由市長辦公室向該診

斷中心洽詢藉由違規狗便中提取 DNA 以鑑識犬籍進而開罰飼主之科學及其可行性，本人隨即主動向該中心分享日本政府建立牛籍、同步保存 DNA 及屠宰分切後肉品中複驗 DNA 以驗證牛隻登記的正確性等技術資訊供參。

四、 2017 年 7 月 19 日參加倫巴第大區松德里奧（Sondrio）衛生局在松德里奧市舉辦之「Emergenze non epidemiche di carattere veterinario」（中譯：與獸醫師特性有關的非流行病性緊急事件研討會），屬於公務獸醫師繼續教育課程之一，會後測驗通過並已取得教育學分數。該課程探討公務獸醫師在地震災害、水災、火災等大規模災害期間所扮演的角色，應就地提供專業建議，以協助民防部門的行動達到高度的生物安全水準，並使各動物牧場維持有效的生物安全措施。還須以獸醫師角度參與審核死亡動物之清運、消毒計畫，盡專業知識之力協助民防部門的工作能具有生物安全效果，有效阻止繼發性的爆發動物傳染病，避免災區內正在復甦的畜牧產業再次遭受疾病的衝擊。

五、 擔任第 2 作者，並提出部分研究成果與研究室同仁共同製作研討會海報「Presenza di metalli in carne e salsiccia suine del mercato italiano: Risultati preliminari（中譯：在義大利市場上生鮮豬肉和豬肉香腸中的重金屬含量：初步結果），於 2017 年 9 月 13-17 日義大利獸醫衛生學家協會（A.I.V.I.）第 27 次全國研討會（於佩魯賈（Perugia）市舉行）被大會接受並發表海報，編號 P006-58。

六、 2017 年 12 月 6-7 日，前往歐洲食品安全局（EFSA，位於義大利帕馬（Parma）市）以觀察員身份參加生物危害及污染專家小組開放會議（117th Plenary meeting of the BIOHAZ Panel），從會議中瞭解該局專家正在針對歐洲禽肉沙門氏桿菌近期的盛行率不降反升的趨勢研擬並更新預防措施，同時將借重全基因體定序技術的優點，將此技術融入相關的監測沙門氏桿菌的活動內。會議中亦討論豬隻屠體體噴灑有機性乳酸及乙酸溶液以降低屠體表面微生物污染的科學意見稿，將在補齊歐盟要求的資料後提交審查。本人亦提出有關肉品安全問題並獲得專家回應。

七、 2018 年 3 月 21 日參訪義大利農業部 Unirelab 研究中心（位於 Settimo Milanese 鎮），該中心為一獸醫學研究機構，1998 年即開始運作並接收樣品，但法律上的正式成立年份是

2003 年，目前有 36 名全職員工，設有三個科學部門（獸醫法醫毒理學實驗室、人醫法醫毒理學實驗室，及獸醫法醫基因學實驗室），每年接收檢驗的樣品超過 20,000 件以上，最大量的業務是接收賽馬的血、尿液並以高精度質譜儀檢驗是否含有比賽禁藥（dopping，主要是興奮劑），其次是檢驗飼料中是否有禁用藥品及其含量。在 2017 年度獸醫法醫毒理學實驗室已檢驗 12,500 件賽馬生物樣品，驗出違規件數佔樣品總數 0.5%。該實驗室肩負並維持高度執法公正性，除實驗儀器及方法不斷精進外，樣品檢驗流程亦全數標準化，檢驗過程具有追溯性並保存完整記錄可稽核。從採樣空瓶開始已經具有追溯性，採樣用空瓶套件已印有唯一條碼，採樣獸醫師對於血液及尿液必須每項目採取一式兩份（paired，成對採樣）並貼上唯一條碼。樣品罐密封貼上封條後，必須在電腦系統掃入採樣條碼將樣品資訊註冊於電腦系統，再送交簽約快遞公司運送至實驗室。實驗室人員檢查包裹防偽性及點收樣品後，立即使用電腦系統掃描條碼以啟動（activate）程序並列印檢驗記錄單，並將一式兩份的樣品隨機置入 A 或 B 組，A 組樣品送入冰庫保存，而 B 組則送交檢驗。若 B 組驗出違規禁藥，檢驗人員將簽名並啟動複驗程序，並從冰庫將 A 組同一條碼樣品取出送入檢驗流程，若複驗驗出違規禁藥，則該匹賽馬將被通知取消資格。人醫法醫毒理學實驗室則是檢驗賽馬的騎師或是雙輪拖車賽馬（trot）的騎師的樣品是否含有禁藥。獸醫法醫基因學實驗室存有賽馬的原始血液樣品（塗在乾燥濾紙）以及基因序列資料庫（儲存在電腦系統），主要業務是接受義大利農業部委託，使用分析儀器鑑識賽馬的基因片段，並利用電腦軟體鑑識親緣關係，供義大利農業部國家賽馬登記資料庫使用，其次的業務是接受委託檢驗食品樣品是否有摻假（例：牛肉中混入馬肉）。本人詢問有關血液樣品是否有保存期限？研究員答以，從 1998 年起保存的血液濾紙到現在都還能檢出同樣的基因片段，因此保存年限應該是高於 20 年。另外，該中心建有能容納 300 匹馬的馬舍，必要時將可以接收馬隻隔離觀察，目前僅保有 5 匹健康馬。除上述例行業務之外，隨著社會需求不斷更新，該實驗室也接受各方請求，在檢驗能力範圍內以洽談收費價格的方式，回應檢驗的需求。本次參訪有助於本人於實驗室內強化實驗過程的追溯性，並確保研究品質。

八、 2018 年 11 月 16 日參加義大利北部高山狩獵地區肉品衛生檢查站所舉辦的 2018 年野生動物獵捕管理與肉品檢查成果研討會（位於 Domodossola 市），據歐盟 Regulation 853/2004 規定，供人食用之肉品必須經過公務獸醫師檢查合格後才能合法銷售，法規範圍涵蓋狩獵生產的肉品。狩獵區的動物係屬於國家財產，只有在公告限定的狩獵期間及狩獵頭數內才可以合法獵捕並製成供人食用的肉品。獵捕人係屬肉品生產業者，因此必須向屠宰衛生主管機關登記並取得編號，且必須將動物送交獸醫檢查站登記；肉品衛生檢查獸醫師會將受檢動物打上屠體編號耳標（禽類則在腳脛繫上金屬號碼牌），記錄捕獲地點 GPS 座標，檢查屠體與內臟，並即時更新統計頭數。高山狩獵區內的屠宰衛生主管機關為主要控制中心，並與狩獵區內的保育學會、農業生產協會、獵捕人協會、憲兵隊、警察局、米蘭大學獸醫學系等單位密切合作並公告必要資訊。高山狩獵區是跨國境的，管理範圍有涵蓋瑞士南部省分。為達成法規所定肉品安全管理的目標，保育學會必須積極使用新科技、公布動物數量監控結果並提報建議獵捕期間及頭數，農業協會持續關注農業損害地區及金額（因為動物密度過高會加速農業損失），獵捕人協會關注可合法獵捕的資訊及建議，憲、警單位關注非法獵捕情形或衝突事件，米蘭大學獸醫學系提供肉品採樣後送檢驗的結果，肉品衛生檢查站公告並回應所有獵捕訊息，並及時更新統計資訊。總結本次聯合研討會所公布之訊息是，過去十年來野鹿的族群越來越大，農業損失金額越來越高，保育學會建議本年度狩獵季節開始時儘量獵取母鹿，在季節後期獵取年輕野鹿，以抑制族群的數目；而獵捕人協會關心獵捕的數量及日期，通常獵捕人偏好成年公鹿，因為公鹿的鹿角可帶來額外收入，母鹿則無；當某區域的獵捕頭數達到上限之後，獵捕人會立即驅車至另一個管區進行狩獵，直至各管區的可獵頭數耗盡為止；肉品衛生檢查站公布 2018 年有 1,400 人登記為獵捕人，2017 年野鹿建議獵捕 2,031 頭，實際檢查 2,158 頭，近 10 年以來所捕野鹿之體長與體重亦逐年減少，除此之外，尚有山羊、野豬、野禽等動物亦屬肉品衛生管理項目。獵捕野豬要不是為了賣野豬肉，要不就是野豬造成農損而允許獵人進入田區獵捕野豬，獵人有責任收集野豬血液、脾臟及橫隔膜供獸醫師檢查，獵人若不收集樣本，就不會符合合法肉品檢查程序，更不會有合法檢查文件，這頭

豬就別賣了。屠體通過公務獸醫檢查站檢查後，取得合格文件，然後再載去銷售。獸醫檢查站有公權力可以對野豬屠體採樣檢查，採樣方式就是死後採樣。獵人獵捕方式大多是用獵槍瞄準野豬後射殺，在義大利北部山區，合法狩獵期間不長，合法狩獵頭數有限量，獵人求快速獲利，先抓先贏，所以用獵槍是最快。在非洲豬瘟檢測結果出爐前，禁止移動屠體。如果採樣檢測到非洲豬瘟疫情，該屠體要銷毀，地方政府會通報並做必要管控，還會把受影響區域給公告關閉起來，這樣一來就禁止人員進入疫區。另外，任何人如果在野外見到死豬屍體，也必須通報地方獸醫服務機關處理、採樣。有關義大利北部山區針對野豬及非洲豬瘟的獵捕管理，由於獵人身份已經事先向政府註冊並取得編號，獵人須將捕獵到的野豬寫入登記表（有獵人基本資料，野豬基本資料，GPS 座標），併同野豬一起送至肉品檢查站檢查。檢查站獸醫師收受野豬屠體後做行政資料登記，屠體被檢查後冰在檢查站內，受公權力管控，檢體則外送做檢驗。行政登記後開立收件三聯單，其中一聯發給獵人帶回家，一聯由檢查站留存，一聯陳送至上級機關。非洲豬瘟的標準檢查期間是 5 個工作日。檢驗單位是後送實驗室（並不是檢查站自己檢測）。5 個工作日內，實驗室會將檢驗結果通知檢查站，檢查站再通知獵人（他收電子訊息，因為獵人已註冊，因此系統就能自動聯絡他）。至於通知檢驗陰性的野豬，獵人可以前往檢查站，出示三聯單為憑據，由檢查站放行，才將野豬從檢查站的冰庫提領出去。

EFSA 對於獵捕野豬有公布生物安全建議：

1. 除非產品標有屠宰檢查合格章，否則不要將來自豬肉或野豬的產品，如新鮮肉類和冷凍肉類，香腸，火腿，豬油，從受感染地區帶到歐盟；
2. 不得以非歐洲國家的豬肉或野豬（不論是新鮮或冷凍的），做成香腸，火腿，豬油等產品；
3. 將任何類型的食物廢物棄置於合適的容器內，不得以任何理由將其用於家豬；
4. 不要將食物垃圾留在野豬可以進入的地方；
5. 及時通知獸醫部門取回死亡野豬；
6. 針對獵人：離開狩獵區前清潔和消毒設備，衣服，車輛和狩獵品；野豬只在指定的

建築中掏內臟；狩獵後避免與家豬接觸；

7. 對於養豬者：尊重生物安全規則，特別是在進入或離開牧場時更換衣服和鞋類，以及避免與其他農場的野豬或豬間接接觸；及時通知獸醫服務機關有關非洲豬瘟的症狀和異常死亡事件。

本次研討會見證了歐洲屠宰衛生法規也可以落實在非工業生產型態且地處偏遠的肉品產業上，由各利益關係人積極溝通以有效實現法規所定來源資訊可追溯及獸醫師執行檢查等依法維護肉品安全之工作，兼顧動物防疫與森林保育需求，並促進區域內肉品產業永續發展。

