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序

人類製造並使用的化學物質 100 年來與日俱增，其中某些物質在較低劑量下經由模擬天然荷爾蒙刺激或抑制體內荷爾蒙的表現而干擾內分泌的正常運作，進而於阻礙生殖、發育等機能，這些物質即為內分泌干擾物質，又稱為「環境荷爾蒙」。由於現在"吃的健康"深植於消費者的觀念，再加上農藥若不當使用不只影響人類，也影響環境，因此本會非常重視有關內分泌干擾之議題，自民國 90 年代即委託相關單位進行農藥之內分泌干擾作用之研究，經過近 10 年之研究，終於在近年來針對疑似具內分泌干擾作用農藥之研究漸露曙光，相關法規或管制策略方向在國內外也不約而同被頻繁討論，因此藉由整合最新科學證據及進展，可利於我國於不遠的未來，形成具內分泌干擾作用政策及法規，達到保障人民、維護環境之最終目的。

鑑於國內外皆有針對具內分泌干擾作用之化學物質訂定監測及限用之相關法規，但疑似具內分泌干擾作用農藥則因證據不充分而尚無明確的管制策略，這也是為什麼近幾年來在國內外不斷被討論的原因。政策法規之形成除須考量國際上針對內分泌干擾作用之法規制定現況，亦需整合最新科學證據及進展。因此了解國際針對內分泌干擾物質之法規制定現況、內分泌干擾物質在我國環境醫學所造成的衝擊實例、我國農藥內分泌干擾之系統性評估與研究策略，並整合我國最新科學及研究結果包括農藥內分泌干擾作用之影響機制、環境影響，進而應用試驗數據之基準劑量評估技術及大數據資訊分析概念評估風險，才能進一步結合及延伸至我國針對疑似內分泌干擾作用農藥提出管理建議，也是凝聚各界意見後，制定政策及法規的不二法門。

為使內分泌干擾的議題能聚焦，在本所同仁及學術界之共同努力之下，不僅深耕我國農藥內分泌干擾作用之相關研究，提供體內及體外模型試驗數據，作為後續評估系統之資料佐證，且經由本次研討會之國內外專家交流及討論，做為加速農藥內分泌干擾之科學合作及法規制定之參考，特將此次研討會論文彙編成冊，期能擴大交流層面，達成民眾、環境與政策管理三贏的目標。

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目錄

Endocrine Disruptors: Scientific and Regulatory Concepts and Approaches (with a specific reference to pesticides)

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Abstract

This discussion presents the toxicological and regulatory issues relating to endocrine disruptors (EDCs). Increasing attention has been given to endocrine disrupting activities of chemicals in the environment. Different views have been expressed regarding the urgency and associated actions by regulators. The situation is complex due to the public health impacts and the diverse views on the levels of scientific evaluation and criteria, along with the associated regulatory consequences in existing but different regulatory framework. In the U.S., the Environmental Protection Agency (U.S. EPA) has set up the Endocrine Disruptor Screening Program (EDSP). In Europe, the European Union (EU) has developed short-, medium- and long-term strategies. Each of the two aspects, scientific criteria for identifying endocrine disruptors and regulatory decision making, carries several options, with the regulatory decisions depending on the scientific evaluations. Some progress has been made and activities are on-going.

Introduction

Increasing global attention has been given to chemicals in the environment that may have endocrine disrupting activities, referred to as endocrine disrupting chemicals (EDCs). Efforts have been made to conduct testing and to provide scientific knowledge on effects of and exposure to the EDCs. Identification of chemicals as potential EDCs with activity on the endocrine system and/or as EDCs with adverse effects on health is challenging with divergent scientific views, and the data, methodology and criteria are still being developed. Regulation of EDCs is complicated by the different existing regulatory framework some of which are defined in legislations. Varying scientific criteria are being considered to serve as a basis for determining regulatory actions.

The endocrine system

The endocrine system consists of glands located throughout the body that produce and release hormones into the body (U.S. EPA 2015). The hormones act as chemical messengers and bind with specific and compatible receptors in or on target cells. The binding alters the cell's existing proteins or turns on genes that will build a new protein, resulting in reactions in the body. The system controls biological processes from conception through different life stages, regulating metabolism, growth and function of the reproductive system, and the development of the brain and nervous system. It is found in all mammals, birds and fish. More than 50 hormones have been identified.

Endocrine disruption

The endocrine system can be disrupted by chemicals that may mimic a natural hormone, block receptors, or stimulate or inhibit the production of hormones (U.S. EPA 2015). Some chemicals have been found to disrupt the endocrine systems of laboratory animals, and compelling evidence shows that endocrine systems of certain fish and wildlife have been affected by chemical contaminants, resulting in developmental and reproductive problems. The relationship of human diseases of the endocrine system and exposure to environmental contaminants is not clearly understood. Endocrine systems are very similar across vertebrate species and endocrine effects are not necessarily species dependent (WHO 2012a). Effects seen in wildlife or experimental animals may also occur in humans if exposure is at a vulnerable time and concentrations leading to alterations of endocrine regulation. Of special concern are effects on early development as these are often irreversible and

may not become evident until later in life.

WHO has provided the following definition for EDCs (2012a, first developed in 2002): “An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations”. In addition, “A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expressed to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations”. Thus there is a distinction between an endocrine disruptor and a potential endocrine disruptor.

In this report by the United Nations Environment Programme (UNEP) and WHO (2012a), the study recommended improving global knowledge of EDCs, reducing potential disease risks, and cutting related costs. These include more comprehensive testing methods to identify possible EDCs, their sources, and routes of exposure; research for more scientific evidence to identify the effects of mixtures of EDCs on humans and wildlife (mainly from industrial by-products) to which humans and wildlife are increasingly exposed; reporting and information on chemicals in products, materials and goods; and collaboration to achieve more data sharing between scientists and between countries to fill gaps in data, primarily in developing countries and emerging economies.

While there are examples of success in banning chemicals to provide health protection, as in the case of lead in children, the scientific data were present many years before the policies were changed and the chemical was banned (WHO 2012b). The question arises as to when the data are sufficient for action. One approach is to make more use of the precautionary principle to ban or restrict chemicals in order to reduce exposure early, even when there are significant but incomplete data and before there is significant and long-lasting harm. At the same time, the situation is addressed at the international level

Science and regulation

Concerns regarding chemicals as EDCs have led to the need to scientifically identify and develop related regulations. Identification involves testing and defining scientific criteria which are then used for developing regulatory options.

International activities

United States

In the U.S., pesticides are technically regulated under existing laws including the Toxic Substances Control Act; Federal Insecticide, Fungicide and Rodenticide Act; Food, Drug and Cosmetic Act; Clean Water Act; Safe Drinking Water Act (SDWA); and Clean Air Act (U.S. EPA 2015). In 1996, the U.S. EPA was required under the authority of the Food Quality Protection Act and the SDWA to address endocrine disruption through establishing a program for screening and testing of chemical substances. In 1998, the agency set up the Endocrine Disruptor Screening Program (EDSP) which is a framework for priority setting, screening and testing of chemicals. An advisory expert panel was set up in 2005. Priority chemical lists were developed for screening under a two-tiered testing scheme. The chemicals are primarily chemicals in drinking water and pesticide programs with a high exposure potential.

EPA currently uses a two-tiered screening program that examines chemicals to determine whether they have the potential to affect endocrine systems. Tier 1 uses a battery of 11 assays (five in vitro, 6 in vivo) for screening the potential to interact with the estrogen, androgen or thyroid hormonal pathways. Tier 2 testing includes multigenerational, longer-term testing across various species (*e.g.*, frog, fish, bird and rat). It is designed to confirm interaction with the endocrine system, identify any adverse endocrine-related effects caused by the substance and establish a quantitative dose-response relationship. The assays need to go through method development, pre-validation, validation on multiple laboratories with emphasis including establishing relevance, and the process involves scientific peer review before regulatory acceptance and implementation. The need for Tier 2 is determined by weighing the evidence from the Tier 1 results and other scientifically relevant data for potential endocrine bioactivity.

Recent results on 52 pesticides from Tier 1 screening have been released. The agency found no evidence for potential interaction with any of the endocrine pathways for 20 chemicals, and potential interaction with one or more pathways for 14 chemicals. The overall evaluation concludes that these 14 chemicals do not pose risks. Of the 18 chemicals that showed potential interaction with the thyroid pathway, 17 also potentially interacted with the androgen pathway, and 14 also potentially interacted with the estrogen pathway. The pesticides included: 2,4-D, abamectin, acephate, acetone, atrazine, benfluralin, bifenthrin, captan, carbaryl, carbofuran, chlorothalonil, chlorpyrifos, cyfluthrin, cyperpethrin, DCPA, diazinon, diclobenil, dimethoate, EPTC, esfenvalerate, ethoprop, fenbutatin oxide, flutolanil, folpet, glyphosate,

imidacloprid, iprodione, isophorone, linuron, malathion, metalaxyl, methomyl, metolachlor, metribuzin, MGK, myclobutanil, norflurazon, o-phenylphenol, oxamyl, PCNB, permethrin, phosmet, piperonyl butoxide, propargite, propiconazole, propyzamide, pyriproxyfen, simazine, tebuconazole, tetrachlorvinphos, triadimefon, and trifluralin.

The U.S. EPA has pointed out that it is important not to equate a chemical's bioactivity with the conclusion of harm to the endocrine system in humans and wildlife. It noted that bioactivity indicates that a chemical has the potential to alter endocrine function, but further testing is needed before one can determine whether the chemical actually alters endocrine function, and whether that altered function produces an adverse outcome in humans and animals. As the Tier 1 data are for screening for the potential to interact with the endocrine system, these are not deterministic for regulatory decision making. New tests for the hormonal systems are under development, and U.S. EPA continues to incorporate new methods involving high-throughput assays and computational toxicology.

Europe

In Europe, no mandatory testing program has been established. The Organization for Economic Cooperation and Development's (OECD) chemical programme has developed a guidance document on the assessment of chemicals for endocrine disruption (2010**).** The European Commission (EC), European Food Safety Authority (EFSA) and ECHA are contributing actively to this programme. WHO has updated the reference document on endocrine disrupting effects (WHO 2012a) first published in 2002.

Various pieces of EU's legislation on chemicals contain specific provisions on EDCs (EC 2011). Currently the main focus for EU and international bodies is to agree on approaches for the identification and assessment of EDCs. EU has acknowledged the phenomenon of ED as a priority area, made funding of related research a priority and developed short, medium and long-term strategies. In 1999, the EC adopted a Communication to the Council and European Parliament on a Community Strategy for Endocrine Disrupters. The strategy addresses the key requirements of further research; international co-operation; communication to the public and appropriate policy action. Recommendations are made for short-, medium- and long-term actions. In 2000, the European Parliament adopted a Resolution on EDCs, emphasizing

the application of the precautionary principle and calling on the Commission to identify substances for immediate action. The Environment Council adopted Conclusions on the Commission Communication in which it stressed the precautionary principle, the need to develop quick and effective risk management strategies and the need for consistency with the overall chemicals policy. The Commission made its 5th report on the progress of the work in 2011.

For the short-term actions a priority list has been established for substances to be investigated further based on possible endocrine disrupting properties. The intention is for this list to be used to prioritize further detailed review of the information, and it is not final and unchangeable. The list was established after an independent review of evidence of endocrine disrupting effects and human/wildlife exposure, and a priority-setting exercise in consultations with stakeholders and the Commission Scientific Committees. Websites are developed for communication to the public, and international workshops were held to discuss testing strategies. The Commission is conducting a feasibility study to examine how the monitoring data could be coordinated and used in a more systematic way.

For the medium-term actions, considerable efforts have been made at national, EU and international level to identify and assess EDCs. Activities within the EU to develop criteria and testing strategies for identification of EDCs have been seen as a consequence of severe restrictions on substances identified as EDCs imposed by several pieces of legislation. The Commission and several Member States have initiated work on possible criteria for identification of EDCs. OECD has developed guidelines for testing potential EDCs. The Endocrine Disruptor Testing and Assessment Advisory Group has initiated work on a guidance document for the assessment of chemicals for endocrine disruption and on a detailed review paper on additional endocrine effects related endpoints.

For long-term actions on legislative actions - implementation of existing legislation, references have been made to Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and the new regulations on plant protection products.

"For Plant Protection Products Regulation (PPPR), substances identified as having ED properties that may cause adverse effects in humans cannot

be authorized under the new PPPR (Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives79/117/EEC and 91/414/EEC….). By December 2013 the Commission is required to present (to the Standing Committee on Food Chain and Animal Health) a draft of the measures concerning specific scientific criteria for the determination of endocrine disrupting properties (in relation to human health impacts) to be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 79(4).“

“Pending the adoption of the scientific criteria for the identification of endocrine disruptors referred to in the preceding paragraph, the PPPR requires that substances that are, or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as Carcinogenic (Category 2) and Toxic for Reproduction (Category 2) shall be considered as having ED properties and accordingly shall not be authorized. In addition, substances, such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties. If on the basis of the assessment of EU or internationally agreed test guidelines or other available data and information, reviewed by the Authority, an active substance is considered to have endocrine disrupting properties that may cause adverse effect in humans, it shall be approved as a candidate for substitution in accordance with Article 24 of the PPPR. Finally, if a substance is deemed to be an endocrine disruptor, it shall not be considered a substance of low risk.”

In 2009, Directorate General Environment, designated by EU to be in charge of regulating EDCs, commissioned a report ‘State of the art assessment of endocrine disruptors’, also known as the Kortenkamp report (2012). The report concluded that any attempt to regulate EDCs would face one major challenge in that there is no such thing as a universal, ready-to-use detection kit for EDs, and that the hormonal system is extremely complex and EDs can hijack it in many different – and largely unknown – ways.

In 2014, the European Commission Directorate General for Health and Food Safety was designated to conclusively define criteria for the identification of EDCs for use in European pesticide and biocide law. In 2015, it organized a one-day

conference, "Endocrine disruptors: criteria for identification and related impacts", in Brussels. The conference was attended by representatives of the EU member states and other countries, European Parliament, business, unions, non-government organisations and the media.
(http://ec.europa.eu/health/endocrine_disruptors/events/ev_20150416_en.htm).

The subject areas discussed at the conference included EU policy on endocrine disruptors, scientific debate on criteria to identify EDCs, impact assessment for defining criteria on endocrine disruptors in the context of the PPPR and the biocidal products regulations (BPR), and the proposed decision matrix for health assessment. The information covered recent major achievements and new on-going work.

The on-going work presented included anticipation of: 7th Environment Action Programme to 2020 - Actions on endocrine disruptors; REACH review of EDCs in authorization, identification of EDCs for Candidate List by 2020; and a workshop on test methods by OECD in Brussels Nov 2015. It also included: uncertainty in RA - public consultation in summer 2015, finalization of draft guidance at the end 2015, testing phase until summer 2016, and finalization at the end of 2016; weight of evidence - public consultation probably 2016, with finalization Sept 2017; biological relevance - probably a public consultation early 2016, and finalization end 2016; and review of non-monotonic dose-response of substances for human risk assessment, probably January 2016. The impact assessment screening study for an estimate of which substances (700) fall under the different policy options (see below) is on-going and an event focusing on the method is planned for fall 2015. A sample of a decision matrix for assessment was presented.

An on-going discussion at the conference and in recent years prior to the conference is the consideration of two aspects: 1) EU criteria to identify EDCs; and 2) regulatory decision making. These two aspects have received divergent views. The health or toxicologic criteria would have a major impact on subsequent regulatory actions on which the toxicologic evaluation would be based.
(http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm)

For regulatory decision making, three (3) options have been proposed: 1) A (baseline). No policy change; 2) B. Introduction of further elements of risk

assessment; 3) C. Introduction of further socio-economic considerations. The decision would follow the evaluation under the toxicologic criteria eventually adopted.

For the toxicologic criteria to identify EDCs which would form the basis and precedes decision making, four (4) Options have been proposed: 1) No policy change and the interim criteria in the BPR and the PPPR would be used; 2) Use the WHO definition to identify EDCs; 3) Use the WHO definition to identify EDCs, plus add additional (3) categories based on strength of evidence (Category I - WHO definition for EDCs; Category II – suspected EDCs; Category III - endocrine active substance; 4) use the WHO definition to identify EDCs, and add potency. Option 4 (b) is added which involves hazard identification and hazard characterization including severity of effects, strength of evidence, reversibility, consistency and potency. These could be used in a decision matrix along with the three categories.

For the no change option where the interim criteria set in the BPR and the PPPR would be applied, substances are or may be considered as EDCs if they are or have to be classified as "carcinogenic category 2" and "toxic for reproduction category 2", or "toxic for reproduction category 2" and "toxic effects on the endocrine organs". Substances not fulfilling above criteria will be considered not EDCs.

It may be noted that identification of EDCs has involved the hazard vs. risk based approaches. The concepts and approaches are also addressed by the WHO and U.S. EPA noting the distinction between potential EDCs vs. EDCs that cause adverse effects.

Continuing efforts are being made as a follow-up of the U.S. EPA’s newly released Tier 1 testing data from the EDTP, and for EU’s priority list and other activities such as those discussed at the recent conference in Brussels.

Summary

Increasing efforts have been made globally to address scientific assessment and regulatory needs relating to environmental chemicals having endocrine disrupting activities. National and international programs have been initiated to generate testing data and to develop strategies, criteria and approaches related to identification, health risk assessment and regulation of EDCs. Some progress have been reported but more work is anticipated. Continuing efforts

are being made to address the needs .

References

1. EC, 2011. Commission Staff Working Paper, 4th Report on the implementation of the "Community Strategy for Endocrine Disrupters". A range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706) European Commission (EC) Brussels, 10.8.2011. SEC (2011) 1001 final.
2. Kortenkamp A., Martin O., Faust M., Evans R., McKinlay R., Orton F., Rosivatz E., 2011. *State of the art assessment of endocrine disrupters. Final report.* European Commission, Directorate-General for the Environment (Project Contract No. 070307/2009/550687/SER/D3).
3. OECD, 2010. Guidance Document on the Assessment of Chemicals for Endocrine Disruption, Version 9. Organization for Economic Cooperation and Development.
4. U.S. EPA, 2015. Endocrine Disruptor Screening Program (EDSP), U.S Environmental Protection Agency. Last updated Sept 15, 2015. http://www.epa.gov/scipoly/oscpendo/index.htm
5. WHO, 2002. *Global assessment of the state-of-the-science of endocrine disruptors.* Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety.
6. WHO, 2012a. State of the science of endocrine disrupting chemicals. An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO. Edited by A Bergman, JJ Heindel, S Jobling, KA Kidd and RT Zoeller.
7. WHO, 2012b. State of the Science of Endocrine Disrupting Chemicals. Summary for Decision Makers. An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization. Edited by Åke Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kidd, R. Thomas Zoeller.

內分泌干擾作用與環境醫學

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摘要

內分泌系統 (Endocrine system)是負責調控體內各種生理功能正常運作的系統，其分泌量或功能過多或過少，都將影響生物體的平衡。內分泌干擾物質 (Endocrine disrupters, EDCs)又稱為「環境荷爾蒙」，根據美國環保署 (US EPA)報告中指出，內分泌干擾物質 (EDCs)是指干擾負責維持生物體內恆定、生殖、發育或行為的內生荷爾蒙之外來物質，影響荷爾蒙的合成、分泌、傳輸、結合、作用及排除。日常生活中暴露內分泌干擾物質 (EDCs)，會干擾體內激素 (荷爾蒙)的平衡、影響內分泌的器官、內分泌回饋機制等，進一步對生殖細胞 (精卵)、內分泌的器官 (生殖腺、甲狀腺等)、生育的結果、代謝疾病、致癌等的不良影響。本節介紹已知之環境中內分泌干擾物質 (EDCs)，包含: 人造雌性激素"己烯雌酚" (diethylstilbestrol, DES)、多氯聯苯 (polychlorinated biphenyls, PCBs)、塑化劑"鄰苯二甲酸酯類" (Phthalate esters, PAEs)和農藥；及其對人體健康的影響。

關鍵詞：內分泌干擾物質、己烯雌酚、多氯聯苯、塑化劑、農藥。

討論

內分泌系統 (Endocrine system)是負責調控體內各種生理功能正常運作的系統之一，由分泌激素 (荷爾蒙)的無導管腺體所組成。人體的內分泌系統，主要包括腦垂腺、甲狀腺、副甲狀腺、胰島腺、腎上腺和性腺 (卵巢、睪丸)等內分泌腺。這些內分泌腺能分泌各種激素 (荷爾蒙)。激素 (荷爾蒙)是一種化學傳導物質，自腺體分泌出來後，藉由體液或進入血液運送到標的器官之受體而產生作用，藉改變體內的化學變化，來協調生理機能；其分泌量必須適量，過多或過少，都將影響生物體的平衡。

根據美國研究統計，睪丸癌、乳癌和尿道下裂症 (Hypospadias)的發生率逐年增加；相反地，精子的數目卻逐年在下降。另亦有研究分析全球，包含：亞洲、澳洲、美歐等多數國家男性睪丸癌發生率也逐漸增加。在台灣，也發現乳癌和前列腺癌的發生率也是逐年攀升。但造成此現象的確切原因還不是很清楚，有可能跟人類在日常生活中暴露內分泌干擾物質 (Endocrine disrupters, EDCs)有關，這些物質干擾體內激素 (荷爾蒙)的平衡、造成激素過高或過低、影響內分泌器官或激素之受體、內分泌回饋機制等，現在更發現內分泌干擾物質 (EDCs)對生殖細胞 (精卵)、內分泌器官 (生殖腺、甲狀腺等)、生育能力 (嬰兒出生的情況)有不良影響，甚至引起代謝性疾病、癌症等疾病。

內分泌干擾物質 (EDCs)又稱為「環境荷爾蒙」，根據美國環保署 (US EPA)報告中指出，內分泌干擾物質 (EDCs)是指外來化學物質透過影響荷爾蒙的合成、分泌、傳輸、結合、作用及排除，干擾負責維持生物體內恆定、生殖、發育或行為的內生荷爾蒙，。這些物質會由空氣、水、土壤、食物等途徑進入體內，對生物體產生作用，甚至危及後代的健康。內分泌干擾物質 (EDCs)對人體健康之影響，會隨類別、年紀和性別而有所不同。一般而言，人類暴露到內分泌干擾物質 (EDCs)時，以胎兒和新生兒最容易受到傷害。目前已有許多文獻報導內分泌干擾物質 (EDCs)對人體之影響，例如：男性繁殖力下降、男性特徵發展缺陷(如:尿道下裂症)、攝護腺癌和睪丸癌的增加；女性生殖力下降、乳癌的增加、子宮內膜異位症等。

許多內分泌干擾物質 (EDCs)，其結構類似生物體內的激素 (荷爾蒙)，可與天然激素 (荷爾蒙)的受體結合，進而促進或抑制生理反應的效果，例如：多氯聯苯 (Polychlorinated biphenyls, PCBs 等物質可與雌性素受體 (Estrogen receptor, ER)結合，產生類似雌性素的作用；而如：殺蟲劑 DDT 的代謝物 DDE 則會與男性激素受體 (Androgen receptor, AR)結合，抑制男性激素作用。但也有些內分泌干擾物質 (EDCs)，其結構不像生物體內的激素 (荷爾蒙)，而是直接影響了生物體內與激素(荷爾蒙)合成有關的酵素，以及破壞激素 (荷爾蒙)及其受體的生成、代謝和細胞信號傳遞等，這些作用稱之為非受體介導途徑。

目前已知之環境內分泌干擾物質 (EDCs)可分為幾大類，包含：農藥 (如：DDT、有機磷農藥和除蟲菊類農藥等)、多環鹵素化合物 (如:戴奧辛和多氯聯苯

等)、塑膠製品 (如:塑化劑和雙酚 A)、家用清潔劑 (如:壬基酚)和重金屬 (如:鉛、汞和鎘)。以下將分別介紹幾種已知之環境中內分泌干擾物質 (EDCs)，及其對人體健康的影響。

一、人造雌性激素”己烯雌酚” (diethylstilbestrol, DES)：

首先，事件為 1938 年英國醫生所合成的人造雌性激素”己烯雌酚” (diethylstilbestrol, DES)，在人體中可誘發類似雌性激素 (動情激素)之功能，被醫界認為是仙丹 (wonder drug)，在 1940~1970 年間至少有 3 百萬女性服用過己烯雌酚 (DES)，其有許多用途，最早被用來治療婦女之流產和早產問題，進而被廣泛應用於協助產後母親停奶、治療小孩的粉刺、攝護腺癌；在畜牧業上，也大量使用己烯雌酚 (DES)，作為家畜之食品添加劑，以加速雞、牛和其他家畜的生長。由於己烯雌酚 (DES)從服藥至發病的時間經過很長，己烯雌酚 (DES)濫用之問題，直到 1970 年間，波士頓醫院的醫生發現，原本只會發生在年紀大於 50 歲之婦女的陰道腺癌 (clear-cell adenocarcinoma, CCA)，居然發生在十多歲左右的年輕女孩；而 8 位患者中，有 7 位被發現她們的母親在懷孕的最初 3 個月時曾服用己烯雌酚 (DES)。經母體暴露於己烯雌酚 (DES)之女性後來亦被發現有生殖困難問題：包括不孕 (infertility)、流產 (miscarriage)、子宮外孕、早產 (preterm delivery)及新生兒或幼兒死亡；其生殖道異常相當常見，如: T 形子宮 (T-shaped uterus)。成年後較易罹患如乳癌等。在動物實驗發現，小鼠較敏感，包括致畸胎性 (teratogenicity)、經胎盤之致癌性 (transplacental carcinogenesis)及導致子代之生殖能力下降。其實直接服用己烯雌酚的婦女，亦較易罹患如乳癌 (增加 27%)。另外，經母體暴露於己烯雌酚 (DES)之男性胎兒其發生尿道下裂症機會比一般男性胎兒高出 10 倍以上。

從己烯雌酚 (DES)的悲劇，人們學到母親暴露到的化學物質能穿透胎盤，干擾胎兒的發育，產生需等到數十年後才看到的可怕結果，這是以往醫界未曾注意到「延遲的長期效應」 (delayed long-term effects)，會到小孩成長到青春期或之後的人生期才發作的效應。更可怕的是，人們不僅需擔憂生下來即顯而易見的畸型，也需擔憂對組織和細胞所造成的隱形影響。

二、多氯聯苯 (polychlorinated biphenyls, PCBs)：

多氯聯苯 (polychlorinated biphenyls, PCBs)耐熱性及電絕緣性良好，化學性質穩定曾被廣泛應用於工業上，如熱媒、變壓器…等，1968 年日本九州福岡地區曾發生集體多氯聯苯中毒，事後調查出工廠在製造米糠油的過程中使用多氯聯苯做為熱媒脫臭，但因熱媒管腐蝕滲出汙染食用油，稱為「油症事件」。而在台灣於 1979 年，彰化某一油脂企業在製造米糠油的過程中，不小心將傳熱介質多氯聯苯自加熱管的裂縫中流出，汙染到米糠油，又稱「台灣油症事件」或「米糠油事件」。1979 年 4 月初，台中的惠明盲校使用米糠油後，師生陸續出現原因不明的皮膚病，皮膚癢、乾燥甚至變黑，還長出大量粉刺及像痘痘的疹子，患病人數日益增加，連附近工廠的員工也出現類似症狀，追查下發現他們都向同一家食品行購買油品；後來檢驗惠明盲校的食用油及

師生們的血液樣本，證實米糠油內含多氯聯苯是造成中毒的原因。

原本認為油症是多氯聯苯中毒引起，後續的研究發現多氯聯苯在反覆加熱的過程中會產生多氯夫喃 (polychlorinated dibenzofurans, PCDFs)，這兩種都是類戴奧辛 (dioxin-like compounds)的內分泌干擾物質 (EDCs)，共同產生危害。皮膚症狀只是中毒初期的症狀，由於這兩種類戴奧辛物質都很安定不易被代謝，容易累積在脂肪組織，無法排出體外；將造成肝臟、生殖、免疫、神經性病變和癌症的發生，甚至影響到後代。

據美國環境保護署 (US EPA)的報告指出，多氯聯苯已被證明具致癌性，其毒害作用還包括內分泌干擾作用。至於對女性生殖系統的影響，長期追蹤平均約 15 歲時暴露的油症患者，發現其結婚後懷孕能力下降，不孕症發生率是一般人的 2 倍；油症親代 25 年前食用受汙染米糠油，其男性子代會出現去雄性化的情況，如：陰莖長度較短、雄性荷爾蒙降低、雌性荷爾蒙較高、精子型態異常率增加、精子活動力下降等。經長期追蹤台灣 30 年及日本 40 年研究數據，發現男性總死亡率增加 20%，男性癌症總死亡率增加 30%，男性肺癌死亡率增加 70%。女性的肝癌死亡率增加 1 倍。多氯聯苯 (PCBs)會導致人體多種病變，且會經由母體胎盤或哺乳傳給胎兒，這些油症兒在出生時會有皮膚發黑、眼瞼浮腫、免疫功能受損等問題。至於在長大之後，則可能出現智力與體力發展遲緩、注意力不集中、攻擊性行為等現象。絕大多數的受害者歷經三十年，仍在與體內的毒素搏鬥。

三、塑化劑"鄰苯二甲酸酯類" (Phthalate esters, PAEs)：

鄰苯二甲酸酯類 (PAEs)，主要用於塑膠製品作為塑化劑 (plasticizers)，可使塑膠具有彈性及延展性。日常生活中的塑膠產品、衣服、玩具、醫療設備、化妝品、地板、壁紙，汽車產品 (如:坐椅、椅套)等都含有鄰苯二甲酸酯類 (PAEs)，而人類使用鄰苯二甲酸酯類 (PAEs)已超過六十年以上。部分鄰苯二甲酸酯類 (PAEs)具有干擾內分泌系統之環境荷爾蒙效應，如:鄰苯二甲酸二(2-乙基己基)酯 (Di-(2-ethylhexyl) phthalate, DEHP)及鄰苯二甲酸二丁酯(Di-*n*-butyl phthalate, DBP)等。因為塑化劑用途廣，對環境造成不小的污染，通常透過飲水、食物鏈及空氣接觸或呼吸進入人體，其中又以食入為主，如：用塑膠容器或包材 (尤其是PVC類)儲存食物時，DEHP會微量溶出殘留在食物中。

鄰苯二甲酸酯類 (PAEs)具有內分泌干擾作用，經常藉由干擾荷爾蒙的調控影響了內分泌系統、神經發育和免疫系統等。在胚胎早期，受到內分泌干擾物的影響最敏感且深遠；而青春期可謂生殖發育第二快速期，女性則於此時乳房發育到達顛峰，所以新生兒、青春期、孕婦、乃至育齡男女，可謂族群中的易感受性族群 。目前一般認為早期暴露鄰苯二甲酸酯類 (PAEs)與氣喘、濕疹發生風險增加有關；與兒童的內、外化行為問題以及自閉傾向等有關；並與男性新生兒生殖器到肛門距離 (anogenital distance, AGD)縮短有關，而AGD為雄性化的指標，其縮短被視為去雄性化的表徵；早期暴露鄰苯二甲

酸酯類 (PAEs)亦與女孩第二性徵加速出現 (乳房發育過早)有關；與男性精子活動能力下降及精子數目減少有關；與婦女懷孕週期縮短有關。目前國際癌症研究機 (IARC)將鄰苯二甲酸二 (2-乙基己基)酯 (DEHP)及鄰苯二甲酸二丁酯 (DBP)定為2B，亦即有限證據顯示為人類可能致癌物。另外，有研究指出糖尿病、代謝症侯群、肥胖等代謝相關疾病與內分泌干擾物質有關；而鄰苯二甲酸酯類 (PAEs)暴露與孕婦其孕期體重增加有正相關趨勢，也和其孩童BMI值有正相關。

四、農藥：

環境中有許多的農藥被證實與內分泌干擾有關，由於內分泌干擾物質可在極低劑量下經由模擬天然荷爾蒙刺激或抑制體內荷爾蒙的表現而干擾內分泌的正常運作，進而阻礙生殖、發育等機能，造成人類健康及生態平衡極大威脅。

近 20 年來，台灣前列腺癌發生率增加 7~8 倍，最近挪威的世代研究分析前列腺癌患者 10 年前血中有機氯殺蟲劑代謝物 Oxychlordane 及 Heotachlor epoxide 含量，發現暴露有機氯殺蟲劑可能與 10 年後前列腺癌發生率有關。動物學家 Dr. Guilette 發現暴露有機氯農藥 (p,p'-滴滴依, p,p'-DDE)的鱷魚，雄性生殖器官很小，生殖能力下降；另有研究發現人類暴露有機氯農藥 (如: Lindane 及 p,p'-滴滴依)會造成精子濃度及活動力下降。近 20 年來多項研究發現，人類精液的量、精子濃度、精子活動力及型態之品質皆下降；使得世界衛生組織 (WHO)在 2010 年下修精液品質各項指標之正常值。有研究分析 8 種農藥暴露與精液品質間的關係，發現暴露拉草 (Alachlor)、IMPY、草脫淨 (Atrazine)這 3 種農藥與精液品質的相關性最大。而原本認為較安全的除蟲菊精 (Pyrethroids)，在仔細檢驗下，其暴露亦被發現會造成精子濃度降低、影響受孕能力、精子 DNA 受損、性染色體減數分裂錯誤率增加、染色體對數錯誤率增加、血中黃體生成素 (LH)及濾泡刺激素 (FSH)增加、動情激素 (E_2)及睪固酮 (Testosterone)降低，進而影響精子的形成。另外，不孕症門診研究亦指出有機磷農藥的暴露亦會影響精液品質。

近年來人類出現愈來愈多與內分泌有關的疾病，包括影響正常發育，如: 不孕症、癌症與畸形，對甲狀腺、腦部、代謝 (與肥胖有關)等功能以及胰島素與血糖恆定的影響。許多日常生活用品內含有分泌干擾物質 (EDCs)，包括農藥、醫藥、塑料添加劑等，在許多食品或其他產品會發現這些物質的殘留物或汙染物。因此，人類與野生動物暴露於內分泌干擾物質 (EDCs)所產生的負面影響及其潛在致病因子是全球性的問題，為了保護人類因暴露內分泌干擾物質 (EDCs)所產生的健康與疾病的問題，建議應提升並培養對內分泌干擾物質 (EDCs)的專業人才，以利進行內分泌干擾物質 (EDCs)的風險評估，制定相關保護措施，以預防內分泌干擾物質 (EDCs)對人類健康造成的不良影響。

參考文獻

1. Chiu-Yueh Yang a , Mei-Lin Yu a , How-Ran Guo a , Te-Jen Lai b , Chen-Chin Hsu c , George Lambert d , Yueliang Leon Guo a,e, 2005 The endocrine and reproductive function of the female Yucheng adolescents prenatally exposed to PCBs/PCDFs Chemosphere 61 (2005) 355–360
2. Guo, Y. L., Lin, C. J., Yao, W. J., *et al* (1994) Musculoskeletal changes in children prenatally exposed to polychlorinated biphenyls and related compounds (Yu-Cheng children). *Journal of Toxicology and Environmental Health*, 41, 83-93.
3. Guo YL, Hsu PC, Hsu CC, Lambert GH. 2000. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. Lancet 356:1240–1241.
4. Huyghe E[1], Matsuda T, Thonneau P. 2003 Increasing incidence of testicular cancer worldwide: a review. J Urol. 170(1):5-11.
5. Klip H, Verloop J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE. 2002 Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. Lancet. ;359(9312):1102-7.
6. Li MC, Chen PC[1], Tsai PC Furue M, Onozuka D, Hagihara A, Uchi H, Yoshimura T, Guo YL 2015.
7. Mortality after exposure to polychlorinated biphenyls and polychlorinated dibenz ofurans: a meta-analysis of twohighly exposed cohorts. Int J Cancer.;137(6):1427-32.
8. Stella Koutros,[1] Hilde Langseth,[2] Tom K. Grimsrud,[2] Dana Boyd Barr,[3] Roel Vermeulen,[4] Lützen Portengen,[4] Sholom Wacholder,[1] Laura E. Beane Freeman,[1] Aaron Blair,[1] Richard B. 2015Hayes,[5] Nathaniel Rothman,[1] and Lawrence S. Eng 2015 Prediagnostic Serum Organochlorine Concentrations and Metastatic Prostate Cancer: A Nested Case–Control Study in the Norwegian Janus Serum Bank Cohort. *Environ Health Perspect*; DOI:10.1289/ehp.1408245
9. Shanna H Swan, Robin L Kruse, Fan Liu, Dana B Barr, Erma Z Drobnis, J Bruce Redmon, Christina Wang, Charlene Brazil, James W Overstreet, 2003 Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect. 111(12): 1478–1484.
10. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL 2005 Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect.; 113(8):1056-61.
11. Titus-Ernstoff L[1], Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker

W, Kaufman R, Noller K, Herbst AL, Colton T, Hartge P. 2001. Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. Br J Cancer. Jan 5;84(1):126-33.

12. Taiwan Cancer Registry 台灣癌症登記中心
http://tcr.cph.ntu.edu.tw/main.php?Page=N1

Endocrine Disruption and Environmental Medicine

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Abstract

The endocrine system secretes variety of hormones and regulates many physiological functions. When hormones secretion or activity is modulated, the homeostasis of some physiological functions will be disturbed leading to some adverse effects, such as reproductive toxicity, developmental toxicity and cancer. In the recent years, exposure to some chemicals has been reported to disturb the endocrine system and associate with some diseases in humans. Among these chemicals are diethylstilbestrol (DES), polychlorinated biphenyls (PCBs), phthalate esters (PAEs) and some pesticides. This section reviews these epidemiological studies for the association between endocrine disruptors and human diseases.

Key words: endocrine disruptor, diethylstilbestrol (DES), polychlorinated biphenyls (PCBs), phthalate esters (PAEs), pesticides.

國內農藥內分泌干擾物質系統性評估

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[1]行政院農藥委員會農業藥物毒物試驗所應用毒理組

中文摘要

內分泌干擾物質為存在於環境中，可改變內分泌系統之功能，造成完整生物體或其子代之不良健康影響。內分泌干擾物質包括了自然存在、合成形成、工業產生等等之化學物質，可造成精子數下降、生殖器官異常及癌症，更甚者影響神經發育；為呼應國際上針對內分泌干擾物質之法規制定潮流，本文不僅回顧歐盟及美國之內分泌干擾物質之評估及檢測，另舉出內分泌干擾作用系統性評估之評估案例，並以此針對我國內分泌干擾物質管理策略提出建議。而物質是否確為內分泌干擾物質之判定評估方法，直至近年 (約 2011 年)國際上才有較具體的結論，主要其是以「證據權重 (weight of evidence; WoE)」為基礎依據之系統性評估方法，評估方法主要以經濟合作暨發展組織 (Organisation for Economic Co-operation and Development, OECD) 及美國環保署 (US Environmental Protection Agency, USEPA)所公告之規範為準，以此系統性評估方法評估各項農藥對 3 種內分泌路徑之干擾作用，包括雄性素、雌性素及甲狀腺系統路徑，其評估結果可作為風險評估或政策擬定之基礎資訊。

關鍵字：內分泌干擾作用、證據權重

前言

內分泌干擾物質為存在於環境中，可改變內分泌系統之功能，影響生物體及其子代和整體族群之健康，依據 Weybridge 定義 [3]，內分泌干擾物質「為外源性物質，且經由改變內分泌功能而造成完整生物體或其子代之不良健康影響」，此定義已被世界衛生組織 (World Health Organization, WHO)所接受及引用。內分泌干擾物質包括了自然存在、合成形成、工業產生等等之化學物質，然而物質是否確為內分泌干擾物質之判定評估，國際趨勢一直以來並非相當明朗，直至今年(2015)美國才公告內分泌物質之評估結果，而歐盟雖於近年多次公告化學物質之內分泌干擾作用之可能性等級，但也須待明年方將公告完整報告及評估方法，由於各國國情不同，因此我國與歐盟或美國的農藥禁限用、製造量、進口量、使用量及暴露量情形皆有所差異，為貼近我國國人之農藥使用狀況及內分泌干擾情形，我國應針對國內高使用量之具內分泌干擾作用疑慮及未經試驗測試農藥進行評估，而評估方法乃依據文獻提出將證據權重整合之系統性評估方法 [3]並利用近年 (約 2011 年)才較具體成形的內分泌干擾物質之判定評估模式，其是以「證據權重 (weight of evidence; WoE)」為基礎依據 [1, 5, 6]，以系統性評估方法，評估上述條件篩選出的農藥，將較直接應用國外所公告之內分泌干擾農藥名單更具法規制定代表性及參考性。

歐盟與美國對疑似內分泌干擾作用農藥評估概況

美國環保署所啟動之「內分泌干擾物質篩選計畫 (Endocrine Disruptor Screening Program, EDSP)」經多年各界努力，於 2009 年公布了 67 種疑似內分泌干擾之化學物質的初步篩選名單，之後另將多種未繼續登記使用之農藥或化學物質排除，時至今年 (2015)7 月 30 日公布關注的 52 種化學物質 (48 種為農藥之主要作用成分，4 種為其他成分)之試驗篩選結果 (表 1 [9])，其中 20 種為非內分泌干擾物質，18 種可能具內分泌干擾作用，需補充階層 2 試驗結果，另外 14 種則經階層 1 試驗後，評估認為試驗證據顯示具內分泌干擾作用，但基於其保護基準劑量 (point of depature)大於內分泌干擾作用劑量，或是該農藥已未再登記使用等原因，使該農藥毋須進一步補充階層 2 試驗。而針對本份篩選結果報告，美國環保署之後便針對此 18 項化學物質及其登記廠商要求後續補充試驗資料。