# 心得及建議事項

一、 建議針對重大危害因素進行屠宰衛生安全偵檢與研發工作及相關追溯活動，以結合檢驗結果，降低屠宰肉品安全風險，保障食肉衛生安全。

二、 我國活豬及屠體運輸車輛已全面裝置即時追蹤系統（GPS），對於追溯來源牧場與肉品流向可以作有效之管控，在發生非洲豬瘟或其他疫病時，亦可對管制區之動物實施移動管制。有關肉品檢查資訊從屠宰場回饋至來源牧場之影響研究成果，融入上列新政策內可彰顯肉品來源資訊透明化管理後產生新的效益，並可輔助農民及運輸人員維持良好防疫措施，保護肉品產業鏈永續發展，應予支持。

三、 屠宰衛生檢查獸醫師在維護肉品安全的重要性扮演重要關鍵角色，良好的在職訓練、溝通技能以及吸收最新資訊，可以確保屠宰衛生檢查業務的有效、常態、一致化地執行，建議在屠檢人員相關課程或會議加強屠後檢查判定案例交流，並發表本研究成果，促進在職人員吸收新興肉品安全問題，為未來作好準備。

# 致謝

本次進修承蒙行政院選送優秀公務人員 105 年度國外進修計畫經費支持，進修期間承蒙行政院人事行政總處劉瀞文、謝奇帆專員、駐義大利臺北代表處高淑雅秘書、行政院農業委員會及本局同仁對進修計畫的執行、諮詢與不時關懷，並適時提供諸多協助，使本次進修過程圓滿，順利取得學位，對個人成長而言亦獲益良多，謹此致上最高之謝意。

# 附錄

本研究成果已獲國際期刊刊登，計 2 篇文章。
已發表文章 1：(https://doi.org/10.1080/19440049.2018.1540889)

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## Levels and distribution of PBDEs and PFASs in pork from different European countries

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**ABSTRACT**

Meat and meat products are included in a great number of human diets. However, the great consumption of meat needs to be controlled for the presence of traces of contaminants. The European Commission has not stated maximum limits for some environmental pollutants such as the perfluoroalkyl substances (PFASs) and polybrominated diphenyl ether (PBDE); the European Food Safety Authority (EFSA) Scientific Panel has recommended that more occurrence data for PFASs in food should be collected to improve the accuracy of future exposure calculations. Therefore, the distribution of PFASs and PBDEs trace contaminants from eight EU Member States were investigated through liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) and Gas Chromatography-Mass Spectrometry (GC-MS/MS). No PFASs were detected, except perfluorooctanoic acid, in only one Austrian sample at the concentration of 0.531 ng $g^{-1}$. PBDEs were detected in 3 out of 77 samples: one from Germany showed the presence of all congeners analysed in the concentration range 0.53–0.77 ng $g^{-1}$, the others, from Netherland and Italy, respectively contained PBDE 153 (0.53 ng $g^{-1}$) and PBDE 100 (0.62 ng $g^{-1}$). The results show that the analysed samples do not pose a risk for human beings in regard to PFASs and PBDEs. Further studies are needed to keep monitoring their presence in foodstuff, as it has been suggested by European Commission.

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GC-MS/MS; LC-HRMS; PBDEs; PFASs; Pork

### Introduction

Generally, food of animal origin plays an important role in determining the exposure of human beings to contaminants of chemical origin (Liem et al., 2000; Pastorelli et al. 2005; Törnkvist et al. 2011; Vogt et al. 2012). Perfluoroalkyl substances (PFASs) and polybrominated diphenyl ether (PBDE) contamination of food is a global issue of environmental pollution. PBDEs are one class of brominated flame retardants (BFRs) that can be released from manufacturing commercial products (e.g. acrylonitrile–butadiene–styrene and polystyrene plastics, polyurethane foams), packaging materials, electronic devices, as computers or televisions. PBDEs can be released into the air, water, and soil at places where they are produced or used, but they have very low water solubility, and when these substances are released to water, they typically bind to sediment (ATSDR, 2011). These substances generally bind strongly to soil particles, and therefore, do not move easily through soil layers (Routti et al. 2015).

PFASs, such as perfluorooctane sulfonate (PFOS), represent a class of compounds showing high thermal, chemical, and biological inertness. Their application began in the early 1950s and, due to their widespread use, they are globally found in the environment, both in animals and in humans (Routti et al. 2015). Many countries, e.g. Germany, French, Denmark and Spain, reported the results of PFASs analysis from human serum samples (Ingelido et al. 2010) and other animals (Chiesa et al. 2018), where they found very low concertation with the average about 15 pg $g^{-1}$ in pork and higher in fish where they reach 45 ng $g^{-1}$ (Table 4). The highest concentrations are found near densely inhabited areas due to discharge of industrial and municipal

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wastewater and fire-fighting operations (Lindstrom et al. 2011; Zacs and Bartkevics 2016).

Perfluorooctanoic acid (PFOA) and PFOS are recognised as endocrine disruptors with reproductive toxicity, and immunosuppression activity (Pèrez et al. 2014); several studies have shown that in experimental animals they have adverse effects including developmental toxicity, neurobehavioral toxicity and lung toxicity, as well as carcinogenic genotoxic potential (EFSA 2012). On the basis of their properties, the EFSA proposed tolerable daily intake (TDI) levels for PFOA (1500 ng $kg^{-1}$ body weight per day) and PFOS (150 ng $kg^{-1}$ body weight per day) (EFSA 2012) due to their adverse effects in experimental animals and due to dietary exposure has been suggested as the main exposure route to PFASs.

Most information regarding toxicity of PBDEs and their metabolites is from animal studies that show developmental neurotoxicity and endocrine disruption (Costa and Giordano 2007; Darnerud 2008). One study examined the effects of PBDEs in humans. The authors detected four congeners (PBDEs 47, 99,100, 153) in greater than 97% of women's serum samples analysed and found significant decreases in fertility associated with PBDE exposure in women (Harley et al. 2010).The EFSA Panel on Contaminants in the Food Chain (EFSA 2011) considers eight PBDE congeners to be of primary interest: PBDE-28, –47, –99, –100, –153, –154, –183 and –209. In 2008, the United States Environmental Protection Agency (EPA, 2009) issued health assessments of four individual PBDE congeners, PBDE-47, –153, –99 and –209, within its Integrated Risk Information System (IRIS) programme. The dominant food category that is exposed to PBDE is food with high fat content, because there is a relationship between the PBDEs levels and the fat content (EFSA 2011). In 2012–2013, a U.S. meat and poultry (beef, pork, chicken, turkey) study reported that the mean summed concentrations of seven PBDE congeners from beef, pork, chicken and turkey were 0.40, 0.36, 0.19 and 0.76 ng $g^{-1}$ lipid weight (lw), suggested that the U.S. consumer daily intake of PBDEs from meat and poultry was 6.42 ng $day^{-1}$ (Lupton and Hakk 2017).

Meat and meat products are included in a great number of human diets. Their regular consumption means a significant intake of proteins and essential micronutrients. In addition, pork meat is used in many countries to produce derivative products (hams and cured meats) with high-qualitative value and relative recognition as Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI) products.

However, the great consumption of pork meat (Table 5) needs to be controlled for the presence of chemical compounds. EU has not stated Maximum Levels (MLs) for PBDEs and PFASs; the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) recommended that more occurrence data for PFASs in food should be collected to improve the accuracy of future exposure calculations (EFSA 2008). Subsequently, the European Commission issued the Commission Recommendation 2010/161/EU on the monitoring of PFASs in food in the Member States (EFSA 2012). It is therefore important, to obtain information on the presence of these pollutants in food, mainly in those products whose consumption is highest (Table 5). EFSA's CONTAM Panel acknowledged that there were significant data gaps on issues such as the contribution of different foodstuffs, among which pork, to human exposure and that further research and data collection would be necessary (EFSA 2008).

Toxicological studies show that PFOS and PFOA are adsorbed after oral exposure and primarily accumulate in the serum, kidney and liver (EFSA 2008). Perfluoroalkyls tend to remain in the body unchanged for long periods. It takes approximately 4 years for the level to halve, so constant exposure could increase the levels in the organism resulting in adverse overcome (ATSDR 2009). People could be exposed to PBDEs in a wide variety of ways, including foods or dusts/soils, air or through skin contact. The toxicokinetic of PBDEs depends on the number and position of the bromine atoms: the more toxic congeners are the lower brominated PBDEs, due their ability to bioaccumulate, mainly in body fat, consequently decabromodiphenyl ether is expected to be less toxic than lower brominated PBDEs. Nowadays, the effects of PBDEs are not all well established and it is not known if PBDEs are carcinogens to human. However, the International Agency for Research on Cancer (IARC) has classified PBDE as a Group 3 carcinogen based on inadequate evidence of carcinogenicity in humans and

inadequate or limited evidence in experimental animals (Agency for Toxic Substances and Disease Registry 2011). So, based on the EFSA recommendation, in this paper we investigated the presence of PFASs through LC-HRMS and PBDE through GC-MS/MS in pork samples from eight EU Member States, to improve the knowledge on data gap of these compounds in literature.

## Materials and methods

### Chemicals and reagents

The $^{13}$C-labelled PFOS (MPFOS) and $^{13}$C-labelled perfluorononanoic acid (MPFNA), which were used as the internal standard (IS) in this study, and the 17 PFASs derivatives the perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), (perfluorobutane sulfonate acid), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorooctane sulfonic acid (PFOS), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorodecane sulfonic acid (PFDS), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutane sulfonate acid (PFBS) and perfluorohexane sulfonic acid (PFHxS), which were used for standard curve constructions, were purchased from Fluka (Sigma-Aldrich, St. Louis, MO, USA). Mixtures of PBDE congeners (PBDE 28; PBDE 33; PBDE 47; PBDE 99; PBDE 100; PBDE 153 and PBDE 154) and 3-fluoro-2,2′,4,4′,6-pentabromodiphenyl ether (FBDE) as IS for PBDEs were purchased from AccuStandard (AccuStandard, Inc. New Haven, CT, USA). The purity of all standards was greater than 98%. Hexane and acetone (special grade for pesticide residue analysis Pestanal) were purchased from Fluka (Sigma-Aldrich, St.Louis, MO, USA). Each solvent is in HPLC or analytical grade. Purified water was supplied from a Milli-Q system (Millipore, Merck KGaA, Darmstadt, Germany). The solid phase extraction cartridges (Oasis WAX 3 mL, 60 mg) were bought from Waters™ (Milford, MA, USA). The ammonium formate, sodium acetate, acetic acid (99.9%) and 25% ammonia solution were purchased from Fluka. QuEChERS materials for the extraction were obtained from Supelco (Sigma-Aldrich, St. Louis, MO, USA); Supel™ QuE Citrate (EN) tubes, containing sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate. magnesium sulphate and sodium chloride were used for the extraction. Supel™ QuE-Z SEP (EN) tubes were used for the clean-up step.

### Standard solutions

To make the stock solution, each of the 17 standard PFASs compounds was prepared at 1 mg mL$^{-1}$ concentration in methanol and stored at −20°C. The working solutions which were diluted from the stock solution at concentrations of 10 and 100 ng mL$^{-1}$ in methanol were freshly prepared before use and stored at 4°C.

Working solutions of PBDEs were prepared by diluting the stock solution in hexane for pesticides and then stored at −20°C. An uncontaminated meat sample (previously checked for the presence of PBDEs and considered blank with a concentration of compounds less than limit of detection (LOD) used as control was selected for all the procedure optimisation steps. For meat fortification, 1.0 g of the control sample was spiked by adding an appropriate volume of the standard working solution to cover the concentration range from 0.5 to 10 ng g$^{-1}$ (five calibration points: 0.5, 1, 2, 5, 10 ng g$^{-1}$) for PBDEs in relation to literature to realise the matrix-matched calibration curves.