而在歐盟之評估概況方面 [10]，歐盟公告之先導篩選名單共約 565 種化學物質，為在第 1 類於體內試驗 (in vivo)中，至少對 1 種生物體具內分泌干擾活性，且具內分泌干擾作用之高可能性之農藥共 19 種 (表 2)，之後歐盟會將於今年公布 400 種農藥及 100 種生物農藥之最終評估結果及評估方法，並於 2016 年 4 月前公布所有篩選之化學物質評估結果、方法及衝擊評估，而整體內分泌干擾物質之結論亦將於 2016 年底前公布。

表 1. 美國環保署出具內分泌干擾作用化學物質篩選評估結果[9]

化學物質名稱	可能影響之內分泌路徑			需進行階層2試驗
	雌性素路徑	雄性素路徑	甲狀腺路徑	
•二、四-地 (2,4-D)				
•阿巴汀 (Abamectin)				
•毆殺松 (Acephate)				
•丙酮 (Acetone)				
•草脫淨 (Atrazine)	○	○		
•倍尼芬 (Benfluralin)	○	○	○	
•畢芬寧 (Bife nthrin)				
•蓋普丹 (Captan)				
•加保利 (Carbaryl)		○		2-1
•加保扶 (Carbofuran)			○	
•四氯異苯腈 (Chlorothalonil)			○	2-2
•陶斯松 (Chlorpyrifos)				
•賽福寧 (Cyfluthrin)				
•賽滅寧 (Cypermethrin)		○		2-1
•大克草 (DCPA)			○	2-2, 2-3
•大利松 (Diazinon)				
•二氯苯腈 (Diclobenil)		○		2-1
•大滅松 (Dimethoate)			○	2-3
•菌達滅 (EPTC)				
•益化利 (Esfenvalerate)			○	
•普伏松 (Ethoprop)			○	
•芬佈賜 (Fenbutatin oxide)				
•福多寧 (Flutolanil)	○	○		2-1
•福爾培 (Folpet)	○			2-1
•嘉磷塞 (Glyphosate)				
•益達胺 (Imidacloprid)				
•依普同 (Iprodione)	○	○		2-1
•異佛爾酮 (Isophorone)				
•理有龍 (Linuron)		○	○	2-1, 2-2, 2-3
•馬拉松 (Malathion)				
•滅達樂 (Metalaxyl)	○	○		2-1
•納乃得 (Methomyl)				
•莫多草 (Metolachlor)			○	
•滅必淨 (Metribuzin)			○	2-2,2-3
•協力克 (MGK 264)	○		○	
•邁克尼 (Myclobutanil)	○	○		2-1

•氟草敏 (Norflurazon)			○	
•鄰苯基苯酚(o-Phenylphenol)	○			2-1
•殺滅 (Oxamyl)				
•五氯硝苯 (PCNB)	○		○	2-1
•百滅寧 (Permethrin)		○		
•益滅松 (Phosmet)			○	
•協力精 (Piperonyl butoxide)				
•殺蟎多 (Propargite)			○	2-2
•普克利 (Propicon azole)	○	○		2-1
•戊炔草胺 (Propyzamide)		○	○	
•百利普芬 (Pyripr oxyfen)		○		
•草滅淨 (Simazine)	○	○		
•得克利 (Tebuconazole)	○	○		2-2
•樂本松 (Tetrachlorvinphos)			○	
•三泰芬 (Triadimefon)				
•三福林 (Trifluralin)				

○：影響此內分泌干擾之路徑；2-1：魚類試驗；2-2：兩棲類試驗；2-3：哺乳類試驗。參考資料來源：美國環保署。

表 2. 歐盟篩選為第 1 類且評估為具內分泌干擾作用高可能性之農藥 [10]

	評估為具內分泌干擾作用高可能性之農藥		
	高使用與製造量者	低使用與製造量者	原資料不足者
藥劑名稱	•免克寧 (Vinclozolin) •錳乃浦 (Maneb) •得恩地 (Thiram) •鋅乃浦 (Zineb) •理有龍 (Linuron (Lorox)) •草脫淨 (Atrazine) •拉草 (Alachlor)	•三福林 (Trifluralin)	•畢芬寧 (Bifenthrin) •賽洛寧 (Cyhalothrin) •第滅寧 (Deltamethrin) •加保利 (Carbaryl) •靈丹 (Lindane (HCHs)) •滴滴涕 (DDT) •芬瑞莫 (Fenarimol) •代森錳 (Mancozeb) •免得爛 (Metiram) •滅必淨 (Metribuzin) •撲滅寧 (Procymidone)

參考資料來源：歐盟

內分泌干擾作用系統性評估及案例

內分泌干擾作用系統性評估方法，基礎是利用證據權重 (weight of evidence; WoE)之概念，合併經濟合作暨發展組織及美國環保署之階層篩選體系所建立。近 50 年，WoE 主要應用於風險評估，但其於不同領域具有不同定義，因此 Weed (2005)對於科學性及政策擬定之發表文獻進行統計，發現 WoE 之定義主要分為 3 大區塊：比喻性質者 (metaphorical)、方法論性質者 (methodological)及理論性質 (theoretical)者[8]。就 WoE 於法規應用而言，美國環保署將 WoE 應用於內分泌干擾物質評估及致癌物質等評估 [4]至此對 WoE 才有較明確之定義，為判定已得的資料，是否支持某物質可造成特異性影響，其特性描述過程稱之 [5]；而其展現方法為以證據作為基礎的研究方法，並評估所收集證據之價值或權重，以支持針對某物質之評估結論 [2]。

為了使評估時 WoE 判定標準一致，美國環保署制定了 WoE 之標準規範及為了確保資料品質等判定時標準一致，也制定了品質判定之標準規範 [6, 7]。整合美國及歐盟之 WoE 概念及經濟合作暨發展組織、美國環保署之內分泌階層試驗，系統性評估包含 6 步驟，並以普克利 (Propiconazole)與陶斯松 (Chlorpyrifos)之評估為例 [7]，步驟 1 為試驗結果蒐集 (Data collecting)，依據美國環保署之階層 1 內分泌干擾作用試驗，收集目標影響指標試驗 (targeted endpoint studies)資料 (表 3)；步驟 2 為個別農藥之資料蒐集 (Relevant data)，收集個別農藥之所有相關試驗及文獻資料，且應包括多重指標試驗 (multi-endpoint studies) (表 4)；步驟 3 為資料可靠性判定，以 ToxRTool 將所蒐集之資料依 Klimisch 氏分類分為 4 等級，並僅採信等級 1、2 之資料；步驟 4 為以經濟合作暨發展組織階層統整資料，如試驗資料間有所矛盾，則以高階層者為依據；步驟 5 為考量多重影響指標，並以

影響路徑統整資料， (如表 5 陶斯松案例)；步驟 6 為結論及判定。依據其 WoE 權重，評估個別農藥之作用模式 (mode of action; MOA)、影響指標 (endpoint)及影響路徑，最後並依據 Weybridge 定義判定個別農藥分屬於內分泌干擾物質、非內分泌干擾物質或資料不足以判定為內分泌干擾物質等 3 族群。依此系統性評估結果，判定陶斯松非為內分泌干擾物質。另外再以普克利 (Propiconazole)為例，評估結果在階層 1 之試驗結果顯示，普克利對雌性素之路經干擾方面，其可能與雌性素受體具反應關係、抑制環化酶與抑制睪固酮生成、影響雌性動物發身與魚類生殖；對雄性素之路徑干擾方面，其具雄性素受體結合反應並具拮抗反應、影響雄性動物生殖與發育、影響魚類生殖；而在甲狀腺系統路徑影響方面，美國環保署判定普克利並無影響；據此科學數據評估判定並考量普克利之保護基準劑量 (point of depature)，公告普克利無須再進行階層 2 哺乳類及鳥類之試驗，但仍需階層 2 之魚類試驗 (表 1)以進一步釐清其內分泌干擾作用之疑慮。

表 3. 陶斯松在美國環保署階層 1 內分泌干擾試驗結果摘要[3]

	試驗方法	MoA*[1]	濃度或劑量	結果	LOEL*[2]	備註
體外 (in vitro)	雌性素受體結合試驗	E(拮抗)	10^{-10}-10^{-3} M	-	無	對雌性素受體無活性
	雌性素受體媒介轉錄試驗	E	10^{-10}-10^{-4} M	+	10^{-5}、10^{-4} M	10-25%之誘發率
	雄性素受體結合試驗	A (拮抗)	10^{-10}-10^{-3} M	+/-	10^{-4}、10^{-3} M	體內試驗較低濃度造成腦及紅血球乙醯膽鹼脂酶減少
	固醇生成試驗	S (雌性素及睪固酮)	10^{-10}-10^{-4} M	+	10^{-5}、10^{-4} M	睪固酮生成增加、雌性素生成減少、體內試驗腦及紅血球乙醯膽鹼脂酶減少
	環化酶試驗	S (雌性素)	10^{-10}-10^{-3} M	-	無	非抑制性
體內 (in vivo)	子宮切除試驗	E	0, 0.5, 1.5, 4 mg/kg/day	-	無	23%增重減少、4 mg/kg/day 紅血球乙醯膽鹼脂酶減少
	Hershberger 試驗	A (拮抗)、S (DHT)	0, 1, 6, 12 mg/kg/day	-	無	腦 (6、12 mg/kg/day)及紅血球 (所有劑量)乙醯膽鹼脂酶降低
	雌性發身試驗	E (拮抗)、S (雌性素)、HPG axis、HPT axis	0, 0.5, 1, 2 mg/kg/day	-	無	腦 (2 mg/kg/day)及紅血球 (所有劑量)乙醯膽鹼脂酶降低
	雄性發身試驗	A (拮抗)、S (睪固酮)、HPG axis、HPT axis	0, 0.5, 1, 2 mg/kg/day	-	無	腦 (2 mg/kg/day)及紅血球 (所有劑量)乙醯膽鹼脂酶降低
	兩棲類變態試驗	HPT axis	0, 0.215, 0.881, 3.68, 13.6 μg/L	-	無	四肢及尾乙醯膽鹼脂酶降低 (3.68、13.6 μg/L)
	魚類短期繁殖試驗	E (拮抗)、A (拮抗)、S (雌性素及睪固酮)、HPG axis	0, 0.251, 0.812, 3.02 μg/L	+	無	腦乙醯膽鹼脂酶降低 (雌性所有劑量；雄性 0.812、3.02 μg/L)

*(1) MoA, mode of action (作用模式)，E: estrogenicity (具雌性素作用)，S: steroidogenesis (雌性素、睪固酮)，A: androgenicity (具雄性素作用)，DHT: dihydrotestosterone (二氫睪固酮)；HPT axis: hypothalamus pituitary thyroid axis (下視丘-腦下垂體-甲狀腺內分泌軸線)；HPG axis: hypothalamus pituitary gonad (下視丘-腦下垂體-性腺內分泌軸線)。

*(2) LOEL, Lowest observed effect level (最低無可見毒害藥量)

參考資料來源： Juberg, 2013。

表 4. 試驗項目與內分泌干擾有關的影響指標。

試驗項目	內分泌影響指標摘要
亞慢性毒性試驗	生長、卵巢重、肝重、腎重、腎上腺重、睪丸重、臨床生化學、各器官組織病理學：子宮、卵巢、甲狀腺、腎、腎上腺、腦下垂體、肝、輸卵管、副甲狀腺、副睪、睪丸、前列腺、貯精囊
慢性/致癌性毒性試驗	生長、卵巢重、甲狀腺重、肝重、腎重、腎上腺重、睪丸重、臨床生化學、各器官組織病理學：子宮、卵巢、甲狀腺、腎臟、腎上腺、腦下垂體、肝、副睪、睪丸、前列腺、貯精囊、包皮腺
發育毒性試驗	生長、子宮腺重、肝重、子代畸形
繁殖毒性試驗	雌性：生長、交配時間、臨床生化學、各器官組織病理學：子宮、卵巢、甲狀腺、腎臟、腎上腺、腦下垂體、肝、輸卵管；雄性：各器官組織病理學：副睪、睪丸、甲狀腺、腎臟、腎上腺、腦下垂體、前列腺、肝臟、貯精囊。
神經發育毒性試驗	生長、陰莖包皮分離年齡

*影響指標應於發育敏感時期觀察，包括產前、產後初期及發身時期

表 5. 陶斯松在經濟合作暨發展組織 (OECD)階層統整資料及影響路徑表[3]

		雌性素路徑			雄性素路徑			
OECD 階層	**試驗方法**	**雌性素活性**	**雌性素拮抗**	**固醇生成**	**試驗方法**	**雄性素活性**	**雄性素拮抗**	**固醇生成**
階層 2	**雌性素受體結合**	-	-	無	**雄性素受體結合**	$+10^{-5}$-10^{-4} M	$+10^{-4}$-10^{-3} M	無
	雌性素受體媒介轉錄分析	10^{-5}-10^{-4} M 輕微反應	無	無				
	固醇生成	無	無	睪固酮下降**、雌性素上升**	**固醇生成**	無	無	睪固酮下降**、雌性素上升**
	環化酶	無	無	非抑制	**環化酶**	無	無	非抑制
階層 3	**子宮激性**	-	無	無	**Hershberger**	-	-	-
階層 4	**雌性發身試驗**	-	-	-	**雄性發身**	-	-	-
	魚類短期繁殖	-	-	-	**魚類短期繁殖**	-	-	-

*對下視丘-腦下垂體-性腺均無影響，**濃度為 10^{1}-10^{2}μM

參考資料來源：Juberg, 2013。

結論

由於我國與歐盟或美國的農藥使用狀況有所異同，現階段針對國內農藥之內分泌干擾作用評估策略以選擇具有內分泌干擾作用疑慮、國內常用、但國內外試驗測試結果不完整者為先，藉由有效地應用系統性評估及舉辦專家討論會議或研討會，整合國內專家意見，最終以農藥之雌性素、雄性素及甲狀腺系統之內分泌干擾作用無可見毒害劑量(no observed adverse effect level, NOAEL)或保護基準劑量與現行法規制定值 (如每日可接受攝食量，acceptable daily intake，ADI)所使用的 NOAEL 值進行比對，進而提供法規制定修訂參考，以確保農藥安全使用及環境安全，達到農產品安心消費的目的。

參考文獻

1. Borgert C. J., Mihaich E. M., Ortego L. S., Bentley K. S., Holmes C. M., Levine S. L., Becker R. A. 2011. Hypothesis-driven weight of evidence framework for evaluating data within the USEPA's Endocrine Disruptor Screening Program. Regulatory Toxicology and Pharmacology 61, 185–191.
2. European Chemical Agency. 2010. How to report weight of evidence. European Chemical Agency. (echa.europa.eu/.../pg_report_weight_of_evidence_en.pdf)
3. Juberg D. R., Gehen S. C., Coady K. K., LeBaron M. J., Kramer V. J., Lu H., Marty M. S. 2013. Chlorpyrifos: Weight of evidence evaluation of potential interaction with the estrogen, androgen, or thyroid pathways. Regulatory Toxicology and Pharmacology 66, 249-263.
4. Krimsky S. The weight of scientific evidence in policy and law. American Journal of Public Health 95, 129-136.
5. Rhomberg L. R., Bailey L. A., Goodman J. E. 2010. Hypothesis-based weight of evidence: A tool for evaluating and communicating uncertainties and inconsistencies in the large body of evidence in proposing a carcinogenic mode of action—naphthalene as an example. Critical Reviews in Toxicology 40, 671–696.
6. USEPA. 2011. Endocrine disruptor screening program: Weight of evidence: Evaluating Results of EDSP Tier 1: Screening to Identify the Need for Tier 2 Testing. (http://www.epa.gov/endo/)
7. USEPA. 2008. Guidelines for ensuring and maximizing the quality, objectivity, utility, and integrity of information disseminated by the environmental protection agency.
8. Weed D. L. 2005. Weight of Evidence: A review of concept and methods. Risk Analysis 25, 1545-1557.
9. www.epa.gov/ingredients-used-pesticide-products/endocrine-disruptor-screening-program-tier-1-assessments
10. www.ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm#priority_list

Endocrine Disruption and Environmental Medicine

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Abstract

Endocrine disruptors, which can change the function of endocrine system and affect organism and offspring health, comprise natural, synthetic and industrial chemicals and result in sperm decreasing, reproductive abnormal, cancer, and neuronal development. Here for determining pesticide endocrine disruptor regulation making trend, first, endocrine disruptor identification and screening status in EU and USEPA is reviewed. Second, systemic evaluation and its applying cases are revealed, and the last, strategies of endocrine disruptors evaluation in our country is introduced. In addition, the evaluation method of how to identify an endocrine disruptor is recently established and concluded in 2011. The basic concept of systemic evaluation is weight of evidence (WoE), and the evaluation methods are based on OECD (Organisation for Economic Co-operation and Development) and USEPA (US Environmental Protection Agency) guidelines. For any pesticide, three endocrine pathway including androgen, estrogen, and thyroid pathway interferences can be evaluated, and these results and information may be used as cornerstone and references for risk assessment or policy making.

Key words: endocrine disruptor, weight of evidence (WoE)

農藥三泰芬對斑馬魚胚胎全轉錄體反應之調控

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摘要

三唑酮（Triadimefon，TDF）為目前常用的農藥之一。在現代農業廣泛的運用下，其三唑酮會殘留在灌溉用水、土壤以及作物中，進而對水生動物造成危害。因此本研究利用了斑馬魚胚胎為實驗檢體，觀察 TDF 對其型態改變和基因體的影響。當處理 2.5、5 與 10 的 TDF 於 24 小時和 72 小時，發現幼魚之外觀有明顯的畸形，並以 RNA-seq 分析斑馬魚胚胎處理 TDF 10 時，其陰性對照組與實驗組的胚胎中之 cDNA 比較，發現有 3591 個基因受到 TDF 所調控，其中包含 3503 個基因上升，88 個基因下降。而在些表現差異的基因分別挑出 20 個最顯著上升或下降的基因，亦發現這些基因與 cytochrome P450 家族蛋白之相關調控、生長、內分泌、代謝有相當大的關係。

因此本研究證明了 TDF 對於水生脊椎動物的之生理影響與分子毒性評估，並瞭解 TDF 所造成之基因體影響，也進一步的釐清可能對人體造成之危害。

前言

三唑酮（Triadimefon，TDF）被廣泛地且長時間的應用於農業上，由於TDF在使用過程中，其農藥殘留會存在於灌溉用水，並具有高度穩定性的特質。目前中國農業已利用TDF來控制及預防作物病害超過二十年餘年(1)。已有許多文獻指出其TDF之毒性機制，例如：破壞真菌之細胞膜的麥角甾醇合成(2)，或是改變動物的求偶激素活性。另外在動物相關試驗中，TDF能抑制CYP51，並顯著的降低小鼠體內的膽固醇與構雄甾烷受體（constitutive androstane receptor ，CAR）之活性(3,4)，或是造成小鼠的肥胖和細胞異常增生(5)。此外，高劑量的TDF（1800ppm）會造成小鼠及大鼠肝細胞腺瘤或甲狀腺濾泡性腺瘤等(5)。

雖然已有相關文獻報導 TDF 對水生動物的危害(6-8)，但對於斑馬魚胚胎之深入毒性分子機制尚未被探討。而斑馬魚胚胎發育到幼魚之過程常被用於研究相關的內分泌干擾機制(9)，亦有研究指出，斑馬魚胚胎發育過程之基因體表現與毒物的代謝機制和人類相似(10,11)，因此觀察 TDF 對於斑馬魚胚胎發育影響可以進一步推測其 TDF 可能對人體的危害性。