## Sample collection

The muscle samples were taken from pigs from the food chain weighing 130–160 kg, and to minimise the damage to the carcass, the muscles used were obliquus internus abdominis and obliquus externus abdominis. Seventy-seven frozen samples from eight different European countries (Austria, Denmark, French, Germany, Holland, Italy, Poland and Spain) were collected. The samples were homogenised and then stored at −20°C; they were defrosted before being analysed. The date of sample collection was from 5 December 2016 to 5 May 2017.

### Sample extraction of PFASS

Weight 1.0 g of homogenised sample into a 15-mL polypropylene screw-cap centrifuge tube. Add

50 µL of internal standard solution (which contains 100 ng $mL^{-1}$ MPFNA and 100 ng $mL^{-1}$ MPFOS in methanol) into the tube, to proceed a final concentration of 5 ng $mL^{-1}$ over the matrix. Shake the tube by hand to mix it with the sample matrix. Add 10 mL of acetonitrile, vortex for 1 min, then put the tube into the water tank with ultrasonication for 30 min at room temperature. Ultrasonicated samples were centrifuged at 4,612 × g, 4°C, for 10 min. Transfer all supernatant liquid into the evaporation flask and dried it with rotary vacuum evaporator at 35°C. Add 10 mL of Milli-Q water into flask and re-suspend the analyte by vortex for 10 s. Load the re-suspended liquid into Waters™ WAX SPE cartridge, which was previously conditioned with 3 mL of 0.05 mL $mL^{-1}$ $NH_4OH$ in methanol, followed with 3 mL of methanol, and 3 mL of Milli-Q water. After the sample finished passing through the cartridge, flush the cartridge with 3 mL of 25 mM acetate buffer pH 4.5 to release proteins and lipids from cartridge, followed with 2 mL of methanol. Elute the cartridge with 3 mL of 0.05 mL $mL^{-1}$ $NH_4OH$ in methanol and transfer the eluted liquid into evaporation flask, then dry it with rotary vacuum evaporator at 35°C. The dried analyte was redissolved in 100 µL of methanol: ammonium formate 20 mM (10:90 v/v) and then transferred into a screw vial for analysis with LC-HRMS.

For the estimation of recovery ratio, use blank pork samples of 1.0 g, divided into group A and B. In Group A, spike into matrix with 50 µL of internal standard solution (which contains 100 ng $mL^{-1}$ MPFNA and 100 ng $mL^{-1}$ MPFOS in methanol) into the tube, to give a final concentration of 5 ng $mL^{-1}$, and with 10 µL of 17 PFASs mixture (each single compound contains 100 ng $mL^{-1}$) to give a final concentration of 1 ng $mL^{-1}$, then run the extraction procedure. In Group B, spike the internal standard solution of 50 µL into matrix, then run the extraction procedure. With the solid phase extraction finished, spike 10 µL of the 17 PFASs mixture into the eluted liquid. Use LC-HRMS to determine concentration of each PFASs, then calculate the ratio of these PFASs between Groups A and B.

For coefficient of variation of intra-day (repeatability), and inter-day (reproducibility) evaluation, use blank pork samples of 1.0 g, spike into matrix with 50 µL of same internal standard solution into the tube, to give a final concentration of 5 ng $mL^{-1}$, and 10 µL of 17 PFASs mixture (each single compound contains 100 ng $mL^{-1}$) to give a final concentration of 1 ng $mL^{-1}$, then run the extraction procedure. Use LC-HRMS to determine the concentration of each PFAS from each tube and calculate the value of each PFAS for the coefficient of variation of intra-day (repeatability) and inter-day (reproducibility).

### *Sample extraction of PBDES*

The extraction of PBDEs was performed using the QuEChERS (quick, easy, cheap, effective, rugged and safe) method. Briefly, 1.0 g of sample was homogenised and transferred to a QuEChERS extraction tube, then a solution containing the ISs (FBDE) was added to the sample to a final concentration of 100 ng $g^{-1}$. A total of 10 mL of acetonitrile was added as extraction solvent; the tube was shaken for 1 min using a vortex and centrifuged for 10 min at 4612 × g at 4°C. Later, the supernatant was transferred to a QuEChERS clean-up tube, shaken and centrifuged at the same conditions described above. The extract was collected, divided into two aliquots and dried under vacuum in a centrifugal evaporator at a temperature of 35°C. The residue was dissolved in 200 µL hexane for the analysis by GC-MS/MS.

## LC-HRMS orbitrap analyses

The LC-HRMS analysis was performed by an HPLC system (Thermo Fisher Scientific, San Jose, CA, USA), consisting of a Surveyor MS quaternary pump with a degasser, a Surveyor AS auto-sampler with a column oven and a Rheodyne valve with a 20-µL loop. Chromatographic separation was carried out using a Synergi Hydro RP reverse-phase HPLC column (150 x 2.0 mm, particle size 4 µm), with a C18 guard column (4 x 3.0 mm; Phenomenex, Torrance, CA, USA). To minimise PFASs background contamination in the system, use stainless steel column tubes and fittings. Moreover, since PFOA and PFOS were always present in the blank of the chromatographic system, we mounted a

**Table 1.** Formula, exact theoretical mass of the parents, diagnostic transitions and validation parameters of the selected PFASs. The electrospray ionisation (ESI) is set as negative.

| Compound* | Name | Formula | Exact mass [m/z] | Transition [m/z] | LOD (pg $g^{-1}$) | LOQ (pg $g^{-1}$) | Recovery (%) | Intra-day CV (%) ($n$ = 5) | Inter-day CV (%) ($n$ = 7) |
|---|---|---|---|---|---|---|---|---|---|
| PFBA | Perfluorobutyric acid | $C_4HF_7O_2$ | 212.9792 | 168.98836 | 10 | 30 | 99 | 6 | 20 |
| PFPeA | Perfluoropentanoic acid | $C_5HF_9O_2$ | 262.97601 | 218.98560 | 10 | 30 | 104 | 15 | 14 |
| PFBS | Perfluorobutane sulfonate acid | $C_4F_9HO_3S$ | 298.94299 | 98.95434 | 5 | 15 | 119 | 19 | 20 |
| PFHxA | Perfluorohexanoic acid | $C_6HF_{11}O_2$ | 312.97281 | 268.98288 | 10 | 30 | 112 | 11 | 15 |
| PFHpA | Perfluoroheptanoic acid | $C_7HF_{13}O_2$ | 362.96962 | 318.97949 | 5 | 15 | 109 | 7 | 10 |
| PFHxS | Perfluorohexane sulfonic acid | $C_6F_{13}HO_3S$ | 398.9366 | 98.95437 | 5 | 15 | 101 | 19 | 20 |
| PFOA | Perfluorooctanoic acid | $C_8HF_{15}O_2$ | 412.96643 | 368.97681 | 8 | 24 | 114 | 8 | 11 |
| PFNA | Perfluorononanoic acid | $C_9HF_{17}O_2$ | 462.96323 | 418.97385 | 20 | 60 | 110 | 8 | 11 |
| PFOS | Perfluorooctane sulfonic acid | $C_8F_{17}HO_3S$ | 498.93022 | 79.95598 | 10 | 30 | 84 | 13 | 17 |
| PFDA | Perfluorodecanoic acid | $C_{10}HF_{19}O_2$ | 512.96004 | 468.97064 | 28 | 84 | 87 | 5 | 9 |
| PFUdA | Perfluoroundecanoic acid | $C_{11}HF_{21}O_2$ | 562.95684 | 518.96729 | 30 | 90 | 87 | 13 | 20 |
| PFDS | Perfluorodecane sulfonic acid | $C_{10}F_{21}HO_3S$ | 598.92383 | 79.95593 | 50 | 150 | 81 | 10 | 15 |
| PFDoA | Perfluorododecanoic acid | $C_{12}HF_{23}O_2$ | 612.95365 | 568.96436 | 5 | 15 | 80 | 12 | 20 |
| PFTrDA | Perfluorotridecanoic acid | $C_{13}HF_{25}O_2$ | 662.95046 | 618.96094 | 30 | 90 | 80 | 8 | 16 |
| PFTeDA | Perfluorotetradecanoic acid | $C_{14}HF_{27}O_2$ | 712.94726 | 668.95795 | 50 | 150 | 83 | 10 | 15 |
| PFHxDA | Perfluorohexadecanoic acid | $C_{16}HF_{31}O_2$ | 812.94088 | 768.95093 | 50 | 150 | 80 | 9 | 13 |
| PFODA | Perfluorooctadecanoic acid | $C_{18}HF_{35}O_2$ | 912.93449 | 868.94507 | 50 | 150 | 80 | 16 | 20 |

* Reported in alphabetic order.

small Megabond WR C18 column (5 cm x 4.6 mm, particle size 10 µm) between pump and injector to delay our analytes by 2 min from those already present in the system.

The mobile phase used for the gradient consisted of a programmed mixture of solvents A (aqueous ammonium formate 20 mM), and B (methanol). The elution started with 10% B, which increased to 40% in 4 min. Subsequently, the mobile phase B was gradually increased to 95% at the 12th minute, which remained constant up to the 18th minute. The initial conditions were reached at the 20th minute, with an equilibration time of 7 min. The run was performed at flowrate of 0.3 mL $min^{-1}$.

The detector was a Thermo Q-Exactive Plus (Thermo Scientific, San Jose, CA, USA), equipped with a heated electrospray ionisation (HESI) source. Capillary temperature and vaporiser temperature were set at 330°C and 280°C, while the electrospray voltage was set at 3.50 kV operating in negative mode. Sheath and auxiliary gas (nitrogen) were set at 35 and 15 arbitrary units, with S lens RF level of 60. Xcalibur 3.0 software (Thermo Fisher Scientific, San Jose, CA, USA) was used to control the HPLC-HRMS system. The exact mass of the compounds was calculated using Qualbrowser program in Xcalibur 3.0 software. Instrument calibration was done every analytical session with a direct infusion of a LTQ Velos ESI Negative Ion Calibration Solution (Pierce Biotechnology Inc., Rockford, IL, USA).

The Full Scan (FS) acquisition was combined with an Independent Data Acquisition (DIA) mode, providing the MS2 spectra for confirmatory response, based on an inclusion list.

The resolving power of FS was set at 70,000 FWHM. In consideration of the molecular weights of our compound list, a scan range of m/z 200–950 was chosen; the automatic gain control (AGC) was set at $1 \times 10^6$ and the maximum injection time was 200 ms. The DIA segment operated in negative mode at 35,000 FWHM.

Detection of analytes was based on retention time of target compounds, on calculated exact mass of the deprotonated molecular ions, and at least one specific and typical fragment (Table 1). The formula of the compounds, with the exact theoretical mass of the parents and the diagnostic transition used to confirm the different PFASs, are reported in Table 1. Acquisition data were recorded and elaborated using Xcalibur™ software from Thermo Fisher.

## GC-MS/MS analyses

Triple quadrupole mass spectrometry (QqQ) in electronic impact (EI) mode was used for the simultaneous detection and quantification of PBDE in meat samples.

A GC Trace 1310 chromatograph coupled to a TSQ8000 triple quadrupole mass detector (Thermo Fisher Scientific, Palo Alto, CA, USA) was used to confirm and quantify contaminant levels in meat

samples by using a fused-silica capillary column Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane (35 m x 0.25 mm i.d., 0.25-µm film thickness, Restek, Bellefonte, PA, USA). The oven temperature program was as follows: initial temperature of 80°C, held for 3 min, and increased to 170°C at 10° C $min^{-1}$; then, increased from 170°C to 190°C at 3° C $min^{-1}$, and raised to 240°C at 2°C $min^{-1}$, before being ramped to 280°C at 3°C $min^{-1}$ and finally from 280°C to 310°C at 10°C $min^{-1}$ and held at this temperature for 5 min. The carrier gas (helium, purity >99.999%) was in constant flow mode at 1.0 ml $min^{-1}$. A volume of 1 µL was injected using a programmed temperature vaporiser injector (PTV) in splitless mode with a 1-min splitless period and the following inlet temperature programme: 80° C (0.05 min), 14.5°C $s^{-1}$ to 200°C (1 min) and 4.5° C $s^{-1}$ to 320°C (12 min – cleaning phase). A baffle liner (2 mm × 2.75 mm × 120 mm, Siltek-deactivated; Thermo Fisher Scientific) was used. The transfer line was maintained at 270°C and the ion source at 250°C. The electron energy and emission current were set to 70 eV and 50 µA, respectively. The scan time was 0.3 s and the peak width of both quadrupoles was 0.7 Da full widths at half maximum. Argon was used as a collision cell gas at a pressure of 1.5 mTorr. The QqQ mass spectrometer was operated in selected reaction monitoring mode (SRM) detecting two–three transitions per analyte. Identification of PBDEs was carried out by comparing sample peak relative retention times with those obtained for standards under the same conditions and the MS/MS fragmentation spectra obtained for each compound.