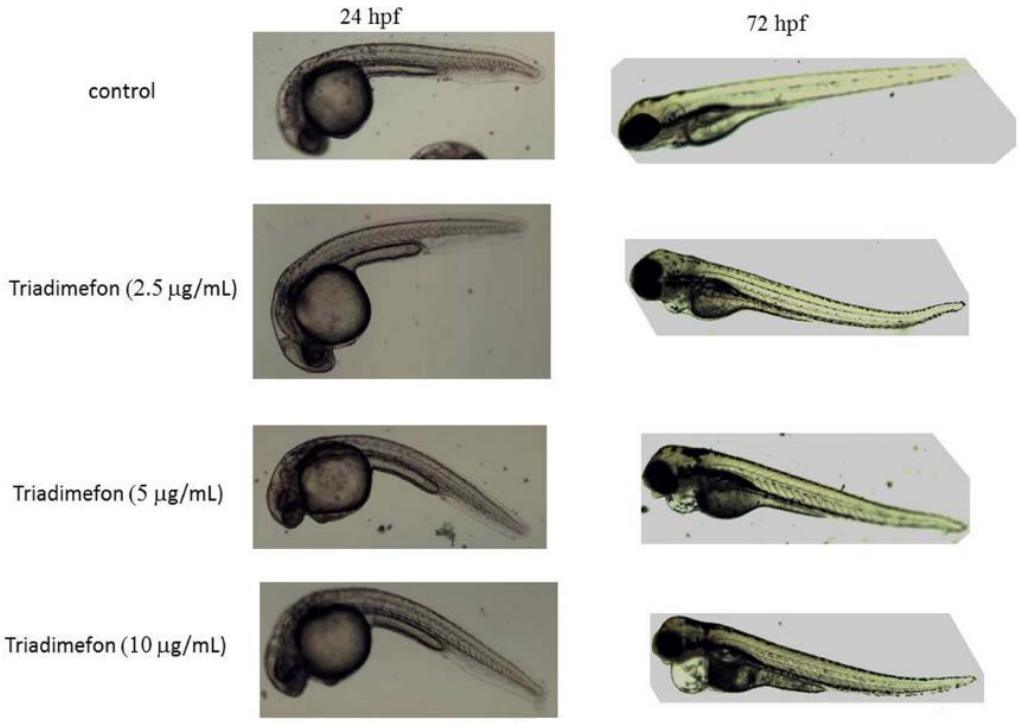
目前觀察基因體變化的方式有很多，而 RNA-seq 為一以高通量（High through put）方式來觀察基因體改變的新技術，並能以短時間且大量的分析其生物或植物的基因變化(12)。因此，本研究利用了 RNA-seq，目的在於：1. 觀察 TDF 對於斑馬魚胚胎發育過程中的基因毒性，並了解其基因體的變化; 2. 受到 TDF 影響之基因體，加以定量，得知其變化的確切數值; 3. 最後將這些受到影響的基因，以相關分子傳遞路徑的方式統整，提供了 TDF 可能造成生物體之生理方面的影響。

結果

1. TDF 造成斑馬魚之型態上的畸形

為了瞭解 TDF 對於斑馬魚型態上的變化，本實驗以不同的時間點分別處理 TDF 於斑馬魚胚胎。結果顯示，當處理 2.5、5 與 10 μg/mL 的 TDF 於 18 小時和 66 小時，發現其頭部大小與尾部有明顯的變小，且心臟異常腫大（Figure. 1）。

Figure. 1



2. 分析對照組與實驗組之 RNA-seq 結果

mRNA 之比較表現量的分析，針對此實驗之 total RNA 並篩選設定最佳的 reads 後，即可以 mapping 方式，與斑馬魚 reference genome 對照組合。本實驗所讀到的 reads 分別為 10,919,177（陰性控制組）及 11,996,068（實驗組），並實際 mapping 回 reference genome，有 74.9%（陰性控制組）與 74.7%（實驗組）成功 mapping 到 reference genome（Table.1）。

Table. 1

Table1. Mapping of clean reads in the zebrafish genomes.

Sample	Input reads	Total mapped	Unique mapped	Multiple position mapped
Control	10,919,177	8,721,571 (79.9%)	8,173,979 (74.9%)	547,592 (5.0%)
+TDF	11,996,068	9,456,232 (78.8%)	8,960,356 (74.7%)	495,876 (4.1%)

比較未處理 TDF 與處理 TDF 之基因體變化，本實驗結果發現共有 3503 個基因表現受到抑制，88 個基因表現異常的上升。並利用 KEGG 分子傳遞路徑之統整軟體，我們將這些 3591 個表現異常的基因做整理，可發現這些改變的基因參與了 28 個相關之分子訊息傳遞路徑（圖表未附）。

針對其表現量上升或下降最顯著的 20 個基因，找出這些基因在生物體之分子功能與機制（Table.2&Table.3）。

Table. 2

Table 2 The 20 most up-regulated DEGs between TDF-free and TDF-treated libraries of zebrafish embryos based on the expressed read frequency

Gene symbol	Log2 ratio(TDF/-TDF)	Description
ppp1r27	6.88	Protein phosphatase 1 regulatory subunit 12B
serpinb1l4	4.64	Serpin peptidase inhibitor
cyp26a1	3.40	Cytochrome P450 26A1
hbbe3	2.85	Hemoglobin beta embryonic-3
slc25a38a	2.71	Solute carrier family 25 member 38-A
cyp2k18	2.62	Cyp2k19 protein
cyp3a65	2.53	Cytochrome P450 3A65
col5a3b	2.47	collagen, type V, alpha 3b precursor
cyp24a1	2.32	1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial
dhrs3a	2.21	Dehydrogenase/reductase (SDR family) member 3a
fabp1b.2	2.15	Fatty acid binding protein 1-B.1
cyp1a	2.09	Cytochrome P450, family 1, subfamily A
gstp2	2.06	Glutathione S-transferase pi 2
arrdc3b	2.04	Arrestin domain containing 3b
dhrs3b	1.99	Dehydrogenase/reductase (SDR family) member 3b
igfbp1b	1.94	Insulin-like growth factor-binding protein 3
fos	1.85	Proto-oncogene c-Fos
cebpb	1.75	CCAAT/enhancer binding protein beta
igfbp1a	1.64	Insulin-like growth factor binding protein-1
mknk2b	1.60	MAP kinase-interacting serine/threonine kinase 2b

Table.3

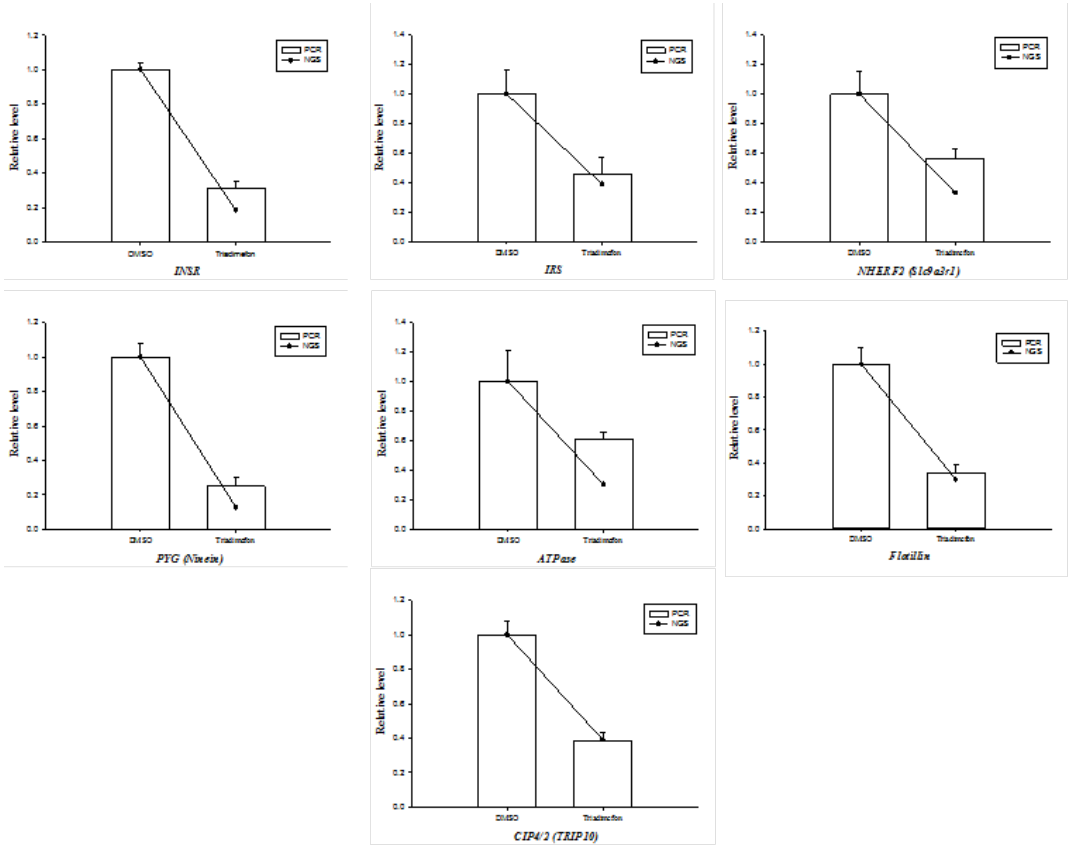
Table 3 The 20 most down-regulated DEGs between TDF-free and TDF-treated libraries of zebrafish embryos based on the expressed read frequency

Gene name	Log2 ratio	Description
dkk2	-6.54	Dickkopf-related protein 2 precurso
ankk1	-6.4	Ankyrin repeat and protein kinase domain-containing protein 1
lrrtm4l1	-6.33	Leucine-rich repeat transmembrane neuronal protein 4
zgc:103438	-6.15	Zgc:103438 protein
si:ch73-367f21.4	-4.9	Zinc finger protein 845-like
pcdh1g32	-4.5	Protocadherin 1 gamma 29 precursor
MIS18BP1	-4.54	Kinetochore-associated protein KNL-2 homolog
il13ra2	-4.47	Interleukin-13 receptor subunit alpha-2 precursor
metap2a	-4.47	Methionine aminopeptidase 2
cldn15b	-4.30	Claudin 15 precursor
sv2c	-4.30	Synaptic vesicle glycoprotein 2C
TNXB	-4.30	Tenascin-like
lipea	-4.22	Hormone-sensitive lipase
st8sia4	-4.22	CMP-N-acetylneuraminate-poly-alpha-2,8-sialyltransferase precursor
trpa1b	-4.22	Transient receptor potential cation channel, subfamily A, member 1b
slc10a2	-4.21	Ileal sodium/bile acid cotransporter
plcg2	-4.10	1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2
rergla	-4.02	Ras-related and estrogen-regulated growth inhibitor-like protein
triap1	-4.02	TP53-regulated inhibitor of apoptosis 1
si:dkey-165n16.1	-4.02	Heat shock protein 67b2-like
cx47.1	-4.02	Connexin 47.1

3. 利用 qRT-PCR 驗證 RNA-seq 之正確性

因 RNA-seq 為高通量的生物定序之技術，因此需利用 qRT-PCR 再次驗證其 RNA-seq 之正確性。研究結果顯示，六個隨機基因（*INSR*、*IRS*、*NHERF2*、*PYG*、*ATPase*、*flotillin*、*CIP4/2*）之檢測，可發現其變化量皆與 RNA-seq 相符（Fig. 2）。

Figure. 2


PCR
NGS
Relative level
DMSO
Triadimefon
INSR
IRS
NHERF3 (Slc9a3r1)
PYG (Nivein)
ATPase
Flotillin
CIP4/2 (TRIP10)

討論與結論

TDF 目前已被證實會引起小鼠肝細胞腺瘤，且在大鼠甲狀腺濾泡中誘導細胞腺瘤形成(13)，並對生物體造成一定的危害。由 RNA-seq 分析結果，表現最顯著差異的 40 個基因（上升與下降各 20 個），其在生物體內有不同的調控與作用機制。例如：維甲酸（Rtinoid acid，RA）在脊椎動物的發展過程中，有助於細胞與細胞間的信號傳遞，並引導胚胎發育的重要因子，而 Cyp26 在 RA 的代謝與表現中扮演了重大的角色(14,15)。Dhrs3a 藉由調控維甲酸受體以保持生物體內 RA 的平衡(16)。CYP 相關之基因，如 CYP2k18、CYP3a65 和 CYP24A1，與 TDF 誘導腫瘤產生有關(8)。

我們還發現了這些表現差異的基因與斑馬魚發育和疾病產生過程中有重要的關聯性，例如：*mknk2b*、*PLCG2*、*rergla*，*arrdc3b* 和 *DKK2*。*Mknk2b* 參與了 MAPK 的訊息傳遞路徑、*Plcg2* 與磷酸肌醇代謝有關、*Rergla* 與 small GTPase 共同調控生物體的生長、*Arrdc3b* 參與了 G 蛋白偶聯受體（G protein coupled receptors，GPCR）的訊號傳遞(17)、*DKK2* 在 Wnt 的訊息傳遞路徑參與了重要的調節等(18)。

RNA-Seq 為現代高通量定序的技術，能夠直接分析大量基因之變化，且目前亦被應用於各種檢體之基因體檢測與定序等(19)。本次實驗結果發現，TDF 對於斑馬魚胚胎之基因體有很大的影響，並利用了 KEGG 將其整理。可發現影響的層面包括了生物的生長、體內的分子訊息傳遞、神經傳導、內分泌系統的干擾等。這些相關生理功能在斑馬魚胚胎接觸到 TDF 後，都有明顯的改變。

在本次研究中，我們分析了斑馬魚胚胎在 TDF 處理下之基因體轉錄反應，但目前尚未有研究針對此部分去探討。因此本次研究結果可提供 TDF 對於斑馬魚胚胎之分子毒性評估，此外，RNA-Seq 可作為一個強大且快速的方法來開發相關生物標誌物的檢測和生物體的基因反應，以利未來在藥物及醫療上的開發。

參考文獻

1. Liu, S., Chang, J., Zhao, Y. and Zhu, G. (2011) Changes of thyroid hormone levels and related gene expression in zebrafish on early life stage exposure to triadimefon. *Environ Toxicol Pharmacol*, **32**, 472-477.
2. Wolf, D.C., Allen, J.W., George, M.H., Hester, S.D., Sun, G., Moore, T., Thai, S.F., Delker, D., Winkfield, E., Leavitt, S. *et al.* (2006) Toxicity profiles in rats treated with tumorigenic and nontumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. *Toxicol Pathol*, **34**, 895-902.
3. Allen, J.W., Wolf, D.C., George, M.H., Hester, S.D., Sun, G., Thai, S.F., Delker, D.A., Moore, T., Jones, C., Nelson, G. *et al.* (2006) Toxicity profiles in mice treated with hepatotumorigenic and non-hepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. *Toxicol Pathol*, **34**, 853-862.
4. Peffer, R.C., Moggs, J.G., Pastoor, T., Currie, R.A., Wright, J., Milburn, G., Waechter, F. and Rusyn, I. (2007) Mouse liver effects of cyproconazole, a triazole fungicide: role of the constitutive androstane receptor. *Toxicol Sci*, **99**, 315-325.
5. Nesnow, S., Ward, W., Moore, T., Ren, H. and Hester, S.D. (2009) Discrimination of tumorigenic triazole conazoles from phenobarbital by transcriptional analyses of mouse liver gene expression. *Toxicol Sci*, **110**, 68-83.
6. Hassold, E. and Backhaus, T. (2009) Chronic toxicity of five structurally diverse demethylase-inhibiting fungicides to the crustacean Daphnia magna: a comparative assessment. *Environ Toxicol Chem*, **28**, 1218-1226.
7. Hermsen, S.A., Pronk, T.E., van den Brandhof, E.J., van der Ven, L.T. and Piersma, A.H. (2011) Chemical class-specific gene expression changes in the zebrafish embryo after exposure to glycol ether alkoxy acids and 1,2,4-triazole antifungals. *Reprod Toxicol*, **32**, 245-252.
8. Lin, C.H., Chou, P.H. and Chen, P.J. (2014) Two azole fungicides (carcinogenic triadimefon and non-carcinogenic myclobutanil) exhibit different hepatic cytochrome P450 activities in medaka fish. *J Hazard Mater*, **277**, 150-158.
9. Zon, L.I. and Peterson, R.T. (2005) In vivo drug discovery in the zebrafish. *Nat Rev Drug Discov*, **4**, 35-44.
10. Chen, Q., Yu, L., Yang, L. and Zhou, B. (2012) Bioconcentration and metabolism of decabromodiphenyl ether (BDE-209) result in thyroid endocrine disruption in zebrafish larvae. *Aquat Toxicol*, **110-111**, 141-148.
11. Kanungo, J., Cuevas, E., Guo, X., Lopez, A.G., Ramirez-Lee, M.A., Trickler, W., Paule, M.G. and Ali, S.F. (2012) Nicotine alters the expression of molecular markers of endocrine disruption in zebrafish. *Neurosci Lett*, **526**, 133-137.
12. van Delft, J., Gaj, S., Lienhard, M., Albrecht, M.W., Kirpiy, A., Brauers, K., Claessen,

S., Lizarraga, D., Lehrach, H., Herwig, R. *et al.* (2012) RNA-Seq provides new insights in the transcriptome responses induced by the carcinogen benzo[a]pyrene. *Toxicol Sci*, **130**, 427-439.

13. Ward, W.O., Delker, D.A., Hester, S.D., Thai, S.F., Wolf, D.C., Allen, J.W. and Nesnow, S. (2006) Transcriptional profiles in liver from mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. *Toxicol Pathol*, **34**, 863-878.
14. Klaunig, J.E. and Kamendulis, L.M. (2004) The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol*, **44**, 239-267.
15. Rydeen, A.B. and Waxman, J.S. (2014) Cyp26 enzymes are required to balance the cardiac and vascular lineages within the anterior lateral plate mesoderm. *Development*, **141**, 1638-1648.
16. Oliveira, E., Casado, M., Raldua, D., Soares, A., Barata, C. and Pina, B. (2013) Retinoic acid receptors' expression and function during zebrafish early development. *J Steroid Biochem Mol Biol*, **138**, 143-151.
17. Alvarez, C.E. (2008) On the origins of arrestin and rhodopsin. *BMC Evol Biol*, **8**, 222.
18. McGraw, H.F., Culbertson, M.D. and Nechiporuk, A.V. (2014) Kremen1 restricts Dkk activity during posterior lateral line development in zebrafish. *Development*, **141**, 3212-3221.
19. Zhang, J.J., Zhou, Z.S., Song, J.B., Liu, Z.P. and Yang, H. (2012) Molecular dissection of atrazine-responsive transcriptome and gene networks in rice by high-throughput sequencing. *J Hazard Mater*, **219-220**, 57-68.

The regulation of transcriptome responses in zebrafish embryo exposure to triadimefon

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Abstract

The residue of triadimefon (TDF) (a pesticide) has become the pollutant in water due to its intensive use in agriculture and medicine and its stability in water leaching from soil and vegetation. In this study, we first performed RNA-seq, a high-throughput method, to analyze the global expression of differential expressed genes (DEGs) in zebrafish embryos treated with TDF (10 μg/mL) from fertilization to 72 h post-fertilization (hpf) as compared to that in the control group (without TDF treatment). Two cDNA libraries were generated from treated and non-treated embryos, respectively. With the vast number of read-mapped genes generated, we observed that many differential genes were expressed between two libraries. The most 20 differentially expressed up-regulated or down-regulated genes were involving in the signaling transduction, the activation of many genes related to cytochrome P450 enzymes, and molecular metabolism. Validation of some selected genes expression confirmed RNA-seq results. The transcriptome sequences were further subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and showed diverse biological functions and metabolic pathways. The data from this study contribute to a better understanding of the potential consequences of fish exposed to TDF, and to evaluate the potential threat of TDF to fish population in the aquatic environment.

Key words: Triadimefon, RNA-seq, Zebra fish, Transcriptome

農藥三泰芬、普克利及得克利對甲狀腺、環化酶及芳香烴受體之影響

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摘要

內分泌干擾物是體外的物質，能夠模仿荷爾蒙而活化或抑制其目標受體的活性，或干擾內分泌的製造、分泌。農藥含有多種結構，而很多農藥的結構巧合的類似於內生性荷爾蒙。環化酶催化雄性激素轉化為雌激素。在本文中，我們專注於對環化酶的表現、活性，及製造雌激素的干擾。

在農藥 difenoconazole (待克利), hexaconazole (菲克利), prochloraz (撲克拉), propiconazole (普克利), tebuconazole 得克利, triadimefon (三泰芬), permethrin (百滅寧), cypermethrin (賽滅寧), fenvalerate (芬化利), diuron (達有龍), and deltamethrin (第滅寧)對於環化酶的表現及活性及其雌激素的製造，在細胞模式或動物模式的影響作用曾經被報導過，但是不同的作者有不同的結果。Imidacloprid (益達胺), iprodione (依普同), quizalofop-p-ethyl (快伏草), ametryn (草殺淨), dimethomorph (達滅芬), fenitrothion (撲滅松), probenazole (撲殺熱), or procymidone (撲滅寧)等農藥則未有與環化酶、雌激素有關的報導。

除了我們的實驗結果，我們整理了過去有關上述農藥干擾環化酶的表現、活性及雌激素的製造的文獻。本文章提供了簡單概要的整理農藥對環化酶、雌激素內分泌干擾的影響。

Introduction

Endocrine disruptors are xenobiotics which can mimic hormones to activate their target receptors or interfere with the endocrine production or secretion. Agriculture chemicals contain versatile structures, and many of their structures are accidentally similar to the endogenus hormones.