The Xcalibur™ processing and instrument control software program and Trace Finder 3.0 for data analysis and reporting (Thermo Fisher Scientific) were used.

### *Analytical performances and method validation*

The validation was carried out following the European Commission SANTE/2015 guideline (European Commission 2015). Recently, SANTE/2015 has been superseded by SANTE/2017 (European Commission 2017). For the PFASs, the method showed high specificity, without interference signals close to the retention time of the analytes, and consequently showed a high signal-to-noise (S/N) ratio in presence of analytes even at concentrations in the order of pg $g^{-1}$. Selectivity demonstrated a good compliance with the relative retention times for each analyte, which in our case were within 2.5% tolerance, with an S/N ratio greater than 3 when compared with the standard solution mix, both in FS and MS2 chromatograms. Moreover, diagnostic fragments showed an ion ratio within the recommended tolerances (European Commission 2002).

The mean recoveries for all analytes ranged between 80% and 117%, indicating the efficiency of the extraction protocol.

By consideration of the S/N ratio of each sample (≥3), the LOD values of 17 PFASs were from 5 pg $g^{-1}$ to 50 pg $g^{-1}$, the limit of quantification (LOQ) values were from 15 pg $g^{-1}$ to 150 pg $g^{-1}$.

Matrix validation curves were linear over the working range demonstrating a good fit for all analytes with an $R^2$ value greater than 0.99. Precision in terms of intra- and inter-day repeatability (Thompson et al. 2010) was calculated using one-way analysis of variance ANOVA, expressed as coefficients of variation (CVs), and was below 19% and 21%, respectively.

For PBDE, the selectivity of the method was evaluated by injecting extracted blank meat samples. The absence of interferences was proved by the lack of peaks with an S/N ratio higher than 3 at the retention times of the target compounds. Pork samples, previously analysed and checked for the absence of all PBDEs, were used as control samples during the optimisation and validation procedure. For the LOQ of the methods, we used the lowest validated spiked level meeting the requirements of recovery within the range of 70–120% and an RSD ≤20%, as defined by the European Commission (European Commission 2002). Finally, the extraction methods were also evaluated for their repeatability, linearity and recovery. Recoveries were calculated at LOQ for all compounds (Table 1). The repeatability as CV% was calculated by analysing six replicates at the same fortification level.

## Results and discussion

### *Method validation parameters*

The methods showed high specificity, without any interference close to the retention time of each

compound, and consequently an S/N ratio great than or equal to 3 in the presence of analytes was confirmed, even at the lowest detectable concentration, demonstrating good selectivity. Matrix validation curves show good linearity over the working range with a good fit ($R^2 > 0.99$) for all compounds. The mean recoveries (from 80% to 119%), with the other validation parameters, are reported in Tables 1 and 2. The CVs % are below 19% and 21%, satisfying the criteria required by the European Commission (EC 2002) and specified by Thompson et al. (2010). Regarding the LOD and LOQ for PFASs and for PBDEs, our satisfactory results show high method sensitivity for the selected compounds both for LC-HRMS and GC-MS/MS analyses.

### Application to pork samples

Overall results in terms of number detected, concentration levels and distribution of contaminants in the pork samples investigated are summarised in Table 3. The results are expressed in fresh weight.

Based on results of 77 samples for PFASs, only PFOA was detected in one Austrian sample with the concentration of 0.531 ng $g^{-1}$. PBDEs were detected in 3 out of 77 samples; only one, coming from Germany, showed the presence of all congeners analysed with the concentration range from 0.53 to 0.77 ng $g^{-1}$. In the other two samples, coming from The Netherlands and Italy, only one congener was detected in each, respectively, PBDE 153 (0.53 ng $g^{-1}$) and PBDE 100 (0.62 ng $g^{-1}$).

Based on our results some conclusions could be made. EU has not stated (MLs) for PBDEs in food, due to the lack of defined risk characterisation for PBDEs in humans, though recent studies have evaluated associations between PBDE concentrations in human tissues (e.g. blood, human milk) and health effects (immunological, reproductive, developmental, genotoxic and carcinogenic effects) (Agency for Toxic Substances and Disease Registry 2011). Comparing our results to the literature, in this work we found very low concentrations compared to other studies for the presence of PBDEs in pork meat coming from Spain (109 ng $g^{-1}$) (Bocio et al. 2003), Catalonia (32.3 ng $g^{-1}$) (Perellò et al., 2009); Sweden (63.6 ng $kg^{-1}$) (Domingo 2004) and (8.074 ng $g^{-1}$) China (Gong et al. 2014).

Due to their lipophilicity, Törnkvist et al. 2011 have shown that the highest contributors to the total PBDEs intake were fish (39%) and dairy products (31%), followed by meat (17%). Vouriner et al. (2012) studied the biomagnification of PBDEs in Atlantic salmon from three areas of the Baltic Sea and they demonstrated that PBDE accumulation is dependent on both age and fat content.

**Table 2.** The retention times (Tr), precursor ions (m/z), product ions (m/z), Collision Energy (V), Recovery (%), LOQ (ng $g^{-1}$) of investigated polybrominated diphenyl ethers (PBDE).

| Compound | Name | Formula | Mass [m/z] | Tr (minute) | Precursor ion [m/z] | Product ion [m/z] | Collision energy (V) | LOQ (ng $g^{-1}$) | Recovery (%) | Intra-day CV (%) ($n = 6$) | Inter-day CV (%) ($n = 6$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PBDE 28 | 2,4,4′-Tribromodiphenyl ether | $C_{12}H_7Br_3O$ | 406.9 | 32.35 | 248 | 139 | 30 | | | | |
| | | | | | **246** | **139** | 30 | 0.5 | 88 | 4 | 8 |
| | | | | | 408 | 246 | 10 | | | | |
| PBDE 33 | 2,3′,4′-Tribromodiphenyl ether | $C_{12}H_7Br_3O$ | 406.9 | 31.95 | 246 | 139 | 30 | | | | |
| | | | | | **248** | **139** | 30 | 0.5 | 89 | 4 | 10 |
| | | | | | 406 | 246 | 10 | | | | |
| PBDE 47 | 2,2′,4,4′-Tetrabromodiphenyl ether | $C_{12}H_6Br_4O$ | 485.8 | 38.52 | 326 | 217 | 30 | | | | |
| | | | | | 328 | 219 | 30 | 0.5 | 91 | 3 | 7 |
| | | | | | **482** | **326** | 20 | | | | |
| PBDE 99 | 2,2′,4,4′,5-Pentabromodiphenyl ether | $C_{12}H_5Br_5O$ | 564.7 | 41.27 | 404 | 297 | 30 | | | | |
| | | | | | 406 | 297 | 30 | 0.5 | 89 | 1 | 5 |
| | | | | | **563** | **404** | 20 | | | | |
| PBDE 100 | 2,2′,4,4′,6-Pentabromodiphenyl ether | $C_{12}H_5Br_5O$ | 564.7 | 42.01 | 404 | 297 | 30 | | | | |
| | | | | | 406 | 297 | 30 | 0.5 | 90 | 7 | 10 |
| | | | | | **564** | **404** | 10 | | | | |
| PBDE 153 | 2,2′,4,4′,5,5′-Hexabromodiphenyl ether | $C_{12}H_4Br_6O$ | 643.6 | 43.70 | 482 | 324 | 30 | | | | |
| | | | | | **484** | **377** | 30 | 0.5 | 93 | 3 | 6 |
| | | | | | 642 | 482 | 20 | | | | |
| PBDE 154 | 2,2′,4,4′,5,6′-Hexabromodiphenyl ether | $C_{12}H_4Br_6O$ | 643.6 | 44.91 | **484** | **324** | 30 | | | | |
| | | | | | 486 | 326 | 30 | 0.5 | 92 | 3 | 2 |
| | | | | | 644 | 484 | 20 | | | | |

* The precursor ion and product ion value reported in **bold** indicates the diagnostic transition.

**Table 3.** Quantification results of compounds from different production areas. The concentrations are expressed in ng $g^{-1}$ fresh weight.

| Production area | No of Sample | Analyte detected | Concentration |
|---|---|---|---|
| Austria | 7 | PFOA ($n$ = 1) | 0.53 |
| Denmark | 8 | n.d. | – |
| France | 8 | n.d. | – |
| Germany | 10 | PBDE 28 ($n$ = 1) | 0.57 |
| | | PBDE 33 | 0.73 |
| | | PBDE 47 | 0.60 |
| | | PBDE 99 | 0.74 |
| | | PBDE 100 | 0.77 |
| | | PBDE 153 | 0.70 |
| | | PBDE 154 | 0.53 |
| Netherland | 8 | PBDE 153 ($n$ = 1) | 0.53 |
| Italy | 20 | PBDE 100 ($n$ = 1) | 0.62 |
| Poland | 8 | n.d. | – |
| Spain | 8 | n.d. | – |

n.d. = Not detected.

Humans can be exposed to PBDEs in a wide variety of ways. The main routes of exposure are from contaminated foods, environment (air, soils) and skin contact with contaminated products. Several studies indicate that infants and toddlers have higher exposures to PBDEs compared to children or adults, due to their smaller weight and their frequent skin contact with floor dust (Agency for Toxic Substances and Disease Registry 2011). Information on PBDE dietary intake is very scarce in literature. It is also important to note that we analysed fresh meat, whereas preparation and different cooking methods can influence the levels of contaminants and so also consumers' exposure. It has been observed that during the cooking process, PBDE losses were higher than other POPs probably due to lipid removal during the process. (Perelló et al. 2009). Pork is widely used in the market, mostly due to its products. Almost all the countries that we have included in our paper have distinctive derived products in which the amount of PBDEs could be increased or reduced by the industrial processing method (Agency for Toxic Substances and Disease Registry 2011). On the basis on our results, we consider that human intake, on the base on our results, does not pose a risk for health. It is reasonable, however, that a risk may be present due to the long exposure to this compound.

PFOA is subject to similar considerations. PFOA is one of a class of chemical compounds that, due to their chemical structure, are very stable in the environment and resistant to biodegradation and hydrolysis (Agency for Toxic Substances and Disease Registry 2009). In living organisms, perfluoroalkyls, unlike PBDEs, bind to protein albumin in blood, liver and eggs, but do not accumulate in fat tissue. Due to their hydrolytic properties, they are more present in water environment and tend to be much more present in fish than other products.

PFOS in our samples did not appear concerning; it was found to be the predominant compound in fish samples (Guerranti et al. 2013; Squadrone et al. 2015; Chiesa et al. 2018), although other studies have found low concentrations in pork (15 pg $g^{-1}$; 0.74 ng $g^{-1}$) (Noorlander et al. 2011; Guerranti et al. 2013).

PFOA was found at low concentration in only one sample, coming from Austria (0.531 ng $g^{-1}$). Our results provide reasons for low concern. Based on what has been reported in the literature, our concentration is less than those found in another study made in Italy (less than 500 pg $g^{-1}$) (Guerranti et al. 2013); Belgium and Spain (55 pg $g^{-1}$) (Ericson et al. 2008; Cornelis et al. 2012); and Norway (15 pg $g^{-1}$) (Giorgi

**Table 4.** The literature data on PFOA, PFOS and PBDE.

| Investigated Compounds | Author | Analytical technique | Sample matrix | Producing area | Concentration or range |
|---|---|---|---|---|---|
| PFOA | Ingelido et al. 2010 | HPLC-MS | Human serum | Italy | 5.77 h.w. |
| | Noorlander et al. 2011 | LC-MS/MS (ESI) | Pork | Netherland | Average 15 w.w. |
| | Domingo 2004 | UPLC-MS/MS | Meat and meat products | Spain | <300 pg $g^{-1}$ f.w. |
| . | Guerranti et al. 2013 | HPLC-MS/MS | Pork | Italy | n.d. <LOD w.w. |
| PFOS | Noorlander et al. 2011 | LC-MS/MS (ESI) | Pork | Netherland | 14 w.w |
| | Domingo 2004 | UPLC-MS/MS | Meat and meat products | Spain | 34 pg $g^{-1}$ f.w. |
| | Guerranti et al. 2013 | HPLC-MS/MS | Pork | Italy | 0.74 w.w. |
| PBDE | Bocio et al. 2003 | GC/MS | Pork and pork products | Spain | 172 w.w. |
| | Perelló et al. 2009 | HRGC/HRMS | Loin of pork | Spain | 7.05 f.w. |
| | Törnkvist et al. 2011 | GC-MS/MS | Meat | Sweden | 0.023 f.w |
| | Gong et al. 2014 | GC/MS | Pork | China | 0.32173 ± 0.75326 w.w |

Analytical technique:
f.w.: fresh weight
h.w.: whole weight
w.w.: wet weight
n.d.: not detected

**Table 5.** Intake of pork meat of European countries (g/capita/day) and fresh water fish.

| Region | Pork intake: year 2000 | Pork intake: year 2013 | Freshwater Fish intake 2000 | Freshwater Fish intake 2013 |
|---|---|---|---|---|
| European Union | 113 | 107 | 6 | 10 |
| Austria | 165 | 144 | 5 | 11 |
| Denmark | 74 | 68 | 8 | 2 |
| France | 103 | 91 | 9 | 12 |
| Germany | 15 | 142 | 6 | 12 |
| Netherland | 149 | 100 | 5 | 8 |
| Italy | 103 | 110 | 5 | 8 |
| Poland | 131 | 127 | 4 | 5 |
| Spain | 175 | 134 | 5 | 11 |

Data sources: Food Supply – Livestock and Fish Primary Equivalent, provided by Food and Agriculture Organization of the United Nations (FAO).

et al. 2013). The highest concentration has been found in fatty fish (1.678 pg $g^{-1}$) (Berger et al., 2009). EFSA Scientific Panel on Contaminants in the Food Chain recommended that more occurrence data for PFASs in food should be collected to improve the accuracy of future exposure calculations (EFSA 2008). Subsequently, the European Commission issued Commission Recommendation 2010/161/EU on the monitoring of PFASs in food in the Member States (EFSA 2012). This paper contributes new knowledge of their presence in foodstuffs. On the basis of these results, there is no risk for human beings, but further studies are needed to keep monitoring their presence in foodstuff, as has been suggested by EU.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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FOOD ADDITIVES & CONTAMINANTS: PART A
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# Presence of emerging contaminants in baby food

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**ABSTRACT**

Food safety becomes imperative when it aims to protect infants. The objective of this study was to investigate the presence of emerging contaminants of which some act as endocrine-disruptors in baby food. Persistent organic pollutants (POPs), perfluoroalkyl substances (PFASs), parabens and antibiotics were analysed in 112 baby food of different categories (meat, fish, vegetables, fruit, cheese). As regard POPs, PFASs and antibiotics, no residues were detected, while one sample showed methyl-paraben (4.14 ng $g^{-1}$), whereas another three contained propyl-paraben (median 1.70 ng $g^{-1}$). Special attention must be paid on parabens metabolites, as 4-hydroxybenzoic acid, the principal parabens metabolite, was detected in all samples (median 176.7 ng $g^{-1}$). It may be present as a degradation product, but also, it can be released from vegetables and fruits during food processing. It is recommended to collect more data on natural vs non-natural occurrence of parabens and metabolites to evaluate the exposure of sensitive population vs ADI published by the European Food Safety Authority and European Medicines Agency.

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## Introduction

Baby food is homogenised food, packaged in sterile conditions, made from fruit, vegetables, fish, meat, or combining different of these matrices, directly ready for use. An alternative to traditional baby food is organic baby food, even if it is more expensive. In general, baby foods are produced by subjecting the selected substances to a sophisticated procedure of homogenisation that makes them digestible for infants between 4–6 months and 2 years old. Infant formulas are very useful in the first months of life, in the so-called weaning phase, when milk is gradually replaced with a practical and functional solution to ensure a complete supply of nutrients. Food safety checks are very important and challenging when the aim is to detect simultaneous residue analysis of different compounds belonging to a wide variety of different classes, in selective foodstuffs both of vegetable and animal origins (Pérez-Ortega et al. 2012), especially to protect a vulnerable and most at-risk population group, such as infants.

On the other hand, the presence of emerging contaminants and/or endocrine disruptors such as persistent organic pollutants (POPs), perfluoroalkyl substances (PFASs), parabens, human and veterinary drugs (e.g. antibiotics) has been recently reported in processed food deriving from environmental contamination and/or farming/crop practices (Chiesa et al. 2018a, 2019).

As the European Food Safety Authority (EFSA) states in its guidance on risk assessment for substances in baby food (EFSA 2017), the immune system in immediate post-natal life is particularly sensitive and exposure to immunotoxicants may result in persistent effects on the immune system that last or appear only long after exposure, but may also occur at lower doses than adult exposure. Different compounds or types of exposure may produce different severities and unpredictable alterations depending on the time of exposure during the immune system development. They may be associated with chronic immunological conditions such as immune deficiency, autoimmunity, inflammation and allergic reactions.

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Although the guidance addresses specifically the risk assessment of infants less than 16 weeks of age, the matters affects infants and young children above 16 weeks of age.

To ensure appropriate nutritional composition and safety of foods for infants and young children, the European Commission has defined specific rules for such foodstuffs.

The Directive 99/39/EC encompasses the specific rules on the presence of pesticide residues in processed cereal-based baby foods and baby foods and requires that this type of food contains no detectable levels of pesticide residues, meaning not more than 0.01 mg $kg^{-1}$, as consumed. In addition, the Directive prohibits the use of certain very toxic pesticides in the production of processed cereal-based baby foods and baby foods and establishes levels lower than the general maximum level of 0.01 mg $kg^{-1}$ for a few other very toxic pesticides.

In addition, the Directive 2006/125/EC, indicates that cereal-based foods and baby foods must also comply with other specific provisions laid down in the relevant measures of EU law on hygiene, on the use of food additives, on the presence of contaminants and on the use of materials intended to come into contact with the products.

As well known, food is considered as a cumulative daily source of parabens and other legislation was established to ensure consumers' safety. A risk assessment of parabens was recommended by the EFSA (2004) and was set an acceptable daily intake (ADI) of 10 mg $kg^{-1}$ body weight (bw)/day for methyl paraben (MeP) and ethyl paraben (EtP), but for a long time safety evaluations have not been defined for other parabens. In recent years, special attention has been paid to propyl-paraben (PrP) and ADI of 1.25 mg $kg^{-1}$ bw was established just a few years ago (European Medicines Agency 2015). The levels of residues that might occur following its utilisation in veterinary products are expected to be too low to impact on industrial food processing and therefore maximum residual limits (MRL) were not set-up, as was declared in EU regulations (European Commission 2015).

The question about paraben presence in processed foods is even more complicated when the parabens transformation products, namely 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid, *p*-HBA), 3,4-dihydroxybenzoic acid (protocatechuic acid, 3,4-DHB), methyl-protocatechuate (OH-MeP) and ethyl-protocatechuate (OH-EtP), are taken into consideration (Xue et al. 2015, 2017). Those (di) hydroxybenzoic acids have been recognised as metabolites of parabens and thus might serve as potential markers of parabens incidence (Wang et al. 2018; Chiesa et al. 2018e). Nevertheless, the parabens are not their unique, exclusive source: *p*-HBA and 3,4-DHB are also naturally present in many plants and vegetables (Tomás-Barberán and Clifford 2000; Kakkar and Bais 2014). Also, both *p*-HBA and 3,4-DHB appear as intermediates in several industrial processes with potential biotechnological applications in food production (Wang et al. 2018), and if not managed properly they could represent a risk for baby food, as well. Additionally, OH-MeP and OH-EtP are recognised as hydroxylation products of MeP and EtP, respectively, and generally, they are produced by biotic and abiotic transformation of many xenobiotics (Xue et al. 2017). There is no available literature data about their origin, level and risk assessment in the baby food.

Salicylic acid, a structural isomer of *p*-HBA, is a compound that is naturally present in foods can cause adverse reactions to persons who are intolerant. Salicylate sensitivity is not as common as other type of food intolerance, but it should be taken into consideration especially when its quantity in baby food is concerned. Studies on the salicylic content of foods are sparse and have produced distinctly different results, giving rise to controversy (Malakar et al. 2017).

As regards veterinary drugs or other class of substances, there is not any current legislation for MRL in baby food, so a zero-tolerance policy is applied establishing that the presence of these compounds is illegal at any level (Aguilera-Luiz et al. 2012).

As regards PFASs, EFSA recommended the analysis of this class of compounds in different food items to assess a reliable risk evaluation, and this appears essential when the highest chronic dietary exposure to perfluorooctanesulfonic acid (PFOS) was estimated for the youngest population groups (EFSA 2018b).

Therefore, in the light of these considerations, the application of these preventive policies require

the development of sensitive analytical methods to determine the presence of these compounds and of their metabolites, useful as markers, at very low concentrations to protect infant health.

There are few works in literature on the multi-residue analysis of emerging contaminants and endocrine-disrupting chemicals in baby food, and those deal with single or only a few classes of compounds, as reported in Table 1, a summary table on the state of art on this topic.

In this regard, our aim was to analyse different baby food on the basis of the matrix type (meat, fish, cheese, vegetables and fruit) for the detection of POPs, PFASs, antibiotics and parabens evaluating the possible direct or indirect contamination of residues, relative to the different breeding/crop practices or environmental contamination, to evaluate infant health risk.

## Material and methods

### Chemicals and reagents

All solvents were purchased from Merck and water was purified by a Milli-Q system (Merck KGaA, Darmstadt, Germany). SupelTM QuE Citrate (EN) tubes and SupelTM QuE-ZSEP (EN) tubes were from Supelco (Sigma Aldrich, St. Louis, MO, USA). The Oasis HLB 3 mL, 60 mg and Oasis WAX 3 mL, 60 mg cartridges were from Waters (Milford, MA, USA). Non-dioxin like-polychlorinated biphenyls (NDL-PCB) (PCB 28; −52; −101; −138; −153 and −180) [congener 209 as internal standard (IS)] and PBDEs (PBDE 28; −33; −47; −99; −100; −153 and −154) [3-fluoro-2,2,4,4,6- pentabromodiphenyl ether (FBDE) as IS] were from AccuStandard (New Haven, USA). Organochlorine pesticides (OCs) (aldrin; α-hexachlorocyclohexane (α-HCH); β-hexachlorocyclohexane (β-BHC); hexachlorobenzene (HCB); dichlorodiphenyldichloroethylene (DDE); dichlorodiphenyltrichloroethane (DDT); dichlorodiphenyldichloroethane (DDD); endosulphan I; endosulphan II; endosulphan sulphate; endrin; heptachlor; heptachlor epoxide; lindane and trans chlordane) were from Restek (Bellefonte, PA, USA). Organophosphorus pesticides (OP): chlorpyriphos diazinon, disulphoton, ethoprophos, mevinphos and phorate, and 4-nonylphenol (IS for OCs and OPs) were from Sigma-Aldrich. The four PAHs: chrysene, benz(a)anthracene, benzo(b)fluoranthene and benzo-(a)pyrene were from Restek (Bellefonte, PA, USA).