Aromatase (cytochrome P450 19, CYP19) is the key enzyme which catalyzes the converting of androgens to estradiol. Aromatase activity is expressed in several tissue sites including the placenta, ovarian granulosa cells, Sertoli and Leydig cells, adipose tissue, several sites in the brain of both sexes, and the preimplantation blastocyte (Lephart and Simpson, 1991).

Because placenta is one of the major tissues producing estrogen, we used human placenta cell line, JEG-3, for the studies of agriculture chemicals. Studies include the analysis of the effects of agriculture chemicals on the aromatase protein expression, aromatase activity, and 17β-estradiol production. The final purpose of this project is to understand whether these agriculture chemicals are endocrine disruptors and disrupts the estrogen production. And then it is possible to predict the effect on the health of human and fetus.

Materials and Methods

Cell line:

JEG-3 is a human choriocarcinoma cell line (人類胎盤的絨毛層癌細胞).
BeWo is a human placental choriocarcinoma cell line. H295R is a human adrenocortical carcinoma cell line. MCF-7 is a breast cancer cell line. R2C is the rat Leydig cell carcinoma cell line. Hepa-1c1c7 cells: mouse hepatoma cells.

MTT cell viability assay:

JEG-3 were plated in 96-well plates at 1×10^4 cells with 0.1 ml medium overnight. Then they were treated with different concentrations of agriculture chemicals for 24 and 48 h. Afterwards, the cells were treated with methylthiazolyldiphenyltetrazolium bromide (MTT) (Sigma) for the assay. The optical densities at 550 nm were measured using an enzyme-linked immunosorbent assay (ELISA) plate reader (BIO-TEK,Winooski, VT). Three or six samples were assayed for each experiment and were repeated at least three times (Su et al., 2008).

Western blot:

JEG-3 cells were plated in 6-well plates at 6×10^5 cells/well with 2 ml medium overnight. At the end of agriculture chemical treatments, cell lysates were collected in lysis buffer (1% NP-40, 0.5mM Tris–HCl (pH 7.5), 0.14M NaCl, 5mM KCl, 5mM EDTA, and 1mM phenylmethylsulfonyl fluoride. Protein lysate at 50 μg of each sample was denatured in

sample buffer and subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). After blotting onto a PVDF membrane, the molecules on the membranes were probed using JEG-3 and GAPDH primary antibodies followed by an HRPconjugated secondary antibody; then they were visualized by an ECL detection system (PerkinElmer Life Sciences, Boston,
(MA). The procedure was adapted from Su et al. (2008).

17*β*-estradiol assay:

The kit of AXSYM system from Abbott was applied to detect estradiol. The procedure is followed as described in the manufacture's instruction.

Aromatase activity assay:

JEG-3 cells were plated in 6-well plates with 2 ml medium overnight. Afterwards, cells were cultured in medium without phenol red and treated with agriculture chemicals. At the end of treatment, culture medium was removed and cells were washed with PBS twice. Then following treatment with none-serum, none-phenol culture medium plus androst-4-ene-3,17-dione [lβ-^{3}H(N)] for 90 minutes, these medium was collected tubes. Chloroform was used to removed the unreacted androst-4-ene-3,17-dione [lβ-^{3}H(N)]. The supernatant was mixed with charcoal, removing the steroids. Then the aqueous part was used to detect radioactivity of water with tritium (Grube et al., 2001).

Results and Discussion

Difenoconazole (待克利)

Only one previous report for the effect of difenoconazole on the aromatase activity is published. Difenoconazole was reported that it is an aromatase inhibitor and slightly inhibits the aromatase activity in H295R cells (Sanderson JT 2002). Difenoconazole inhibits f rainbow trout aromatase activities in a dose-dependent manner in brain and ovarian microsomes (Hinfray et al., 2006).

Our results indicate that difenoconazole (1 μM) highly decreased the proliferation of JEG-3 and MCF-7 cells. In contrast, difenoconazole increased aromatase protein expression on both JEG-3 cells and MCF-7 cells. High dose of difenoconazole (10 μM) decreased aromatase protein expression, which may be due to its toxicity n cellular function. Difenoconazole increased aromatase activity at low dose, but not at high dose in JEG-3. Difenoconazole increased the secretion of 17β-estradiol at low dose (1 μM), but not at high dose (3 μM) in JEG-3.

Hexaconazole (菲克利)

Only one previous report for the effect of hexaconazole on the aromatase activity is published. Hexaconazole **decreased** aromatase activity close to cytotoxic concentrations in H295R cells (Sanderson JT 2002).

Our results indicate that hexaconazole (5μM) decreased the proliferation of JEG-3 and MCF-7 cells. In contrast, hexaconazole (5μM) increased aromatase protein expression on both JEG-3 cells and MCF-7 cells. High dose of hexaconazole (10 μM) decreased aromatase protein expression in JEG-3 cells, but not in MCF-7. Hexaconazole dose-dependently increased aromatase activity in JEG-3. Hexaconazole time course- and dose-dependently increased the secretion of 17β-estradiol in JEG-3, but decreased at high dose (20 μM).

Prochloraz (撲克拉)

A fluorimetric assay indicates that prochloraz is a potent aromatase inhibitor (Trösken et al., 2004**).** It is reported that prochloraz slightly increased aromatase expression and estradiol production in JEG-3 cells (Rieke et al., 2014). In contrast, Laville and his colleagues reported that prochloraz inhibits aromatase activity, but has no effect on aromatase gene transcription and does not cause cytotoxicity, as verified by the MTT viability assay, after exposure to JEG-3 cells (Laville et al., 2006). However, prochloraz inhibits aromatase in the rat Leydig cell carcinoma cell line R2C and the human adrenocorticocarcinoma cell line H295R (Heneweer et al., 2004). Sanderson's group also shows that prochloraz is a potent aromatase inhibitor and inhibits aromatase activity in H295R (Sanderson et al.,2002). In addition, Higley's group indicates that prochloraz dose-dependently decreases aromatase activity and the production of 17β-estradiol in H295R cells (Higley et al., 2010). Kjærstad and his colleagues reported that prochloraz reduced 17β-estradiol production in H295R cells and inhibits the response induced by both 17β-estradiol in MCF-7 cells (Kjærstad et al., 2010). It is also reported that prochloraz reduced 17β-estradiol production in a dose-dependent manner in H295R cells, but does not affect cell viability (Hecker et al., 2006). Prochloraz did not affect estradiol or estrone production in BeWo cells in monoculture (Thibeault et al. 2014). It is interesting that prochloraz also stimulates CYP1A1, mediated by AhR (Rieke et al., 2014). Prochloraz, inhibited the response induced by 17β-estradiol in both the proliferation and the ER transactivation assay in MCF-7 cells (Andersen et al., 2002).

Our results indicate that prochloraz (5μM) decreased the proliferation of JEG-3 and MCF-7 cells. Prochloraz (5μM) increase aromatase protein expression at 6 and 12 h, but decreased after 24 hours treatment in JEG-3 and MCF-7 cells, which may be due to the cytotoxicity on cells. However, prochloraz dose-dependently highly decreased aromatase activity, and time course- and dose-dependently decrease the secretion of 17β-estradiol in JEG-3 cells.

Prochloraz also reduced 17β-estradiol concentration in an *ex vivo* brown trout (Salmo trutta fario) gonad culture (a Marca Pereira et al., 2011). *Ex vivo* ovary cultures of juvenile brown trout (Salmo trutta fario) were exposed for 2 days to prochloraz inhibited ovarian E2

production, but prochloraz did not influence the *ex vivo* expression of aromatase (a Marca Pereira et al., 2014). Prochloraz inhibits *in vitro* aromatase activity in brain and ovarian homogenates from the fish and plasma 17β-estradiol production of fathead minnow (Pimephales promelas) (Ankley et al., 2005). Prochloraz depresses *ex vivo* ovarian production and plasma concentrations of 17β-estradiol in female fathead minnows fish (Ankley et al., 2009). Prochloraz reduces the 17β-estradiol production in H295R cells and fathead minnow ovary explants (Villeneuve et al., 2007). However, H295R cells were about 100-fold more sensitive to the reduction in 17β-estradiol and T production than the ovary explants (Villeneuve et al., 2007). *Ex vivo* ovarian aromatase expression and 17β-estradiol production was significantly reduced in female fathead minnows (Pimephales promelas) (Skolness et al., 2011).

Propiconazole (普克利)

Propiconazole was reported for its regulation on the aromatase activity in JEG-3 cells (Kjeldsen LS et al., 2013; Laville N, 2006; Sanderson JT 2002; Vinggaard et al., 2000). However, results in these reports are different. Propiconazole was reported that it weakly induces the aromatase activity in JEG-3 cells by Kjeldsen **(**Kjeldsen LS et al., 2013**)**. In contrast, propiconazole was reported to weakly inhibits the aromatase activity in JEG-3 cells by Vinggaard and Laville (Vinggaard et al., 2000; Laville N, 2006). Propiconazole slightly inhibits aromatase activity in H295R cells (Sanderson JT 2002). For mix 5 consisted of bitertanol, propiconazole (B01), cypermethrin (B05), malathion and terbuthylazine increase in estradiol was seen as well, indicating increased aromatase activity in H295R cells (Taxvig et al., 2013). However, no individual one of these agriculture chemicals was tested for estradiol secretion. Our results indicate that propiconazole did not distinctly influence the proliferation of JEG-3 cells, but it increases aromatase protein expression and aromatase activity. In addition, propiconazole increase the secretion of 17β-estradiol in JEG-3 cells.

Tebuconazole 得克利

Sanderson and his colleagues showed that **tebuconazole** decreases aromatase activity close to cytotoxic concentrations, indicating that tebuconazole did not have effect on aromatase activity in H295R cells (Sanderson et al., 2002). There was no previous report of tebuconazole in the experiment using JEG-3 cells. Our results indicate that tebuconazole did not influence the proliferation of JEG-3 cells, but it increases aromatase protein expression and aromatase activity. In addition, tebuconazole increase the secretion of 17β-estradiol in JEG-3 cells.

Triadimefon (三泰芬)

Vinggaard and his colleagues reported that triadimefon weakly inhibits the aromatase activity in JEG-3 cells (Vinggaard et al., 2000). However, our results indicate that triadimefon

at high concentration (10 μM) slightly decreased the proliferation of JEG-3 cells, but it (20 μM) time-course dependently increased aromatase protein expression and aromatase activity. In addition, triadimefon (10 μM) increased the secretion of 17β-estradiol in JEG-3 cells.

permethrin 百滅寧

Permethrin induces estrogen-responsive gene mRNA expressions, including aromatase isoform cyp19b, in embryo-larval zebrafish (Jin et al., 2009).

Our results indicate that treatment of permethrin slightly increased the expression of aromatase protein in JEG-3 cells.

Cypermethrin 賽滅寧

Cypermethrin weakly induced the estrogen receptor transactivity, but not induce aromatase activity in human choriocarcinoma JEG-3 cells (Kjeldsen et al., 2013). In contrtast, Laville and his colleague reported that cypermethrin induces aromatase activity, but not aromatase mRNA in JEG-3 cells (Laville et al., 2006).

Our results indicate that **cypermethrin** did not regulate the expression of aromatase protein in JEG-3 cells.

Fenvalerate (芬化利)

Fenvalerate respectively decreases and mildly up-regulates the level of 17β-estradiol in the cerebral cortex of males and females exposed to fenvalerate (Liu et al., 2011). However, pubertal fenvalerate exposure had **no effect on the expression** of **aromatase** in cerebral cortex of males and females (Liu et al., 2011).

Our results indicate that fenvalerate did not distinctly regulate the expression of aromatase protein in JEG-3 cells.

Biuron (達有龍)

Diuron does not affect the aromatase activity in JEG-3 cells (Vinggaard et al., 2002). Our results indicate that diuron did not regulate the expression of aromatase protein in JEG-3 cells.

Imidacloprid aromatase (益達胺)

The effect of imidacloprid on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that imidacloprid did not distinctly regulate the expression of aromatase protein in JEG-3 cells.

Deltamethrin (第滅寧)

Deltamethrin slightly increased **the** aromatase activity in human placental microsomes

(Andersen HR et al., 2002). However, the effect of deltamethrin on the expression of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that deltamethrin increases the expression of aromatase protein in JEG-3 cells, although it decreased JEG-3 cell proliferation.

Iprodione (依普同)

The effect of iprodione on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that iprodione decreased the expression of aromatase protein in JEG-3 cells.

Quizalofop-p-ethyl (快伏草)

The effect of quizalofop-p-ethyl on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that quizalofop-p-ethyl decreased aromatase protein expression in JEG-3 cells.

Ametryn (草殺淨)

The effect of ametryn on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that treatment of ametryn increased the expression of aromatase protein in JEG-3 cells.

Dimethomorph (達滅芬)

The effect of dimethomorph on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that fenitrothion did not regulate the expression of aromatase protein in JEG-3 cells.

Fenitrothion (撲滅松)

The effect of fenitrothion on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that fenitrothion did not regulate the expression of aromatase protein in JEG-3 cells.

Probenazole (撲殺熱)

The effect of probenazole on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that probenazole distinctly decreased aromatase expression in JEG-3

cells.

Procymidone (撲滅寧)

The effect of procymidone on the expression and activity of aromatase or 17β-estradiol secretion was not reported.

Our results indicate that treatment of procymidone for 12 and 24 h caused a minor increase in aromatase protein expression in JEG-3 cells. Treatment of procymidone for 48 h distinctly increased aromatase expression.

References

1. Andersen HR, Vinggaard AM, Rasmussen TH, Gjermandsen IM, Bonefeld-Jørgensen EC. 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. . Toxicol Appl Pharmacol.179:1-12.
2. Ankley GT, Bencic DC, Cavallin JE, Jensen KM, Kahl MD, Makynen EA, Martinović D, Mueller ND, Wehmas LC, Villeneuve DL. 2009. Dynamic nature of alterations in the endocrine system of fathead minnows exposed to the fungicide prochloraz. Toxicol Sci.112:344-353.
3. Ankley GT, Jensen KM, Durhan EJ, Makynen EA, Butterworth BC, Kahl MD, Villeneuve DL, Linnum A, Gray LE, Cardon M, et al. 2005. Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (Pimephales promelas). Toxicol Sci.86:300-308.
4. Grube B, Eng E, Kao Y, Kwon A, Chen S. 2001. White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. J Nutr.131:3288-3293.
5. Hecker M, Newsted JL, Murphy MB, Higley EB, Jones PD, Wu R, Giesy JP. 2006. Human adrenocarcinoma (H295R) cells for rapid in vitro determination of effects on steroidogenesis: hormone production. Toxicol Appl Pharmacol.217:114-124.
6. Heneweer M, van den Berg M, Sanderson JT. 2004. A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase. Toxicol Lett.146:183-194.
7. Higley EB, Newsted JL, Zhang X, Giesy JP, Hecker M. 2010. Assessment of chemical effects on aromatase activity using the H295R cell line. Environ Sci Pollut Res Int.17:1137-1148.
8. Hinfray N, Porcher J, Brion F. 2006. Inhibition of rainbow trout (Oncorhynchus mykiss) P450 aromatase activities in brain and ovarian microsomes by various environmental substances. Comp Biochem Physiol C Toxicol Pharmacol.144:252-262.
9. Jin Y, Chen R, Sun L, Wang W, Zhou L, Liu W, Fu Z. 2009. Enantioselective induction of estrogen-responsive gene expression by permethrin enantiomers in embryo-larval zebrafish. Chemosphere.74:1238-1244.

10. Kjeldsen L, Ghisari M, Bonefeld-Jørgensen E. 2013. Currently used pesticides and their mixtures affect the function of sex hormone receptors and aromatase enzyme activity. Toxicol Appl Pharmacol.272:453-464.
11. Kjærstad MB, Taxvig C, Nellemann C, Vinggaard AM, Andersen HR. 2010. Endocrine disrupting effects in vitro of conazole antifungals used as pesticides and pharmaceuticals. Reprod Toxicol.30:573-582.
12. Laville N, Balaguer P, Brion F, Hinfray N, Casellas C, Porcher J, Aït-Aïssa S. 2006. Modulation of aromatase activity and mRNA by various selected pesticides in the human choriocarcinoma JEG-3 cell line. Toxicology.228:98-108.
13. Liu P, Meng X, Wang H, Ji Y, Zhao M, Zhao X, Xu Z, Chen Y, Zhang C, Xu D. 2011. Effects of pubertal fenvalerate exposure on testosterone and estradiol synthesis and the expression of androgen and estrogen receptors in the developing brain. Toxicol Lett.201:181-189.
14. Mason JI, Carr BR, Murry BA. 1987. Imidazole antimycotics: selective inhibitors of steroid aromatization and progesterone hydroxylation. Steroids.50:179-189.
15. Pereira MLaM, Eppler E, K.L. Thorpe1 JRW, Burkhardt-Holm P. 2014. Molecular and cellular effects of chemicals disrupting steroidogenesis during early ovarian development of brown trout (Salmo trutta fario). Environ Toxicol.29:199-206.
16. Pereira MLaM, Wheelerb JR, Thorpea KL, Burkhardt-Holm P. 2011. Development of an ex vivo brown trout (Salmo trutta fario) gonad culture for assessing chemical effects on steroidogenesis. Aquat Toxicol.101:500-511.
17. Rieke S, Koehn S, Hirsch-Ernst K, Pfeil R, Kneuer C, Marx-Stoelting P. 2014. Combination effects of (tri)azole fungicides on hormone production and xenobiotic metabolism in a human placental cell line. Int J Environ Res Public Health.11:9660-9679.
18. Sanderson JT, Boerma J, Lansbergen GWA, Berg Mvd. 2002. Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. Toxicol Appl Pharmacol.182:44-54.
19. Skolnessa SY, Durhanb EJ, Garcia-Reyeroc N, Jensenb KM, Kahlb MD, Makynenb EA, Martinovic-Weigeltd D, Perkinse E, Villeneuveb DL, Ankley GT. 2011. Effects of a short-term exposure to the fungicide prochloraz on endocrine function and gene expression in female fathead minnows (Pimephales promelas). Aquat Toxicol.103:170-178.
20. Su JG, Liao PJ, Huang MC, Chu WC, Lin SC, Chang YJ. 2008. Aldo-keto reductase 1C2 is essential for 1-nitropyrene's but not for benzo[a]pyrene's induction of p53 phosphorylation and apoptosis. Toxicology.244:257-270.
21. Taxvig C, Hadrup N, Julie Boberga MA, Bossi R, Bonefeld-Jørgensen EC, Vinggaard AM. 2013. In vitro-in vivo correlations for endocrine activity of a mixture of currently used pesticides. Toxicol Appl Pharmacol.272:757-766.

22. Thibeault A-AH, Kathy Deroy CV, Sanderson JT. 2014. A unique co-culture model for fundamental and applied studies of human fetoplacental steroidogenesis and interference by environmental chemicals. Environ Health Perspect.122:371-377.
23. Trösken ER, Scholz K, Lutz RW, Völkel W, Zarn JA, Lutz WK. 2004. Comparative assessment of the inhibition of recombinant human CYP19 (aromatase) by azoles used in agriculture and as drugs for humans. Endocr Res.30:387-394.
24. Villeneuve DL, Ankley GT, Makynen EA, Blake LS, Greene KJ, Higley； EB, Newsted JL, Giesy JP, Hecker M. 2007. Comparison of fathead minnow ovary explant and H295R cell-based steroidogenesis assays for identifying endocrine-active chemicals. Ecotoxicol Environ Saf.68:20-32.
25. Vinggaard AM, Hnida C, Breinholt V, Larsen JC. 2000. Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. Toxicol In Vitro.14:227-234.

Endocrine disruption of agriculture chemicals in aromatase expression and estradiol production

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Abstract

Endocrine disruptors are xenobiotics which can mimic hormones to activate their target receptors or interfere with the endocrine production or secretion. Agriculture chemicals contain versatile structures, and many of their structures are accidentally similar to the endogenus hormones. Aromatase catalyzes the converting of androgens to estradiol. In this article, we focus on the interference with expression and activity of aromatase, and estradiol production.

The effects of agriculture chemicals, difenoconazole, hexaconazole, prochloraz, propiconazole, tebuconazole, triadimefon, permethrin, cypermethrin, fenvalerate, diuron, and deltamethrin, on the expression or activity of aromatase or estradiol production in cells or in animals were reported in the previous publications, but many of them have different results from different authors. None of imidacloprid, iprodione, quizalofop-p-ethyl, ametryn, dimethomorph, fenitrothion, probenazole, or procymidone, was reported for their effect on aromatase or estradiol.

In addition to our results, we review the published reports related to the research in effects of agriculture chemicals on the expression and activity of aromatase and 17β-estradiol production. This article provide a concise review for the endocrine disruption of agriculture chemicals related to aromatase and estradiol production.

Key words: Agriculture chemicals, aromatase, aromatase assay, endocrine disruptor, 17β-estradiol.

應用基準劑量技術評估 4 種疑似內分泌干擾農藥之風險

呂水淵

行政院農藥委員會農業藥物毒物試驗所

摘要

基準劑量分析軟體(benchmark dose soft, BMDS)係由美國環保署所開發，目前仍不斷精進分析軟體的內容，但大抵不影響分析結果，可供分析基準劑量下限值(benchmark dose lower bound, BMDL)，其 BMDL 值相當於 NOAEL 的應用。執行 101-104「應用基準劑量分析方法探討常用農藥對人體健康風險評估」，及 102-104 委辦計畫「類荷爾蒙農藥對雌、雄大鼠發身與甲狀腺功能影響評估」分別建立 BMD 操作方法與完成 29 個常用農藥 BMDL 值，並完成 BMD 操作方法手冊草案及評估待克利、菲克利、撲克拉、普克利、得克利、三泰芬、芬化利、第滅寧及益達胺等 9 種疑似類荷爾蒙農藥。101 至 104 年基準劑量分析結果，其 BMDL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為毆殺松 7.61、100 及 0.08、加保扶 0.61、100 及 0.006、陶斯松 0.0036、100 及 0.00004、納乃得 0.121、100 及 0.0012、托福松 0.171、100 及 0.0017、貝芬替 70.9、1000 及 0.07、撲滅松 0.433、100 及 0.0043、丁基加保扶 66、100 及 0.66、芬普尼 0.011、100 及 0.00011、巴拉刈 0.86、100 及 0.0086、蓋普丹 48、100 及 0.48、滅大松 0.007、100 及 0.00007、硫敵克 6、100 及 0.06、阿巴汀 0.029、100 及 0.00029、依普同 13、100 及 0.13、三泰芬 28、100 及 0.28、甲基鋅乃浦 2、100 及 0.02、普拔克 5、100 及 0.05、甲基陶斯松 0.049、100 及 0.00049、安丹 74、100 及 0.74、二福隆 25、100 及 0.25、依芬寧 16、100 及 0.16、大克蟎 4、100 及 0.04、護矽得 0.405、100 及 0.0045、畢芬寧 15、100 及 0.15、芬瑞莫 3、100 及 0.03、裕必松 0.35、100 及 0.0035、依滅列 3、100 及 0.03 及普伏松 0.46、100 及 0.0046 等 29 種。Tier I 荷爾蒙干擾試驗取得之 BMDL 值，$BMDL_{EDC}$ /$BMDL_{Chr.}$或 $BMDL_{EDC}$ /$NOAEL_{Chr.}$<1 時應進行 Tier II 荷爾蒙干擾試驗，以確認其風險性；若 Tier II 荷爾蒙干擾試驗取得之 BMDL 值，上述比值<1 時，應下調其 ADI 值。9 個疑似類荷爾蒙農藥之雌、雄大鼠血清中荷爾蒙濃度進行基準劑量分析，在雌、雄大鼠發身與甲狀腺功能測試，結果顯示，就血清中荷爾蒙濃度而言，三泰芬(T3)、芬化利(male and female E2、male and female TSH、aromatase)、益達胺(LH)、第滅寧(FSH、T3)及撲克拉(T)值符合 BMD 劑量反應關係，其餘藥劑荷爾蒙濃度不符合劑量反應關係，除撲克拉荷爾蒙干擾作用之 BMDL 值與慢毒性試驗 NOAEL 比值大於 1 外，其餘四個藥劑則均小於 1，其荷爾蒙干擾作用風險值得注意。但就組織重而言，前述 9 個疑似類荷爾蒙農藥除第滅寧外，其餘藥劑內分泌干擾之 NOAEL 值遠大於慢毒性試驗 NOAEL 值，其風險性低。因此有必要釐清上述藥劑對荷爾蒙濃度影響程度。

關鍵詞：內分泌干擾物質、基準劑量

前言

美國環保署在操作手冊指出，進行基準劑量分析前提為農藥慢毒性或亞慢毒性動物試驗數據必需符合基準劑量分析模式，因此目前 NOAEL 仍然是估算 ADI 值重要途徑。動物試驗數據如能符合 BMD 分析，則 BMDL 值將會較一般 NOAEL 值更具代表性，且目前美國環保署及歐盟亦有風險評估報告是以 BMDL 值代替 NOAEL 值。目前歐美國家並未全面採用 BMD 值，進行 ADI 估算，但若劑量情況合適，NOAEL 又無法取得或不如 BMDL 值合適，評估大多會採用此一估值，或並列方式呈現。現行 BMD 分析技術在於輔助 NOAEL 評估農藥 ADI 值，雖然 NOAEL 有下列缺點：(1)受制於動物試驗設計不同影響；(2)無法說明劑量反應估計值的變異；(3)無法說明劑量反應曲線的斜率; (4)有時候動物試驗無法取得 NOAEL 值，雖然可間接使用最低可見毒害劑量 (lowest- observed- adverse- effect level, LOAEL)，但因並非全部動物試驗一定可適合劑量關係模型，但絕大部份動物試驗可取得 NOAEL 或間接取自 LOAEL，因此全世界尤其歐美相關規範仍未修改以 NOAEL 為主的 ADI 估值方式。雖然國際上未全面採用 BMDL 值，但在 USEPA 評估報告與 European Food Safety Authority (EFSA) Journal 則可見到同時考量 BMDL 與 NOAEL 值之評估報告，European Food Safety Authority(2009) 在評估三唑類農藥急性 RfD 值(ARfD)同時並列 NOAEL 與 BMDL 值，但在評估慢性 ADI 值時則僅採用 NOAEL 值，而不採用 BMDL 值，其理由為：評估肝毒性方式可來自在不同三唑藥藥劑及不同試驗中各種不同評估指標，以及某些情況下，肝毒性評估所用的試驗動物除大鼠小鼠及狗外另有其他動物，因此不適合用 BMDL 值來表示。此理由顯然並不是因為值本身不能用，而是因為在評估同一類藥劑時，要對此一類多個藥劑下結論而非針對單一藥劑下結論，以及因肝毒性評估因不同指標不應單一考量某一藥劑及試驗數據。美國環保署重評大滅松報告指出，所以採用 BMDL 值是因為 NOAEL/LOAEL 未能考量動物試驗劑量反應關係，因此採用 BMDL 值。

討論

執行 101-104「應用基準劑量分析方法探討常用農藥對人體健康風險評估」，及 102-104 委辦計畫「類荷爾蒙農藥對雌、雄大鼠發身與甲狀腺功能影響評估」分別建立 BMD 操作方法與完成 29 個常用農藥 BMDL 值，並完成 BMD 操作方法手冊草案及評估待克利、菲克利、撲克拉、普克利、得克利、三泰芬、芬化利、第滅寧及益達胺等 9 種疑似類荷爾蒙農藥。現分述如下：101-104「應用基準劑量分析方法探討常用農藥對人體健康風險評估」，基準劑量分析與傳統 NOAEL 之比較，本次 30 個農藥蒐集到亞慢及慢毒性資料均屬非連續型模式，以美國環保署 2.4 版 BMD 分析軟體進行分析，評估項目包括模式表現與模式適切度。評估參數包括 AIC 數值、Chi-square residual 及其 P 值。符合條件需求為(1)AIC 數值越小較佳，(2)Chi-square residual 需絕對值小於 2，(3)其 P 值越大較佳等項目。101 年試驗分析結果，傳統 NOAEL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為，毆殺松 0.25、10 及 0.03、加保利 15、2000 及 0.008、加保扶 0.22、100 及 0.002、四氯異苯腈 3、100 及 0.03、嘉磷塞 175、

100 及 0.175、陶斯松 1、100 及 0.01、納乃得 3、100 及 0.03、托福松 0.06、100 及 0.0006、貝芬替 2.5、100 及 0.03 及撲滅松 0.5、100 及 0.005。101 年進行 10 種常用農藥 BMD 分析值，有多筆資料者取其最小者。BMDL、SF 及 RfD 分別為，毆殺松 7.61、100 及 0.08、加保利 104.43、500 及 0.209、加保扶 0.61、100 及 0.006、四氯異苯腈 192.4、1000 及 0.2、嘉磷塞 6001、1000 及 6、陶斯松 0.0036、100 及 0.00004、納乃得 0.121、100 及 0.0012、托福松 0.171、100 及 0.0017、貝芬替 76、1000 及 0.07 及撲滅松 0.433、100 及 0.0043。

102 年試驗分析結果，蒐集 30 種常用農藥之基準劑量分析其亞慢或慢毒毒理資料庫蒐集工作，定出其傳統 NOAEL 值、安全係數及參考劑量(RfD)值。由於所取得 30 種常用農藥之毒理資料，有些無法符合基準劑量分析條件要求，總計 13 個農藥毒理資料無法符合要求，剩下 17 個可符合分析要求，但即使數據符合，各種模式之劑量-反應關係不一定可得最佳狀態。本試驗分析結果，傳統 NOAEL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為，大滅松 0.2、10 及 0.02、益達胺 5.7、100 及 0.057、草殺淨 7.2、100 及 0.072、毆殺滅 0.095、10 及 0.009、賽滅寧 5、100 及 0.05、百滅寧 5、100 及 0.05、馬拉松 0.2、10 及 0.02、芬化利 1.85、100 及 0.02、益滅松 1.3、100 及 0.01、撲克拉 0.9、100 及 0.01、菲克利 0.5、100 及 0.005、佈飛松 1、100 及 0.01、芬佈賜 2.5、100 及 0.03、固殺草 2.1、100 及 0.02、芬滅松 0.083、100 及 0.00083、滅大松 0.1、100 及 0.001、滅達樂 8、100 及 0.08、大利松 0.025、10 及 0.002、丁基拉草 1、100 及 0.01、巴拉刈 0.45、100 及 0.005、蓋普丹 12.5、100 及 0.125、免賴得 10、500 及 0.02、福賽得 300、100 及 3、普克利 4、100 及 0.04、巴克素 2.2、100 及 0.022、達馬松 0.02、10 及 0.002、雙特松 0.04、100 及 0.0004、拉草 1、100 及 0.01、愛殺松 0.05、10 及 0.005、培丹 105、100 及 0.1。基準劑量分析結果，僅巴拉刈、蓋普丹及滅大松等三農藥可得 BMDL 值，其 BMDL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為巴拉刈 0.86、100 及 0.0086、蓋普丹 48、100 及 0.48、滅大松 0.007、100 及 0.00007。

103 年試驗分析結果，蒐集 31 種常用農藥之基準劑量分析其亞慢或慢毒毒理資料庫蒐集工作，定出其傳統 NOAEL 值、安全係數及參考劑量(RfD)值。由於所取得 31 種常用農藥之毒理資料，剩下 3 個可符合分析要求，本試驗數據蒐集結果，傳統 NOAEL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為，錳乃浦 4.8、100 及 0.05、福瑞松 0.05、100 及 0.0005、丁基滅必蝨 NA(未知)、NA、NA、甲基多保淨 8、100 及 0.08、施得圃 12.5、100 及 0.125、芬殺松 NA、NA 及 0.007、撲殺熱 NA、NA、NA、賽達松 0.29、100 及 0.003、殺丹 1、100 及 0.01、賓克隆 20、100 及 0.2、普硫松 NA、NA 及 0.0007、丙基喜樂松 NA、NA 及 0.0007、鹼性氯氧化銅 NA、NA、NA、快得寧 NA、NA、NA、丁基加保扶 1、100 及 0.01、亞賜圃 10、100 及 0.1、樂滅草 NA、NA 及 0.0036、滅芬草 NA、NA、NA、三氯松 NA、NA 及 0.0045、滅必蝨 NA、NA 及 0.0007、聚乙醛 2、100 及 0.02、普拉草 NA、NA、NA、達有龍 1.75、250 及 0.007、伏寄普 1、100 及 0.01、三賽唑 5、100 及 0.05、亞滅培 7、100 及 0.07、待克利 1、100 及 0.01、第滅寧 1、100 及 0.01、芬普尼 0.025、100 及 0.0002、達滅芬 5、100 及 0.05、益收生長素 0.5、10 及 0.05。基準劑量分析結果，僅丁基加保扶及芬普尼

等二農藥可得 BMDL 值，其 BMDL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為丁基加保扶 66、100 及 0.66、芬普尼 0.011、100 及 0.00011。

104 年試驗分析結果，蒐集 37 種常用農藥之基準劑量分析其亞慢或慢毒毒理資料庫蒐集工作，定出其傳統 NOAEL 值、安全係數及參考劑量(RfD)值。本試驗數據蒐集結果，傳統 NOAEL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為，腈硫醌 1、100 及 0.01、滅普寧 NA、NA 及 NA、依滅草 NA、NA 及 NA、殺紋寧 NA、NA 及 NA、免速達 NA、NA 及 NA、新殺蟎 0.75、100 及 0.008、硫敵克 3、100 及 0.03、護粒松 0.25、100 及 0.003、腐絕 3、10 及 0.3、阿巴汀 0.2、100 及 0.002、依普同 6、100 及 0.06、三泰芬 2.5、100 及 0.03、甲基鋅乃浦 0.74、100 及 0.007、護賽寧 2.5、100 及 0.02、普拔克 25、100 及 0.2、得克利 3、100 及 0.03、甲基陶斯松 0.1、100 及 0.001、撲滅寧 15、100 及 0.2、安丹 0.2、10 及 0.02、二福隆 2、100 及 0.02、三亞蟎 0.25、100 及 0.003、得芬諾 21、100 及 0.02、芬普寧 3、100 及 0.03、脫克松 6.5、100 及 0.07、依芬寧 3.1、100 及 0.03、克芬蟎 2、100 及 0.02、大克蟎 0.22、100 及 0.002、本達樂 5、100 及 0.05、布芬滅蝨 0.9、100 及 0.01、護矽得 0.14、100 及 0.001、畢芬寧 1.5、100 及 0.02、芬瑞莫 1.2、100 及 0.01、裕必松 0.625、100 及 0.006、可滅鼠 NA、NA 及 NA、平克座 3、100 及 0.03、依滅列 2.5、100 及 0.03 及普伏松 0.04、100 及 0.0004 等 37 種常用農藥之亞慢性或慢性毒理試驗資料。基準劑量分析結果，其 BMDL 值 (mg/kg/day)、安全係數 (SF)及參考劑量(RfD) (mg/kg/day)分別為硫敵克 6、100 及 0.06、阿巴汀 0.029、100 及 0.00029、依普同 13、100 及 0.13、三泰芬 28、100 及 0.28、甲基鋅乃浦 2、100 及 0.02、普拔克 5、100 及 0.05、甲基陶斯松 0.049、100 及 0.00049、安丹 74、100 及 0.74、二福隆 25、100 及 0.25、依芬寧 16、100 及 0.16、大克蟎 4、100 及 0.04、護矽得 0.405、100 及 0.0045、畢芬寧 15、100 及 0.15、芬瑞莫 3、100 及 0.03、裕必松 0.35、100 及 0.0035、依滅列 3、100 及 0.03 及普伏松 0.46、100 及 0.0046 等 17 種。就本試驗蒐集資料結果可知，若農藥動物試驗符合劑量反應關係，以 BMD 分析結果將較傳統 NOAEL 值更具代表性，因 BMD 分析考量到全部試驗劑量表現，而非如傳統 NOAEL 值僅考量某一劑量點。相反地，若試驗結果無劑量反應關係時，完全無法進行 BMD 分析，傳統 NOAEL 值為唯一可用方法。若試驗劑量反應關係不佳時，BMD 分析值亦不佳，傳統 NOAEL 值為較具代表性數值。因此在訂定對人類健康風險參考劑量時應同時考量兩種方法，相輔相成。

102 年類荷爾蒙農藥待克利、菲克利及撲克拉對雌大鼠發身與甲狀腺功能影響評估試驗，結果顯示，對雌大鼠血清中雌素二醇(17β-estradiol, E_2)濃度無明顯影響。103 年類荷爾蒙農藥普克利、得克利及三泰芬對雌大鼠發身與甲狀腺功能影響評估試驗，結果顯示，炔雌醇(17α-ethynylestradiol, EE) 5 mg/kg/day 顯著降低血清中黃體生成素(luteinizing hormone, LH)濃度。三藥劑均未影響血清中 LH 濃度。EE 顯著降低血清中激濾泡素(follicular stimulating hormone, FSH)濃度。三藥劑均未影響血清中 FSH 濃度。EE 顯著增加雌大鼠血清中 E_2，而三藥劑顯著降低其濃度。EE 對雌大鼠血清中環化酶(aromatase)無明顯影響而三藥劑則顯著降低其濃度。EE 或普克利及得克利均不影響三碘甲狀腺素(triiodothyroxine, T3)濃度但三泰芬則顯著增加其濃度。EE 與三藥劑均不影響血清中甲狀腺素(thyroxine, T4)濃度。EE 顯著增加血清中激甲狀腺素濃度而三藥劑則

否。104 年類荷爾蒙農藥芬化利、第滅寧及益達胺對雌大鼠發身與甲狀腺功能影響評估試驗，結果顯示，EE 顯著增加血清中 E_2 濃度，降低血清中睪固酮(testosterone, T)與 LH 濃度，增加激甲狀腺素(thyroid stimulating hormone, TSH)濃度，對三碘甲狀腺素(triiodothyroxine, T3)、甲狀腺素(thyroxine, T4)、FSH 及 aromatase 等濃度無明顯影響。芬化利、第滅寧及益達胺等三藥劑均增加血清中 E2 濃度，但均降低 T3、T4、TSH 及 aromatase 等濃度，對 T、LH 及 FSH 等濃度均無明顯影響。就甲狀腺組織絕對與相對重而言，三藥劑除第滅寧降低甲狀腺不含氣管相對重外無明顯影響，但三藥劑均降低雌大鼠 T3、T4、TSH 及 aromatase 等濃度。

102 年類荷爾蒙農藥待克利、菲克利及撲克拉對雄大鼠發身與甲狀腺功能影響評估試驗，結果顯示，對雄大鼠血清中 T 濃度無明顯影響。103 年類荷爾蒙農藥普克利、得克利及三泰芬對雌大鼠發身與甲狀腺功能影響評估試驗，結果顯示，處理人工合成睪固酮(testosterone propionate, TP) 0.4 mg/kg/day 顯著降低血清中黃體生成素(luteinizing hormone, LH) 濃度； 雄性素受體拮抗劑 Flutamide 3 mg/kg/day 則顯著增加血清中 LH 濃度；三藥劑均不影響血清中 LH 濃度。處理 TP 0.4 mg/kg/day 顯著降低血清中 FSH 濃度；而 Flutamide 3 mg/kg/day 及三藥劑則無明顯影響。處理 TP 或 Flutamide 對雄大鼠血清中 aromatase 濃度無明顯影響，三藥劑顯著降低雄大鼠血清中環化酶濃度。處理 TP 增加血清中睪固酮濃度但統計不顯著，而 Flutamide 則顯著增加其濃度。得克利與三泰芬顯著降低雄大鼠血清中 T 濃度但普克利雖亦降低但統計不顯著。處理 TP 或 Flutamide 對雄大鼠血清中 T3 濃度無明顯影響，普克利或得克利則顯著增加其濃度，但三泰芬雖也增加但統計不顯著。處理 TP 或 Flutamide 或三藥劑均不影響血清中甲狀腺素(thyroxine, T4)及 TSH 濃度。104 年類荷爾蒙農藥芬化利、第滅寧及益達胺對雄大鼠發身與甲狀腺功能影響評估，結果顯示，處理 TP 0.4 mg/kg/day 顯著降低血清中 LH 與 FSH 濃度，對 E_2、T3、T4、TSH、T 及 aromatase 無明顯影響； Flutamide 3 mg/kg/day 則顯著增加血清中 T 濃度，對 E2、LH、FSH、T3、T4、TSH 及 aromatase 等無明顯影響；芬化利增加 T3 與 TSH，對 E2、T、LH、FSH、T4 及 aromatase 等無明顯影響；第滅寧增加 T3、TSH、FSH 及 aromatase，降低 T 濃度，低劑量增加而中劑量降低 E2 濃度、對 T4 與 LH 無明顯影響；益達胺降低 T4、LH 及 T 濃度，增加 E2、T3、TSH、FSH 及 aromatase 等濃度。綜合而言，三藥劑除第滅寧降低甲狀腺不含氣管相對重外對無明顯影響，但三藥劑均增加雄大鼠 T3 與 TSH 濃度，芬化利對 T、LH、FSH、T4 及 aromatase 等無明顯影響；第滅寧增加 FSH 及 aromatase，降低 T 濃度，對 T4 與 LH 無明顯影響；益達胺降低 T4、LH 及 T 濃度，增加 FSH 及 aromatase 等濃度。

Tier I.