PFASs: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorobutane sulphonic acid (PFBS), perfluoroheptanoic acid (PFHpA), PFOA, perfluorohexane sulphonate (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), PFOS, perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUnDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), and perfluorooctadecanoic acid (PFODA) were from Chemical Research 2000 Srl (Rome, Italy) and ISs perfluoro-[1,2,3,4,5-$^{13}$C5]nonanoic acid (MPFNA) and perfluoro-[1,2,3,4-$^{13}$C4]octanesulfonic acid (MPFOS).

Antimicrobial agents: amoxycillin, ampicillin, benzylpenicillin, cefquinome, ceftiofur, cefalexin, ciprofloxacin, chloramphenicol, chlortetracycline, cloxacillin, danofloxacin, dicloxacillin, dimetridazole, doxycycline, enrofloxacin, florfenicol, florfenicol amine, flumequine, furaltadone, furazolidone, lincomycin, lomefloxacin, marbofloxacin, nalidixic acid, nitrofurazone, oxolinic acid, oxytetracycline, ronidazole, spiramycin, sulphadiazine, sulphathiazole, sulfadimethoxine, sulphadimidine, sulfamerazine, tetracycline, thiamphenicol, tiamulin, tilmicosine, tinidazole, trimethoprim, tylosin and enrofloxacin-d5 (IS) were from Merck.

Parabens: MeP, EtP, propyl-(PrP), butyl-(BuP) and benzylparaben (BzP), 4-hydroxybenzoic acid (pHBA), 3,4-DHB, OH-MeP and OH-EtP including 4-fluorobenzoic acid (4-FB) used as IS, were from Merck (KGaA, Darmstadt, Germany).

### Standard solutions

For stock and working solutions, kept at −20°C, hexane was used as solvent for GC-MS/MS and methanol for HPLC-HRMS analyses.

### Sample collection

The total number of collected samples was 112. In detail: 45 meat (veal, swine, horse, lamb, rabbit, chicken, turkey), 13 fish (plaice, salmon, sea bream, hake, trout, bass, cod), 47 fruit (apple, pear, plum, blueberry, apricot, peach, mixed

**Table 1.** Literature summary on emerging contaminants and endocrine-disrupting chemicals detected in baby food.

| Reference | Compounds | Baby food typologies | Extraction Technique | Detection techniques | LOD/LOQ CCα/CCβ (ng $g^{-1}$) | Min and Max Conc. detected (Application) (ng $g^{-1}$) |
|---|---|---|---|---|---|---|
| **Antibiotics** | | | | | | |
| Gentili et al. (2004) | Sulphonamides | Bovine (veal and beef), Porcine (pig and ham), Poultry meat (chicken and turkey), | ASE | LC-ESI-MS/MS | LOD: 0.4–1.7<br>LOQ: 1.2–5.1 | <LOQ- 3.5 |
| Díaz-Alvarez (2009) | Quinolones<br>Fluoroquinolones | Chicken meat and vegetables | Ultrasound-assisted extraction + solid-phase extraction | HPLC-UV | LOD: 30–110<br>LOQ: 100–350 | No application |
| Rodriguez et al. (2011) | Fluoroquinolones | Baby food purées ham, chicken, turkey, lamb, beef, sole, hake | MISPE (molecularly imprinted solid phase extraction) | LC-FLD (liquid chromatography with fluorescence detection) | $CC_{\alpha}$:11–19<br>$CC_{\beta}$:18–32 | n.d.-3 |
| Aguilera-Luiz et al. (2012) | Multiresidue veterinary drugs | Meat-based baby food and powdered milk-based infant formulae | QuEChERS | UHPLC-MS/MS | CCα:0.5–16.2<br>CCβ:1.4–22.4 | <5–25.2 |
| Jia et al. (2014) | MULTI-RESIDUES (333 veterinary drugs and pesticides included antibiotics, OCs and OPs) | Baby food (93 including VBF, MBF, CBF, FBF and PMBIF) | QuEChERS | UHPLC-Q-Orbitrap | CCα:0.01–5.35<br>CCβ:0.01–9.27 | 1.45–22.34 |
| Nasr et al. (2014) | Macrolides (Tylosin and josamycin) | (chicken muscles, chicken liver, bovine muscles, liver, milk and eggs) Chicken-based baby food and baby formulae | Liquid–liquid extraction | MLC–monolithic method with UV | LOD: 1100–3000<br>LOQ:3600–9900 | No application |
| Nebot et al. (2014) | Tetracyclines | Meat/vegetables | Liquid–liquid extraction | HPLC-MS/MS | LOQ: 5.0 | 5.0–9.0 |
| Vakh et al. (2018) | Fluoroquinolones | Chicken, beef or turkey | Automated magnetic dispersive micro-solid phase extraction | HPLC-FLD | LOD: 1.5–3.0<br>LOQ: 5.0–10.0 | No application |
| **Persistent Organic Pollutants – POPs (PBDEs, PCBs, OCs, PAHs, OPs)** | | | | | | |
| Pandelova et al. (2011) | PCBs<br>OCs | Fruits, vegetables, meat, fish, | ASE for PCBs<br>Soxhlet extraction for OCs | HRGC/HRMS | LOD: 0.0005<br>LOQ: 0.0035 | 0.001–0.04 |
| Jeong et al. (2014) | PBDEs | Homemade baby food | Soxhlet extraction | HRGC/HRMS | LOQ: 0.0001–0.01 | 0.245–6.00 |
| Jeong et al. (2014) | OCs<br>PCBs | Homemade baby food | Soxhlet extraction | HRGC/HRMS | LOD: 0.00012–0.0015<br>LOQ: 0.0004–0.005 | 0.00028–3.338 |
| Liu et al. (2014) | PBDEs | Baby food (formula, cereal, and puree) | ASE | GC/MS | - | n.d.- 0.94 |
| Schecter et al. (2010) | PBDEs | Meat based baby food (Ham/veal, beef) | Soxhlet extraction | HRGC-HRMS<br>GC–ECD | LOD: 0.0002–0.1 | 0.102–3.156 |
| Notardonato et al. (2018) | OPs | Freeze-dried products (chicken, rabbit, turkey) and soft baby foods (chicken, rabbit, sea bream, plaice) | Ultrasound–vortex-assisted DLLME (liquid–liquid microextraction) | GC–IT/MS | LOD: 0.2–4.7<br>LOQ: 2.3–8.5 | <LOQ |
| Toms et al. (2016) | PBDEs<br>OCPs<br>PCBs | Fruit-, vegetable-, meat-, fish- and dairy-based baby foods | ASE | GC/MS | LOD: 0.0001–0.0005 | <LOD–0.095 |
| Lorán et al. (2010) | PCBs | Processed cereal baby food, meat (chicken, beef and lamb), fish (sole and hake) | Soxhlet extraction | HRGC coupled to Ion Trap MS/MS | 0.1–0.5 | 0.03–0.29 |
| Leandro et al. (2005) | OCs and OPs | Fruit and rice, fish and pasta, potato and pork | QuEChERS | Large volume injections LVI–GC–MS/MS<br>LC-MS<br>GC-MS | 0.5–10 | No application |
| Fontcuberta et al. (2008) | OCs | Not specified | Liquid extraction | GC/MS | LOQ: 5–10 | n.d |
| Dobrinas et al. (2011) | OCs | Fruit, vegetable, meat–vegetable + fish–vegetable based purée | Soxhlet extraction | GC-ECD | - | <LOD – 304 |
| Radford et al. (2014) | Ops | Vegetable and fruit | Solid phase extraction | HPLC-MS/MS | LOD: 0.18–2.7 | 0.08–1.43 |

(*Continued*)

**Table 1.** (Continued).

| Reference | Compounds | Baby food typologies | Extraction Technique | Detection techniques | LOD/LOQ CCα/CCβ (ng $g^{-1}$) | Min and Max Conc. detected (Application) (ng $g^{-1}$) |
|---|---|---|---|---|---|---|
| Al-Zahraa et al. (2016) | OCPs, OPPs | Fruit-vegetables and rice cereal-based baby foods | QuEChERS | GC/MS | LOD:0.0001–0.0191 | n.d.- 13.97 |
| Santonicola et al. (2017) | PAH | Meat (chicken, turkey, calf, pig, lamb, horse) and fish (trout, flounder, salmon, hake, sea bass, gilthead bream) | Liquid extraction | HPLC-FD | LOD: 0.005–0.11 | n.d – 72.88 |
| **Perfluoroalkyl Substances – PFASs** | | | | | | |
| Ullah et al. (2012) | PFASs | Vegetables, meat, and fish | Liquid extraction +SPE C18 + SPEC8 | HPLC/HRMS (qTOF) | LOD 0.0018–0.2<br>LOQ 0.006–0.066 | n.d.-1.84 |
| Lorenzo et al. (2016) | PFASs | Meat, poultry, fish, offal, vegetables and fruit | Liquid extraction +SPE Strata X | UHPLC-MS/MS. | LOD 0.75–4.5<br>LOQ 3.75–15.00 | 0.017–5.013 |
| **Parabens** | | | | | | |
| Chiesa et al. (2018e) | Methyl- (MeP), ethyl-, propyl-, butyl-, benzylparaben, 4-hydroxybenzoic acid (pHBA) | Fish and fish products (including baby food) | Simple liquid-liquid extraction | LC-HRMS | LOD 0.65–3.50<br>LOQ 2.15–11.70 | pHBA 27.40–94.00 |

**Abbreviations**: Accelerated Solvent Extraction (ASE); cereal-based food (CBF); liquid–liquid equilibrium (LLE); meat-based food (MBF); magnetic dispersive solid phase extraction (MDSPE); powdered milk-based infant formulae (PMBIF); Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS); (non-fatty based on fruit (FBF); Solid Phase Extraction (SPE); vegetable baby food (VBF).

fruit) and vegetable (legumes, zucchini, carrots, potatoes, sweet potato, tomato, broccoli, peas, spinach, mixed vegetables) and 7 cheese baby food. They were from different commercial Italian brands, present in the international market, and bought in different Italian supermarkets. Moreover, 11 samples of different matrices were bought in some supermarkets of Serbia, to extend the international scope. The sample details are specified in Table 2.

### *Sample treatment protocol for PoPs*

Two gram samples were extracted by the QuEChERS protocol described in Chiesa et al. (2018a).

### *Sample treatment protocol for PFASs*

Two gram samples were extracted as in our previous works (Chiesa et al. 2018b).

### *Sample treatment protocol for antibiotics*

One gram samples were extracted as described by Chiesa et al. (2017), (2018c) and (2018d).

### *Sample treatment protocol for parabens and metabolites*

The sample procedure performed for parabens is reported by Chiesa et al. (2018e).

### *GC-MS/MS analyses for POPs*

The instrument was a GC Trace 1310 chromatograph coupled to a TSQ8000 triple quadrupole mass detector (Thermo Fisher Scientific, Palo Alto, CA, USA) with electronic impact (EI) mode set in selected reaction monitoring mode (SRM). The column was a fused-silica capillary Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane (35 m x 0.25 mm i.d., 0.25 µm film thickness, Restek, Bellefonte, PA, USA). The oven temperature program and all operation parameters were the same as for our previous work (Chiesa et al. 2018a). Xcalibur software was used to control instrument and Trace Finder 3.0 for data processing (Thermo Fisher Scientific).

**Table 2.** Sample collection details according to food categories.

| Meat | Fish | Fruit/vegetables | Cheese |
|---|---|---|---|
| Veal | Plait | Apple | Cheese (bovine milk) |
| Swine | Hake | Plum | |
| Horse | Plait and potatoes* | Pear | |
| Lamb | Trout and vegetables* | Pear and blueberry | |
| Rabbit | Bream and vegetables* | Apple and blueberry | |
| Chicken | Bream and potatoes* | Apple and banana | |
| Turkey | Bass and vegetables* | Apple and peach | |
| Veal and ham | Cod and potatoes* | Apple and apricot | |
| Chicken and carrots* | Cod and vegetables* | Banana and kiwi | |
| Chicken with green beans and zucchini* | Salmon and vegetables* | Mixed fruit | |
| Veal and vegetables* | | Carrot and apple | |
| Veal and carrots* | | Legumes | |
| Veal and potatoes* | | Zucchini | |
| Veal, broccoli and carrots* | | Broccoli | |
| Veal, potatoes and mushrooms* | | Carrots, potatoes and zucchini | |
| Turkey, corn and potatoes* | | Sweet potato and carrots | |
| | | Tomato and vegetables | |
| | | Peas and spinach | |
| | | Mixed vegetables | |
| **Total n = 45** | **n = 13** | **n = 47** | **n = 7** |

*for mixed categories, meat and fish represented the major component as declared in the label.

### *LC-HRMS orbitrap analyses for PFASs, antibiotics, and parabens*

A Q-Exactive Orbitrap equipped with a heated electrospray ionisation source (HESI) was used. The HPLC system was a Surveyor MS quaternary pump (Thermo Fisher Scientific, San Jose, CA, USA) with a Synergi Hydro-RP reverse-phase HPLC column (150 × 2.0 mm, i.d. 4 µm) and a C18 guard column (4 × 3.0 mm; Phenomenex, Torrance, CA, USA). The mobile phase used for PFASs was a gradient of aqueous NH4COOH (20 mM) and MeOH; for antibiotics and parabens separation a binary mixture of aqueous HCOOH (0.1%) and MeOH was used. All the parameters are described in our previous works (Chiesa et al. 2018a, 2018d, 2018e).

For each analytical method, we combined a full scan (FS) with a data-independent acquisition (DIA), providing the $MS^2$ spectra for confirmatory analysis.

Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) acquired and elaborated data.

### *Validation parameters*

Antibiotic validation was assessed following the Commission Decision guidelines 657/2002/CE, while for the other analytes SANTE/11813/2017 guidelines were followed. All the validation parameters are described in our previous works (Chiesa et al. 2018a, 2018d). Regarding parabens, our analytical procedure published earlier (Chiesa et al. 2018e) was followed strictly, including also the determination of validation parameters for 3,4-DHB, OH-MeP and OH-EtP that were not previously elaborated.

### *Statistical evaluation*

Preliminary statistical evaluation (Shapiro-Wilk Test) revealed that data were not normally distributed. Therefore, non-parametric Kruskal-Wallis One Way analysis followed by all pairwise multiple comparison processes (Dunn's method) were used to check the differences between the medians of the datasets. Statistical analyses were performed using Sigma Stat (Statistical Analysis System, version 12.5) software (Jandel Scientific GmbH, Erkrath, Germany). A *P*-value of 0.05 was set as statistically significant.

## Results and discussion

No POPs were found in samples analysed. In literature, one of the compounds detected with highest frequency were PCBs, where concentrations ranged up to 95 pg $g^{-1}$ (Toms et al. 2016), 0.03 ng $g^{-1}$ and 0.29 ng $g^{-1}$ for fish and gluten-free cereals products (Lorán et al. 2010), 7.78–270 pg $g^{-1}$ (Jeong et al. 2014) while negligible PCB levels were detected in another study, in line with our results (Table 1). Literature results showed PBDEs were found with

median concentrations at 21 pg $g^{-1}$ in United States samples and 36 pg $g^{-1}$ in Chinese samples (Liu et al. 2014). In one study, conducted on homemade Korea samples, PBDEs were found with highest frequency in 90% of samples at concentrations from 24.5 to 6000 pg $g^{-1}$(Jeong et al. 2014), higher than those found in commercial formulae from the United States where median concentration were 1725 pg $g^{-1}$ for meat samples, 283 pg $g^{-1}$ fish, 31.5 pg $g^{-1}$ in dairy products (Schecter et al. 2004). The lower levels were found in European products, with whom our results are in line suggesting a safety of the products. Moreover, according to European Community in 2006 (European Commission 2006), baby food should be free of pesticides residues and EFSA panel set a Maximum Residue Level of 0.01 mg $kg^{-1}$ in food for infant, as consumed (EFSA 2018a). In one study conducted in Spain (Fontcuberta et al. 2008), the authors observed a gradual disappearance of regulated chlorinated organic pesticides from 1989–2000 period and 2001–2006 period, suggesting that this could reflect an improvement of worldwide regulation (Fontcuberta et al. 2008). In our study, no pesticides residues were found and this reflects what has been reported in other studies (Fontcuberta et al. 2008) on the progressive lower detection of pesticides as a consequence of the improvement of industrial processes and regulation. So, on the base of our results, a growing enhancement of regulation could be linked to an improvement of product safety and therefore an absence of contaminants (EFSA 2018a).

As regard PFASs, none were detected, demonstrating that this kind of contamination in the different baby food analysed may currently not be of concern. In particular if we compare our results to the few studies present in literature, in that of Ullah et al. (2012) the detection frequency (percentage detects) for the 13 investigated PFASs was 77% in fish, 64% in meat, 49% in vegetables at concentrations below the respective minimum detectable level of 7 to 20 pg $g^{-1}$, and could thus only be estimated semi-quantitatively. Quantifiable concentrations of several PFASs were found in pig liver and fish and the highest level of PFOS (1.8 ng $g^{-1}$) was quantified in fish from The Netherlands, if compared to 13 pg $g^{-1}$ found in those from Bangladesh. In the study of Lorenzo et al. (2016), PFBA and PFOA were detected in 100% of analysed samples with concentrations up to 5013 ng $g^{-1}$, followed by PFDA (83%) up to 387 ng $g^{-1}$ and PFOS detected only in 17% of the samples and they stated they can derive from the production chain since many parts of the equipment were made of perfluoroalkylated materials.

As regard antibiotics, also in this case we found no residues in any analysed baby food. In the study of Gentili et al. (2004), among 30 analysed infant foods for sulphonamides, one, whose formulation was based on veal meat, was positive to sulfamethizole (1.4 ng $g^{-1}$) and other two samples were <LOQ. In the work of Aguilera-Luiz et al. (2012) only one meat baby food sample out of 21 showed the presence of levamisole at 9.5 ng $g^{-1}$. In the work of Nebot et al. (2014) 31 baby food samples containing between 15% and 20% beef analysed for tetracyclines, only 3 samples showed doxycycline with concentrations between 5 and 9 ng $g^{-1}$, one tetracycline (5.4 ng $g^{-1}$) and another chlortetracycline (7.2 ng $g^{-1}$). In the other few works reported in Table 1, no compounds were present.

Parabens affect reproductive or endocrine endpoints at high concentrations in both male and female immature experimental animals, and with exposure, both boys and girls may be at risk of endocrine disruption. Oestrogenic effects in boys may increase the risk for incomplete masculinisation resulting in decreased sperm quality. In girls, an increased oestrogenic load may increase the risk of early puberty, and premature mammary development (Boberg et al. 2010). The great majority of samples enrolled in this study did not reveal measurable levels of parabens (Table 3), except one plum preparation that contained 4.14 ng $g^{-1}$ of MeP and one apple, one pear and one turkey sample that contained PrP at the concentrations of 1.2, 3.4 and 1.33 ng $g^{-1}$, respectively. Although having such low incidence, this kind of contamination should not be underestimated as it is not clear what might be the origin of those two parabens discovered randomly in 4 out of 112 samples (<3.5%). The range of concentration detected herein corresponds to the daily intake which is 2–3 orders of magnitude (about 1000 times) below ADI recommended by European Medicines Agency (2015) which was set at 1.25 mg $kg^{-1}$.

**Table 3.** Concentration levels (ng $g^{-1}$) of parabens and their analogues/possible metabolites in all baby food sample analysed.

| | MeP[a] | EtP | PrP | BtP | BzP | p-HBA | 3,4-DHB | OH-MeP | OH-EtP |
|---|---|---|---|---|---|---|---|---|---|
| Positive (%) | 1 (0.9%) | n.d. | 3 (2.7%) | n.d. | n.d. | 112 (100%) | 86 (76%) | 10 (8,9%) | 3 (2.7%) |
| Mean | 4.14 | n.d. | 1.70 | n.d. | n.d. | 321.7 | 162.2 | 3.7 | 7.5 |
| Median | / | n.d. | 1.33 | n.d. | n.d. | 176.6 | 10.1 | 2.1 | 7.3 |
| Min | / | n.d. | 1.20 | n.d. | n.d. | 14.4 | 2.1 | 0.8 | 7.2 |
| Max | / | n.d. | 3.24 | n.d. | n.d. | 2149 | 3638 | 14.4 | 8.2 |
| Percentile 25% (Q1) | / | n.d. | 1.33 | n.d. | n.d. | 93.9 | 3.3 | 1.1 | 7.2 |
| Percentile 75% (Q3) | / | n.d. | 3.24 | n.d. | n.d. | 455.9 | 52.6 | 4.6 | 8.2 |

[a] Refer to text (materials and methods section) for full names of the abbreviated compounds.
*n.d. = not detected.

Special attention needs to be directed towards PrP because legislation concerning this compound has been rather confusing in the past and an ADI has been recommended recently (European Medicines Agency 2015). PrP is an antimicrobial preservative used in veterinary medicinal products, and it was previously classified as additive E216. As a result of EFSA's re-evaluation (2004) of parabens with E numbers E214-E219, the E classification of PrP (and its sodium salt) were successively suspended. This decision was based on the scientific data indicating that administration of PrP to male rats resulted in adverse effects on the hormonal system and male reproductive functions. It is therefore recommended to collect more occurrence data for parabens and transformation products to conduct a thorough exposure and safety assessment.

Unfortunately, the literature data regarding parabens' occurrence in processed food intended for infants' diet is rather limited, apart from the preliminary results reported by our group that concerns exclusively baby food containing fish (Chiesa et al. 2018e) where no parabens were detected.

*p*-HBA was found in all samples which is why results obtained here in regard to different type of infant food preparation were obtained. It is well-established that *p*-HBA does not exclusively derive as degradation product and potential indicator of parabens treatment, but it is also naturally present in many vegetables (Tomás-Barberan and Clifford, 2000). Indeed, when samples from four food groups were taken into consideration there were evident differences in the *p*-HBA level (Figure 1). The vegetable samples possessed an extremely high amount of *p*-HBA most probably due to the endogenous origin of *p*-HBA, with preparations based on carrot and plum showing the highest levels. However, the reason why samples that consisted of meat only, contained a substantial amount of this metabolite is uncertain (n = 36, median = 89,3, 25–75 ng $g^{-1}$ percentile = 50.9–99.1 ng $g^{-1}$). One possible explanation might lay down in the fact that those samples were subjected to more elaborate technological processes (such as cooking) including the addition of water treatment that might be source of parabens, as well. Also, it remains to be defined what would be the safe levels of *p*-HBA because regardless of its origin it has been reported (independently from other parabens) to exhibit oestrogenic activity (Boberg et al. 2010). Actually, *p*-HBA is used as a flavouring additive, with no safety concern declared at current levels of intake.