荷爾蒙干擾試驗取得之 BMDL 值，$BMDL_{EDC}$ /$BMDL_{Chr.}$或 $BMDL_{EDC}$ /$NOAEL_{Chr.}$<1 時應進行 Tier II 荷爾蒙干擾試驗，以確認其風險性；若 Tier II 荷爾蒙干擾試驗取得之 BMDL 值，上述比值<1 時，應下調其 ADI 值。為進行荷爾蒙干擾作用風險評估，利用基準劑量分析軟體，進行上述 9 個疑似類荷爾蒙農藥在雌、雄大鼠發身與甲狀腺功能測試中大鼠組織重與血清中荷爾蒙濃度分析，結果顯示，就血清中荷爾蒙濃度而言，三泰芬(T3)、芬化利(male and female E2、male and female TSH、aromatase)、益達胺(LH)、第滅寧(FSH、

T3)及撲克拉(T)值符合 BMD 劑量反應關係，其餘藥劑荷爾蒙濃度不符合劑量反應關係，詳細數值如表所示。除撲克拉荷爾蒙干擾作用之 BMDL 值與慢毒性試驗 NOAEL 比值大於 1 外，其餘四個藥劑則均小於 1，其荷爾蒙干擾作用風險值得注意。但就組織重而言，前述 9 個疑似類荷爾蒙農藥除第滅寧外，其餘藥劑內分泌干擾之 NOAEL 值遠大於慢毒性試驗 NOAEL 值，其風險性低。因此有必要釐清上述藥劑對荷爾蒙濃度影響程度。

表 1. 經由慢毒性 NOAEL 與 BMDL 及內分泌干擾作用 BMDL 值評估 5 種疑似類荷爾蒙風險

Pesticide	[1]EDC tests (mg/kg/day)				Chronic test (mg/kg/day)		[2]$BMDL_{EDC}$ /$BMDL_{Chr.}$	[3]$BMDL_{EDC}$ /$NOAEL_{Chr.}$	Source
	$BMDL_h$	$BMDL_t$	$NOAEL_h$	$NOAEL_t$	BMDL	NOAEL			
Imidacloprid	3(LH)		< 10 (m, f)	30(m) 60(f)		6		3/6	Dir 08/116
Iprodione					0.13	0.06			Dir 03/v92
Propiconaozle			< 15 (m, f)	150(m, f)		4			Dir 03/70
Tebuconazole			< 15 (m, f)	50(m) 150(f)		3			EFSA08
Triadimefon	0.1(T3)		< 10 (m, f)	30(m) 100(f)	28	3	0.1/28	0.1/3	JMPR2004
Difenoconazole			100 (m,f)	100(m) 10(f)		1			Dir 08/69
Hexaconazole			150 (m,f)	150(m) 15(f)		0.5			JMPR1990
Prochloraz	16(T)		50(m) 150(f)	50(m) < 15(f)		1		16/1	EFSA11
Fenvalerate	0.04(m,TSH)		< 1 (m, f)	5(m) 20(f)		2		0.04/2	JMPR1986
	1(f,E2)							1/2	
	2(m,E2)							2/2	
	5(aromatase)							5/2	
	0.4(f,TSH)							0.4/2	
Deltamethrin	0.85(T3)		< 0.3 (m, f)	1(m) 3(f)		1		0.85/1	Dir 03/5
	0.76(FSH)							0.76/1	
Carbendazim					76	2			Dir 06/135
Flusilazole					2.77	0.2			Dir 06/133

[1]EDC tests: tests for endocrine disrupting chemical

[2]$BMDL_{EDC}$ /$BMDL_{Chr.}$: BMDL for endocrine disrupting chemical /BMDL for chronic toxicity

[3]$BMDL_{EDC}$ /$NOAEL_{Chr.}$: BMDL for endocrine disrupting chemical /NOAEL for chronic toxicity

結論

利用基準劑量分析軟體，進行上述 9 個疑似類荷爾蒙農藥在雌、雄大鼠發身與甲狀腺功能測試中大鼠組織重與血清中荷爾蒙濃度分析，結果顯示，就血清中荷爾蒙濃度而言，三泰芬(T3)、芬化利(male and female E2、male and female TSH、aromatase)、益達胺(LH)、第滅寧(FSH、T3)及撲克拉(T)值符合 BMD 劑量反應關係，其餘藥劑荷爾蒙濃度不符合劑量反應關係，詳細數值如表所示。除撲克拉荷爾蒙干擾作用之 BMDL 值與慢毒性試驗 NOAEL 比值大於 1 外，其餘四個藥劑則均小於 1，其荷爾蒙干擾作用風險值得注意。但就組織重而言，前述 9 個疑似類荷爾蒙農藥除第滅寧外，其餘藥劑內分泌干擾之 NOAEL 值遠大於慢毒性試驗 NOAEL 值，其風險性低。因此有必要釐清上述藥劑對荷爾蒙濃度影響程度。

參考文獻

1. Allen, B. C., Kavlock, R. J., Kimmel, C. A., Faustman, E. M. 1994a. Dose-response assessment for developmental toxicity: II. Comparison of generic benchmark dose estimates with NOAELs. Fund. Appl. Toxicol. 23:487-95.
2. Allen, B. C., Kavlock, R. J., Kimmel, C. A., Faustman, E. M. 1994b. Dose-response assessment for developmental toxicity: III. Statistical models. Fund. Appl. Toxicol. 23: 496-509.
3. Allen, B. C., Strong, P. L., Price, C. J., Hubbard, S. A., Daston, G. P. 1996. Benchmark dose analysis of developmental toxicity in rats exposed to boric acid. Fund. Appl. Toxicol. 32: 194-204.
4. Alexeeff, G. V., Lewis, D. C., Ragle, N. L. 1993. Estimation of potential health effects from acute exposure to hydrogen fluoride using a ‘ benchmark dose’ approach. Risk Analysis 13: 63-9.
5. Auton, T. R. 1994. Calculation of benchmark doses from teratology data. 1994. Regul. Tox. Pharmacol. 19: 152-67.
6. Benchmark dose software (BMDS) http://www.epa.gov/ncea/bmds/
7. Bhattacharya, R., Lin, L. 2010. An adaptive nonparametric method in benchmark analysis for bioassay and environmental studies. Stat. Probab. Lett. 80: 1947-53.
8. Davis, J. A., Gift, J. S., Zhaz, Q. J. 2011. Introduction to benchmark dose methods and U.S. EPA’s benchmark dose software (BMDS) version 2.1.1. Toxicol. Appl. Pharmacol. 254: 181-191.
9. EPA. (2011). OPPTS 890.1500 Endocrine Disruptor Screening Program Test Guidelines. Pubertal development and thyroid function in intact juvenile/peripubertal male rats assay.
10. European Food Safety Authority. 2009. Scientific opinion on risk assessment for a selected group of pesticides from the triazole group to test possible methodologies to assess cumulative effects from exposure through food from these pesticides on human health. EFSA journal 7:1167
11. Izadi, H., Grundy, J. E., Bose, R. 2011. Evaluation of the benchmark dose for point of departure determination for a variety of chemical classes in applied regulatory settings. Risk Anal. Doi: 10.1111/j.1539-6924.2011.01732.
12. Lachenmeier, D. W., Kanteres, F., Rehm, J. 2011. Epidemiology-based risk assessment using the benchmark dose/margin of exposure approach: the example of ethanol and liver cirrhosis. Int. J. Epidemiol. 40: 210-8.
13. Murata, K., Karita, K., Horiguchi, H., Iwata, T., Hirose, A. 2011. Application of the benchmark dose approach to epidemiological endopoints with clinical standards. Sangyo Eiseigaku Zasshi 53: 67-77.
14. Oberg, M. 2010. Benchmark dose approaches in chemicals health risk assessment in

relation to number and distress of laboratory animals. Regul. Toxicol. Pharmacol. 58 : 451-4.

15. Suwazono, Y. Uetani, M., Akesson, A., Vahter, M. 2010. Recent applications of benchmark dose method for estimation of reference cadmium exposure for renal effects in man. Toxicol. Lett. 198: 40-3.
16. Suwazono, Y., Nogawa, K., Uetani, M., Nakada, S., Kido, T., Nakagawa, H. 2011. Application of the hybrid approach to the benchmark dose ofurinary cadmium as the reference level for renal effects in cadmium polluted and non-polluted areas in Japan. Eenviron. Res. 111:312-4.
17. Thomas, R. S., Clewell, H. J. 3rd, Allen, B. C., Wesselkamper, S. C., Wang, N. C., Lambert, J. C., Hess-Wilson, J. K., Zhao, Q. J., Andersen, M. E. 2011. Application of transcriptional benchmark dose values in quantitative cancer and noncancer risk assessment. Toxicol. Sci. 120:194-205.
18. USEPA. 2009. Pubertal development and thyroid function in intact juvenile/peripubertal female rats. USEPA Guideline for the testing of chemicals OPPTS 890.1450.
19. USEPA. 2009. Pubertal development and thyroid function in intact juvenile/peripubertal male rats. USEPA Guideline for the testing of chemicals OPPTS 890.1500
20. US Environmental Protection Agency Office of Pesticide Programs. 2008. Revised Interim Reregistration Eligibility Decisions for Dimethoate.
21. Weihua, Z., Ekman, J., Almkvist, Å., Saji, S., Wang, L., Warner, M and Gustafsson, J. Å. 2002. Involvement of androgen receptor in 17β-estradiol-induced cell proliferation in rat uterus. Biol. Reprod. 67: 616-623.
22. Wolf, C. J., Hotchkiss, A., Ostby, J. S., LeBlanc, G. A. and Gray Jr., L. E. 2002. Effects of prenatal testosterone propionate on the sexual development of male and female rats: A dose response study. Toxicol. Sci. 65: 71-86.

Risk assessment of four suspected endocrine disrupting pesticides with benchmark dose analysis on human health

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Abstract

Benchmark dose soft (BMDS) is developed and improved consistently by USEPA. This soft supplied the BMDL which is equally to NOAEL in dose response analysis. We carried out two plans, “risk assessment of widely used pesticides with benchmark dose analysis on human health.” from 2012 to 2015 and “Studies on the effect of hormone-like pesticides on pubertal developmental and thyroid function in male intact juvenile/peripubertal rats.” from 2013 to 2015. We set sup BMD analysis manual and finished 29 BMDL values for widely used pesticides. Also we achieved the 5 BMDL values from 9 suspected endocrine disrupting chemicals (EDC). Results of BMD plans from 2011 to 2015 with BMDL, SF (safety factor), and RfD (reference dose) showed that acephate 7.61, 100 and 0.08, carbofuran 0.61, 100 and 0.006, chlorpyrifos 0.0036, 100 and 0.00004, methomyl 0.121, 100 and 0.0012, terbufos 0.171, 100 and 0.0017, carbendazim 76, 1000 and 0.07, fenitrothion 0.433, 100 and 0.0043, carbofulfan 66, 100 and 0.66, fipronil 0.011, 100 and 0.00011, paraquat 0.86, 100 and 0.0086, captan 48, 100 and 0.48, methidathion 0.007, 100 and 0.00007, thiodcarb 6, 100 and 0.06, abamectin 0.029, 100 and 0.00029, iprodione 13, 100 and 0.13, triadimefon 28, 100 and 0.28, propineb 2, 100 and 0.02, propamocarb HCl 5, 100 and 0.05, chlorpyrifos-methyl 0.049, 100 and 0.00049, propoxur 74, 100 and 0.74, diflubenzuron 25, 100 and 0.25, Etofenprox 16, 100 and 0.16, dicofol 4, 100 and 0.04, flusilizole 0.27, 100 and 0.027, bifenthrin 15, 100 and 0.15, fenarimol 3, 100 and 0.03, phosalone 0.35, 100 and 0.0035, imazalial 3, 100 and 0.03, ethoprophos 0.46, 100 and 0.0046. Results of EDC BMDL for 9 suspected EDC pesticides showed that triadimefon (triiodothyroxine, T3), fenvalerate (male and female 17ß-estradiol, thyroid stimulating hormone, aromatase), imidacloprid (luteinizing hormone LH), deltamethrin (follicular stimulating hormone, FSH) and prochlroaz (testosterone, T) fit the model and the others did not. If $BMDL_{EDC}$ /$BMDL_{Chr.}$ or $BMDL_{EDC}$ /$NOAEL_{Chr.}$ from EDC Tier I test was small than 1 then the Tier II test was necessary to run. In the same way if $BMDL_{EDC}$ /$BMDL_{Chr.}$ or $BMDL_{EDC}$ /$NOAEL_{Chr.}$ from Tier II test was small than 1 then the ADI should be reduced. EDC risk assessment showed that four pesticides were to be concerned except prochloraz in terms of hormone effects. On the contrary EDC risk assessment showed that it is safe for these suspected EDC pesticides except deltamethrin in terms of effect of tissue weight. Based on above hormone effects from these nine pesticides

are needed to be further investigated.

Key words: endocrine disrupting chemical (EDC), benchmark dose (BMD)

Toxicoinformatics Tools for Studying Endocrine Disruption Effects of Pesticides

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Abstract

Toxicoinformatics has been an essential tool for setting priorities for toxicity testing. Databases were firstly developed to manage the enormous experimental data generated by toxicology studies. Informatics techniques were subsequently applied to identify structural and physicochemical patterns for targets important for endocrine disruption. A number of ligand- and structure-based prediction models based on the identified patterns and protein 3D structures have been developed for predicting interactions with specific targets. Finally, chemical-protein interaction profiles can be utilized to infer affected functions, pathways and diseases generating hypothesis for further experimental investigation. Several publicly available toxicoinformatics tools useful for endocrine disruption research were selected as follows. Databases of EDKB, EADB and EDCs DataBank were designed to manage data of endocrine disruption activities and 3D structures. SwissTargetPrediction, PASS, OpenVirtualToxLab and PharmMapper based on chemical similarity, quantitative structure-activity relationship, protein-ligand docking and pharmacophore methods, respectively, are representative systems for target prediction. For the inference of affected pathways, ChemDIS and CTD provide an automatic tool for the systematic analysis of enriched gene ontology terms, pathways and disease terms based on chemical-gene/protein interaction profiles. This work will give an overview of the abovementioned tools for studying endocrine disruption effects of pesticides.

Key words: toxicoinformatics, endocrine disruptor, database, target prediction, chemical-disease inference.

Introduction

Modern high-throughput techniques generate enormous experimental data. The processing and management of the data is out of the scope of traditional softwares. The evolving field of toxicoinformatics presents new techniques for the data management, pattern recognition and prediction. Various online databases were created for the management of experimental data generated by toxicology studies and served as primary data sources for pattern recognition and development of prediction models by machine learning techniques. The underlying patterns of physicochemical and structural properties could be identified for further experimental investigation. In addition to the patterns useful for better understanding of the mode of action, the well-established prediction models could largely accelerate endocrine disruption research by setting priorities for testing and generating hypothesis for further study.

Despite of numerous published toxicoinformatics tools that are publicly available as web servers or softwares for general toxicology studies, only a few of the tools are suitable for endocrine disruption research of pesticides. To give an overview of toxicoinformatics tools for endocrine disruption research of pesticides, several types of tools were reviewed in this study as shown in Table 1, including databases, ligand- and structure-based tools for target prediction, and the chemical-disease inference system. Examples were also given to demonstrate the ability of the toxicoinformatics tools.

Databases for endocrine disruption research

Among the publicly available toxicology databases, there are three databases with special emphasis on the endocrine disruptors including Endocrine Disruptor Knowledge Base (EDKB) (Ding *et al.*, 2010), Estrogenic Activity Database (EADB) (Shen *et al.*, 2013) and EDCs DataBank (Montes-Grajales and Olivero-Verbel, 2015).

EDKB is a biological activity database developed by National Center for Toxicological Research (NCTR) collecting *in vitro* and *in vivo* experimental data from their own assays and literatures for more than 3,000 chemicals. Various assays were curated in EDKB including estrogen receptor binding, androgen receptor binding, uterotropic activity, cell proliferation, and reporter gene assays. For each record, the chemical structure, name, molecular formula, CAS number, assay type and results are available with hyperlinks to literature source and related databases including TOXNET (Fowler and Schnall, 2014) and ChemIDplus (Tomasulo, 2002). The data could also be utilized to develop QSAR models to predict estrogen and androgen activity (Devillers, 2009).

Instead of collecting all endocrine related assays, EADB focuses on estrogenic activity and is also developed by NCTR. There are more than 18,000 estrogenic-activity data points available in EADB with more than 1,200 binding assays, reporter-gene assays, cell-proliferation assays, and *in-vivo* assays in 11 different species. Similar information of

chemical structure, name, molecular formula, CAS number, assay type, result and hyperlinks to databases and literatures are included in EADB. Both EDKB and EADB are available as desktop softwares rather than web services. Users will have to install the softwares to access the databases.

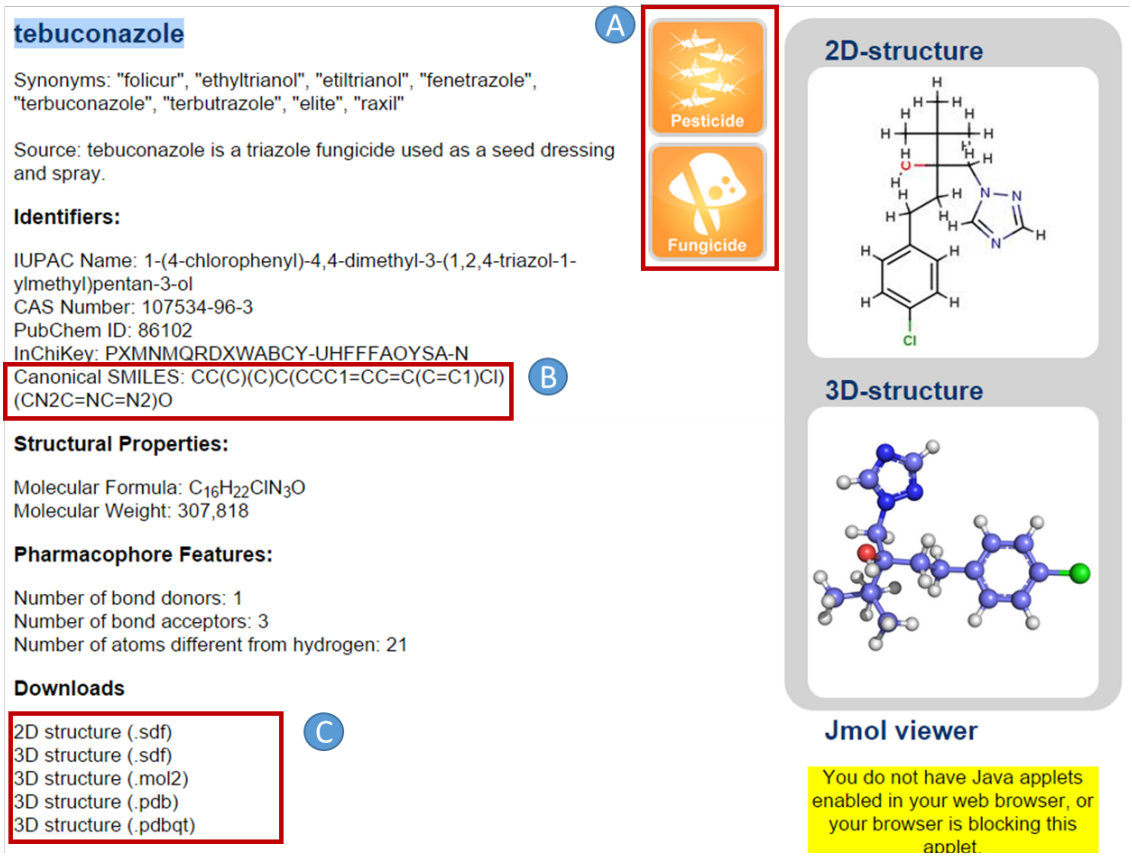


Figure 1. Tebuconazole in the EDCs DataBank. A) Categories of tebuconazole. B) SMILES is a string representation of the chemical structure of tebuconazole. C) 2D and 3D structures can be downloaded as input for target prediction tools

EDCs DataBank is a freely available online database collecting 3D structures of chemicals complied from the EU list of potential endocrine disruptors and TEDX list. A total of 615 molecules are available in EDCs DataBank including pesticides, natural and industrial products, cosmetics, drugs and food additives with hyperlinks to toxicology databases of TOXNET (Fowler and Schnall, 2014), ACToR (Judson *et al.*, 2008; Judson *et al.*, 2012) and ToxCast. Four file formats of 3D structure (mol2, pdb, pdbqt and sdf) are downloadable for each molecule from EDCs DataBank. The structure files could be submitted to webservers and softwares for target prediction. Figure 1 shows the example page of tebuconazole.