Regarding 3,4-DHB, recent studies indicate its potential to act as a protective antioxidant polyphenolic compound against various diseases including neoplasms (Xie et al. 2018) while the findings about the positive correlation between its urinary concentration and childhood obesity call for caution (Xue et al. 2015). The differences between infant food based on meat or vegetables/fruit is also apparent when the amount and distribution of 3,4-DHB is concerned (Table 3, Figure 2): the median level (with $25^{th}$-$27^{th}$ percentile) in 22 meat/meat+veg samples was 3.4 ng $g^{-1}$ (1.8–4.6 ng $g^{-1}$) vs 38 ng $g^{-1}$ (5.2–177.8 ng $g^{-1}$) for all 46 fruit and vegetables preparations. Considerable variability within each class and limited number of fish/fish+veg samples disabled any statistical confirmation regarding the fact that fish/fish+veg samples contained notably lower levels when compared with veg/fruit samples. Cheese samples did not reveal any measurable amount of 3,4-DHB. Extremely high contents of 3,4-DHB were found in all three pure plum specimens (2148, 2471 and 3638 ng $g^{-1}$). A high concentration of 3,4-DHB was found in one sample

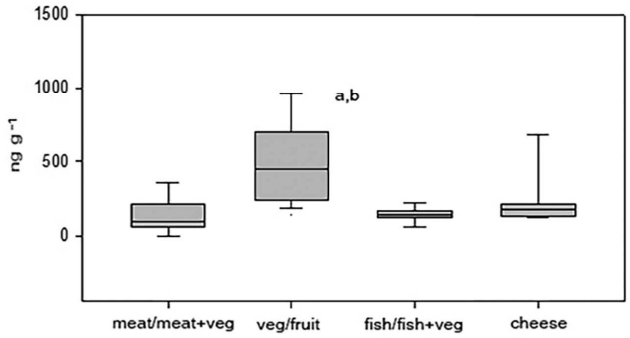


Figure 1. Distribution of *p*-HBA according to baby food category: animal, vegetable/fruit, cheese and mixed matrix for which meat or fish represented the major component as declared on the label. Data are reported as median with 25th–75th percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks, followed by Dunn's test for pairwise multiple comparison procedures: a – stands for $p < .001$ when meat/meat +vegetables samples were compared with vegetables/fruit preparation; b – stands for $p < .001$ when fish/fish +vegetables samples were compared with vegetables/fruit preparation.

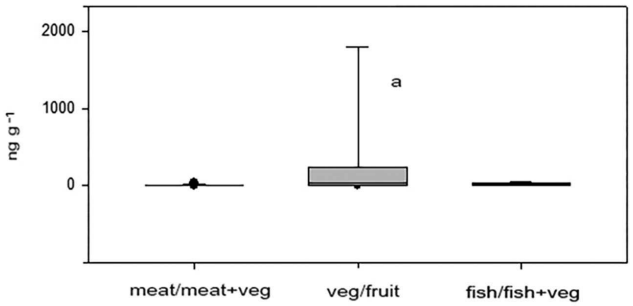


Figure 2. Distribution of 3,4-DHB in the samples where it was detected. N (meat/meat+veg) = 22; N (veg/fruit) = 46; N (fish/fish+veg) = 9. Data are reported as median with $25^{th}$–$75^{th}$ percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks that revealed statistical significance (a stands for $p < .001$ vs meat/meat+veg group).

that was plum-apple homogenate (943.2 ng $g^{-1}$). The endogenous origin of 3,4-DHB in those samples is apparent, as plum has been shown to contains a substantial amount of polyphenolic compounds, 3,4-DHB included (Kakkar and Bais 2014). The same samples contained OH-EtP and also here their natural origin as part of polyphenolic pertinence is more plausible. Random occurrence of OH-MeP in meat and vegetable also points towards its endogenous origin. On the other hand, a very important finding concerning OH-MeP is highlighted by its frequent appearance in preparations that contained fish as a main constituent: 7 of 13 fish samples showed OH-MeP presence. Considering that OH-MeP is the main hydroxylated MeP derivate in aquatic biota (Xue et al. 2017) the content of OH-MeP especially in infant food preparation based on fish (without any other ingredient) might be a reliable indicator of parabens contamination.

The analysis conducted for parabens confirmed the presence of salicylic acid in all infant food samples and its distribution is presented in Figure 3. This is due to the addition of ingredients rich in salicylates, such as vegetables where salicylates are naturally present in high quantities (Malakar et al. 2017). Plant salicylates have an important role against pathogens, herbivores, and abiotic stresses,

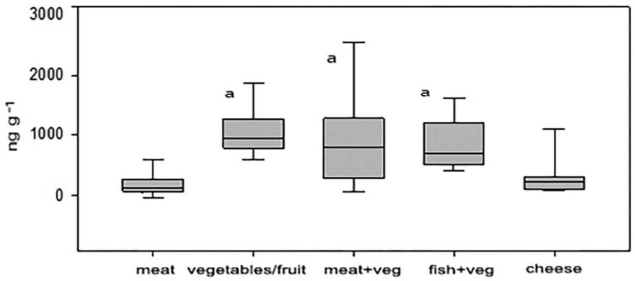


Figure 3. Distribution of salicylic acid according to baby food category: meat, vegetable/fruit, mixed meat + vegetables, mixed fish + vegetable and cheese. Data are reported as median with 25th–75th percentile range. Comparison was done using Kruskal-Wallis One Way Analysis of Variance on Ranks that revealed statistical significance (a stands for $p < .001$ vs meat group).

mediating physiological and biochemical processes. Several studies reported the beneficial action of the salicylates on the human health, due to the anti-inflammatory and antioxidative activities (Malakar et al. 2017). However, the concentration of salicylic acid is species-dependent and different plants could produce high amounts of these substances that could be a potential health risk (Cunningham 2010), especially for infants as particularly vulnerable category. In this regard, infants are a matter of concern because some of them may have adverse reactions to even a small quantity of salicylates. Salicylates are well-known food additives and therefore an analytical strategy that would distinguish between naturally occurring and industrially introduced salicylic acid is needed for further investigation. This is especially because of increased incidence of allergic reaction to salicylate. Our data regarding the salicylic acid concentration in food items for infant diet are the first of this kind; therefore it was not possible to make a comparison with similar studies. Our results indicate the much lower content compared to fresh food items as recently was reported by Kęszycka et al. (2017). Therefore, it remains to be elucidated whether the concentration found in the samples enrolled in this study represents a safety risk for some paediatric categories and in which extend food processing influences its final quantity.

## Conclusions

POPs, PFASs or antibiotics were not detected and all samples were compliant with European legislation. Confirmation of negative data is also important, particularly for the indications and needs dictated by EFSA and other competent authorities in expanding a database on residual analyses of emerging contaminants in different types of food for a reliable risk assessment. On the other hand, some parabens and their metabolites, which are classified as endocrine disruptors, were detected at trace levels and significantly below the ADI recommended by EFSA and the European Medicines Agency. This study shows the importance of collecting more data on the occurrence of parabens and transformation products to assess exposure and possible health impact for sensitive populations such as infants and young children.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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# PRESENZA DI METALLI IN CARNE E SALSICCIA SUINE DEL MERCATO ITALIANO: RISULTATI PRELIMINARI.


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## INTRODUZIONE

Negli ultimi decenni, la crescente globalizzazione ed industrializzazione ha causato un progressivo incremento dei livelli di contaminanti chimici nell'ambiente, quali plastificanti, pesticidi, diossine e metalli. In particolare, questi ultimi, risultano di interesse in termini di sanità pubblica. il Piombo, infatti, potrebbe causare gravi danni cerebrali e renali e aborti spontanei nelle donne in gravidanza; il Mercurio disfunzioni del sistema nervoso come tremori, irritabilità, problemi di memoria, peggioramenti della visione e dell'udito; il Cadmio malattie renali e problemi respiratori ed il Cromo disturbi respiratori quali tosse, asma, reazioni allergiche e potrebbe indurre neoplasie epatiche e renali per esposizione cronica a Cr(VI) (1).
Scopo del presente lavoro è indagare la presenza dei cinque metalli in carni suine provenienti da 8 differenti paesi Europei (Fig.1) e salsicce suine, presenti sul mercato italiano.

## MATERIALI E METODI

**Campioni.** Da dicembre 2016 a marzo 2017 raccolti 20 campioni di muscolo suino (Fig.1) e 23 di salsiccia prodotta in Italia con carni suine (origine EU).
**Preparazione.** 0.5 g + 5 mL di $HNO_3$ al 65% e 0.5 mL di $H_2O_2$ al 30%. In recipienti 3M-TFM®. Digestione in microonde (Milestone Inc., Shelton, CT, USA) ad alta temperatura (200 ° C; 30'). Campioni e bianco (soluzione senza matrice aggiunta) portati a 50 mL, con acqua ultrapura. Un mL di soluzione posto in provetta di 15 mL e portato a 10 mL con acqua ultrapura.
**Analisi.** ICP-MS, Agilent 7500ce (Agilent Technologies, CA, USA).
**Validazione.** Regolamento della Commissione Europea 333/2007 (2)

Figura 1: Paesi di origine della carne suina e numero di campioni

**RISULTATI**
Valori espressi in µg/Kg

| | Pb | | Hg | | Cd | | Ni | | Cr | |
|---|---|---|---|---|---|---|---|---|---|---|
| | carne | salsiccia | carne | salsiccia | carne | salsiccia | carne | salsiccia | carne | salsiccia |
| Positivi | 22 | 20 | 23 | 20 | 23 | 20 | 23 | 20 | 22 | 20 |
| Media ± DS | 167±142 | 162±125 | 49.3±24.9 | 56± 61.5 | 7.53±1.82 | 17.4± 18.9 | 550±148 | 722±183 | 130±119 | 533±173 |
| 1°quartile | 79.0 | 81.9 | 37.1 | 36.6 | 6.14 | 10.4 | 493 | 625 | 71.0 | 426 |
| Mediana | 99.9 | 111 | 42.6 | 42.4 | 7.09 | 11.2 | 528 | 670 | 93.8 | 496 |
| 3°quartile | 227 | 255 | 50.6 | 47.8 | 9.01 | 14.6 | 596 | 737 | 121 | 608 |
| Massimo | 643 | 541 | 146 | 315.3 | 11.4 | 93.1 | 939 | 1413 | 560 | 924 |

## DISCUSSIONE e CONCLUSIONI

| Pb | Hg | Cd | Ni | Cr |
|---|---|---|---|---|
| | TWI=1.3 mg/kg pc | | TDI= 0.0028 mg/kg pc<br>$BMDL_{10}$= 0.0011 mg/kg pc | TDI=0.3 mg/kg pc |
| MRL 0.1 mg/kg carne | TL=0.27 mg/kg carne | MRL 0.05 mg/kg carne | TL=0.39 mg/kg carne<br>$TL_A$=0.15 mg/kg carne | TL=42 mg/kg carne |
| 21campioni>MRL | 1campione>TL (0.32 mg/kg) | 1campione>MRL (0.09mg/kg) | 42campioni>TL<br>42campioni>$TL_A$ | Nessuno>TL |
| TWI= tolerable weekly intake; TDI= tolerable daily intake; $BMDL_{10}$= Lower benchmark dose at 10% extra risk (for the incidence of systemic contact dermatitis); MRL= Maximum residue limit | | | | |
| TL= tolerance level=TDI x body weight/0.5 kg meat (from EFSA standard food basket) | | | | |

Nella tabella sono riportati gli MRL indicati dall'Unione Europea e, in mancanza di questi, i TL calcolati in base ai limiti soglia raccomandati dall'EFSA. Il Cromo non ha mai superato il TL, il Cadmio ed il Mercurio hanno superato il limite solo in un campione dei 43 analizzati. In merito al Piombo, il 51% dei campioni testati si sono mostrati **fino a 5 volte** superiori al limite, evidenziando un rischio espositivo per adulti e bambini. Il Nichel ha mostrato sia un rischio di tossicità cronica che di insorgenza di dermatiti cutanee di natura allergica nella quasi totalità dei campioni.

Questi dati preliminari indicano quindi un ridotto o nullo rischio espositivo per Mercurio, Cadmio e Cromo da assunzione di carne suina e derivati. Il Piombo, ma soprattutto il Nichel evidenziano la possibilità di un rischio cronico per il consumatore di carne suina. Il consumo di carne suina, inoltre può indurre reazione allergiche in soggetti Ni-sensibili.


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