Table 1. Toxicoinformatics tools for endocrine disruption research.

Tool	Description	URL

Database		
EDKB	EDKB collects >3,000 chemicals bioassay data including binding to estrogen/androgen receptors, uterotropic activity, cell proliferation and reporter gene assays experimental data	http://www.fda.gov/ScienceResearch/BioinformaticsTools/EndocrineDisruptorKnowledgebase/default.htm
EADB	>18,000 estrogenic-activity data are available in EADB with >1,200 binding, reporter-gene, cell-proliferation and in-vivo assays in 11 species	http://www.fda.gov/ScienceResearch/BioinformaticsTools/EstrogenicActivityDatabaseEADB/default.htm
EDCs DataBank	3D structures of 615 chemicals are curated from the EU list of potential endocrine disruptors and TEDX list with hyperlinks to databases of TOXNET, ACToR and ToxCast	http://edcs.unicartagena.edu.co
Target Prediction		
SwissTarget-Prediction	Prediction is made by searching for similar compounds in its database consisting of 280,000 bioactive compounds for >2,000 targets from 5 different organisms (Ligand-based prediction)	http://www.swisstargetprediction.ch
PASS Online	QSAR models are available for prediction of >4,000 targets and bioactivities of organic compounds (Ligand-based prediction)	http://www.pharmaexpert.ru/passonline/
OpenVirtual-ToxLab	Input compounds will be computationally docked into 3D structures of 16 targets related to endocrine disruption and its binding affinities will be calculated (Structure-based prediction)	http://www.biograf.ch/data/projects/OpenVirtualToxLab.php
Pharm-Mapper	More than 7,000 receptor-based pharmacophore models corresponding to 1,627 drug targets are available for target prediction (Structure-based prediction)	http://59.78.96.61/pharmmapper
Inference of affected functions, pathways and diseases		
ChemDIS	ChemDIS integrating databases of PubChem, STITCH, GO, KEGG, Reactome, DO and DOLite is useful for inferring potential affected functions, pathways and diseases based on chemical-protein interactions	http://cwtung.kmu.edu.tw/chemdis/
CTD	Inference is inferred based on manually curated chemical-gene/protein interactions with integration of GO, KEGG, Reactome and MEDIC	http://ctdbase.org

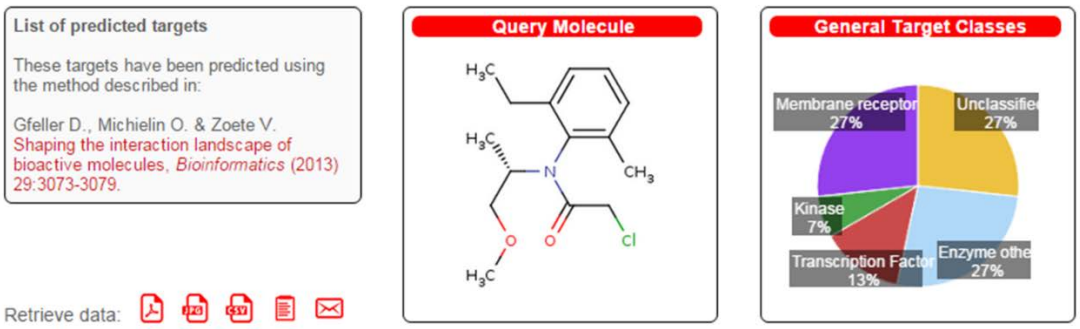
Target prediction tools

The identification of interacting targets is an essential step for characterizing endocrine disruptors. Although there have been a lot of tools developed for target prediction of small molecules, the comprehensiveness of target prediction tools is still an unsolved problem due to the lack of complete data for every gene or protein, i.e. prediction models are not available for a large number of genes or proteins. Users should carefully interpret the predicted results. Informatics tools for target prediction can be categorized into ligand- and structure-based methods according to the utilized information. Ligand-based methods utilize information concerning only chemical itself without target structure information, while structure-based methods utilize both chemical and target structure information. Since the target structure information is scarce, the number of structure-based target models is far less than that of ligand-based target models. Four tools are selected mainly based on the usability of tools and availability of prediction models for endocrine disruption-related targets including SwissTargetPrediction (Gfeller *et al.*, 2014), PASS Online (Filimonov *et al.*, 2014), OpenVirtualToxLab (Vedani *et al.*, 2015) and PharmMapper (Liu *et al.*, 2010).

SwissTargetPrediction is a webserver for ligand-based target prediction. Given a small molecule, SwissTargetPrediction predict targets by searching for targets associated with similar compounds from its database consisting of 280,000 bioactive compounds for more than 2,000 targets from 5 different organisms. The similarity search is based on a measure of both 2D and 3D similarity. The prediction ability of similarity-based methods is largely limited by the size of database that only similar molecules could be predicted. Figure 2 shows the predicted targets for metolachlor.

To better predict unseen molecules, quantitative structure-activity relationship QSAR was introduced to identify physicochemical and structural patterns for discriminating active and inactive molecules. The patterns, e.g. a functional group and hydrophobicity, could be useful for predicting a new molecule with low similarity to training set. The freely accessible web resource PASS Online is designed to predict more than 4,000 bioactivities and targets of organic compounds in a ligand-based manner that only chemical structures are required for prediction. The prediction models of PASS Online were developed by analyzing the relationship between more than 300,000 organic compounds and bioactivity data. Important targets and bioactivity for endocrine disruption such as androgen receptor, estrogen receptor, CYP19A1, antithyroid and endocrine disruptor are included in PASS Online serving as a useful resource for endocrine disruption research.

In contrast to the ligand-based methods utilizing only chemical information, structure-based methods incorporate target 3D structures to calculate the fitness of a chemical to the binding cavity of a target. In that way, structure-based methods enable the prediction of interacting targets without sufficient known interacting compounds for developing ligand-based models.



Target	Common name	Uniprot ID	ChEMBL ID	Probability*	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	MAPT	P10636	CHEMBL1293224		127 / 15	Unclassified
Muscleblind-like protein 1	MBNL1	Q9NR56	CHEMBL1293317		126 / 12	Unclassified
Muscleblind-like protein 2 *(by homology)*	MBNL2	Q5VZF2			126 / 12	Unclassified
Muscleblind-like protein 3 *(by homology)*	MBNL3	Q9NUK0			126 / 12	Unclassified
Monoglyceride lipase	MGLL	Q99685	CHEMBL4191		7 / 2	Enzyme
Estrogen receptor *(by homology)*	ESR1	P03372	CHEMBL206		15 / 1	Transcription Factor
Estrogen receptor beta	ESR2	Q92731	CHEMBL242		15 / 1	Transcription Factor
Tyrosyl-DNA phosphodiesterase 1	TDP1	Q9NUW8	CHEMBL1075138		57 / 2	Enzyme
Sterol O-acyltransferase 1	SOAT1	P35610	CHEMBL2782		1 / 40	Enzyme
Sterol O-acyltransferase 2 *(by homology)*	SOAT2	O75908	CHEMBL4465		1 / 40	Enzyme
DNA-dependent protein kinase catalytic subunit	PRKDC	P78527	CHEMBL3142		16 / 1	Ser_Thr Kinase
Mu-type opioid receptor	OPRM1	P35372	CHEMBL233		32 / 20	Membrane receptor
Delta-type opioid receptor	OPRD1	P41143	CHEMBL236		32 / 20	Membrane receptor
Kappa-type opioid receptor	OPRK1	P41145	CHEMBL237		34 / 14	Membrane receptor
Nociceptin receptor *(by homology)*	OPRL1	P41146	CHEMBL2014		33 / 14	Membrane receptor

* Probabilities have been computed based on a cross-validation. They may therefore not represent the actual probability of success for any new molecule (see FAQ).

Figure 2. Predicted targets for metolachlor from SwissTargetPrediction.

OpenVirtualToxLab, a freely accessible version of their commercial VirtualToxLab software for no-profit organizations, is a docking-based target prediction software with special emphasis on endocrine disruption research. Currently, 16 models are available in OpenVirtualToxLab including 10 nuclear receptors (androgen, estrogen α, estrogen β, glucocorticoid, liver X, mineralocorticoid, peroxisome proliferator-activated receptor γ, progesterone, thyroid α, thyroid β), four members of the cytochrome P450 enzyme family (1A2, 2C9, 2D6, 3A4), a cytosolic transcription factor (aryl hydrocarbon receptor) and a potassium ion channel (hERG). Chemical 3D structure is required for prediction. A remote computer cluster is responsible for the time-consuming computation. Unlike the ligand-based methods giving similarity or likelihood score, the docking-based method generate binding affinity value that could be potentially useful for further investigation. According to the calculated binding affinity, the interaction between a chemical and a target will be scored as strong, moderate, weak or no binding. The built-in function of 3D structure viewer can be

utilized to visualize binding modes. For each chemical, a ToxPot score will be calculated by combining all results from each target representing its overall toxicity.

PharmMapper is a structure-based target prediction tool consisting of more than 7,000 receptor-based pharmacophore models corresponding to 1,627 drug targets. Pharmacophore is the spatial arrangement of features responsible for binding to a receptor. An in-house database collecting 3D complex structures of target and ligand was developed and served as training dataset for developing pharmacophore models using LigandScout (Wolber and Langer, 2005), an automatic tool for generating pharmacophore models from 3D complex structures. Given a chemical, PharmMapper will find the best matching pharmacophore models and output target information.

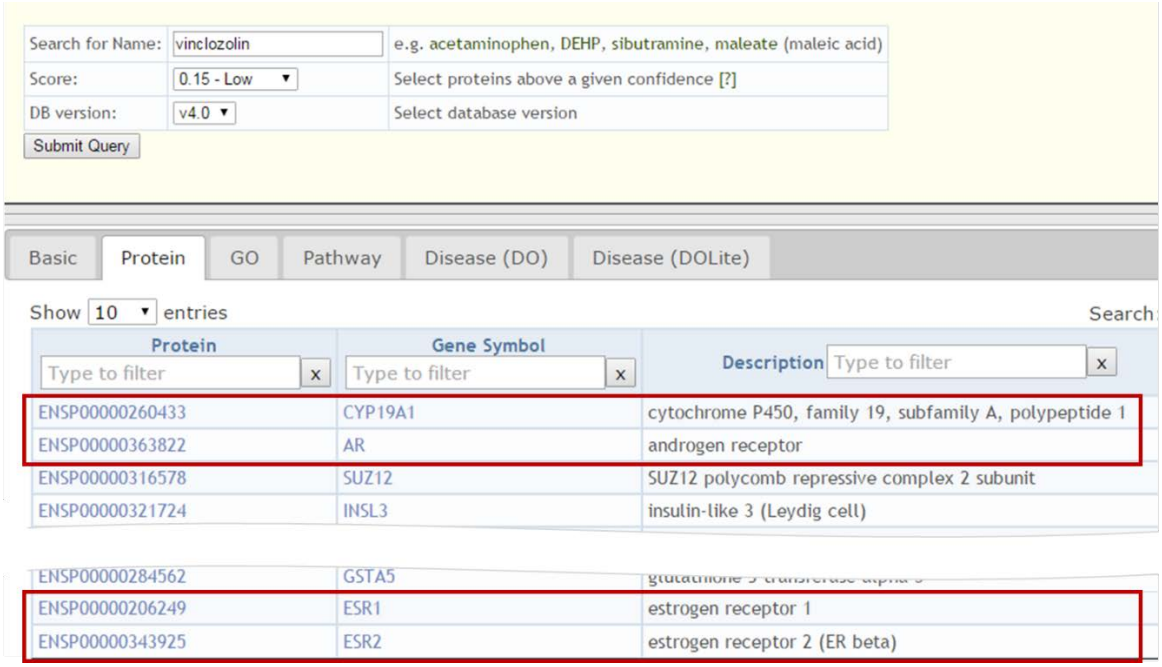


Figure 3. The interacting proteins of vinclozolin from ChemDIS. Marked records are endocrine disruption related proteins including CYP19A1, AR, ESR1 and ESR2.

Chemical-disease inference system

The aforementioned target prediction tools aim to identify interacting targets important for endocrine disruptions. The subsequent toxicity tests of characterizing clear adverse effects related to endocrine disruption is still time-consuming. To further accelerate the study of adverse effects, ChemDIS system is recently proposed to infer potentially affected functions, pathways and diseases (Lin *et al.*, 2014; Tung, 2015). ChemDIS is a web-based inference system integrating multiple information resources of PubChem (Bolton *et al.*, 2008), STITCH (Kuhn *et al.*, 2014), Gene Ontology (GO) (Ashburner *et al.*, 2000), KEGG (Kanehisa *et al.*, 2014), Reactome (Croft *et al.*, 2014), Disease Ontology (DO) and DOLite (Du *et al.*, 2009; Kibbe *et al.*, 2014). With the user-friendly interface, only chemical name is required for inference. Given a chemical, ChemDIS firstly identify all known interacting proteins and

analyze the affected functions, pathways and diseases by applying hypergeometric tests to identify significantly enriched terms among the interacting proteins. ChemDIS is designed to study multi-target effects and is capable of generating testable hypothesis for further experimental investigation that could facilitate the endocrine disruption study of pesticides. The analysis results of vinclozolin is shown in Figure 3 and 4 as an example.

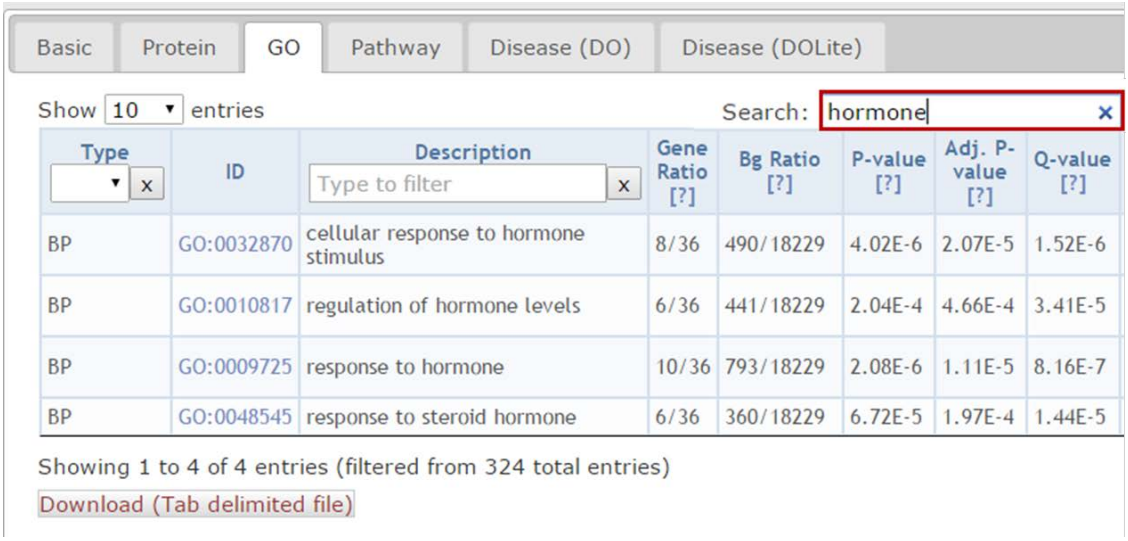


Figure 4. The hormone-related functions potentially affected by vinclozolin from ChemDIS.

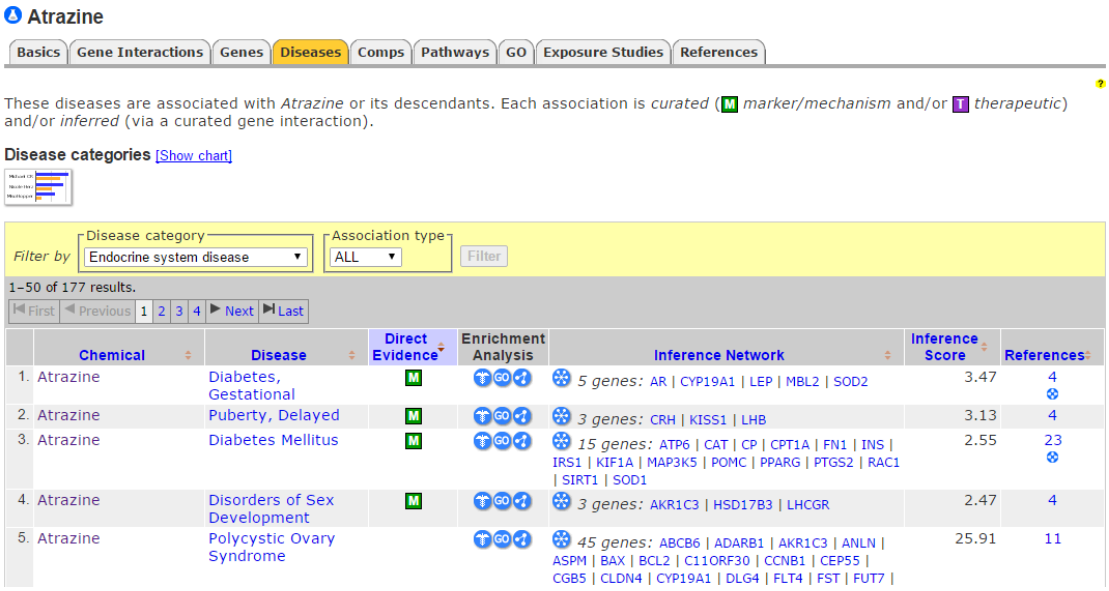


Figure 5. The inferred endocrine system diseases associated with atrazine from CTD.

Comparative Toxicogenomics Database (CTD) (Davis *et al.*, 2015) is a versatile database offering useful information and tools for endocrine disruption research. The fundamental difference between CTD and ChemDIS is the interaction data utilized to make inference. In contrast to ChemDIS utilizing chemical-protein interaction data from STITCH database, CTD uses manually collected chemical-gene/protein interaction data from >100,000 selected

literatures providing a unique toxicogenomics resource. Enrichment analysis of GO, KEGG and Reactome predicts the affected functions and pathways. For chemical-disease inference, the enrichment analysis of CTD is based on its own MEDIC terms rather than the DO terms utilized in ChemDIS. In addition to the inference functions, exposure data is recently incorporated and visualization tools are developed to improve the usefulness and usability.

Conclusion

Toxicoinformatics is an emerging field generating more and more useful tools. In this work, we reviewed several selected tools that could be helpful for endocrine disruption research including databases, tools for target prediction and chemical-disease inference systems. All of them are publicly accessible and user-friendly as softwares or webservers. Please note that toxicoinformatics is a powerful tool that has been used for setting priorities for testing and experimental investigation, however, its prediction may not be suitable for filling data gaps for risk assessment. It is expected that more toxicoinformatics tools will be developed for endocrine disruption research.

Acknowledgement

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References

1. Ashburner M., Ball C.A., Blake J.A., Botstein D., et al., 2000. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25: 25-29.
2. Bolton E.E., Wang Y., Thiessen P.A., Bryant S.H., 2008. Chapter 12 - PubChem: integrated platform of small molecules and niological activities. In: Ralph, A.W., David, C.S. (Eds.), Annual Reports in Computational Chemistry. Elsevier, pp. 217-241.
3. Croft D., Mundo A.F., Haw R., Milacic M., et al., 2014. The Reactome pathway knowledgebase. Nucleic Acids Res. 42: D472-477.
4. Davis A.P., Grondin C.J., Lennon-Hopkins K., Saraceni-Richards C., et al., 2015. The Comparative Toxicogenomics Database's 10th year anniversary: update 2015. Nucleic Acids Res. 43: D914-920.
5. Devillers J., 2009. Endocrine disruption modeling. CRC Press.
6. Ding, D., Xu, L., Fang, H., Hong, H., et al., 2010. The EDKB: an established knowledge base for endocrine disrupting chemicals. BMC Bioinformatics 11 Suppl 6: S5.
7. Du P., Feng G., Flatow J., Song J., et al., 2009. From disease ontology to disease-ontology lite: statistical methods to adapt a general-purpose ontology for the test of gene-ontology associations. Bioinformatics 25: i63-68.

8. Filimonov D.A., Lagunin A.A., Gloriozova T.A., Rudik A.V., et al., 2014. Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. Chem. Heterocycl. Comp. 50: 444-457.
9. Fowler S., Schnall J.G., 2014. TOXNET: information on toxicology and environmental health. Am. J. Nurs. 114: 61-63.
10. Gfeller D., Grosdidier A., Wirth M., Daina A., et al., 2014. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 42: W32-38.
11. Judson R., Richard A., Dix D., Houck K., et al., 2008. ACToR--Aggregated Computational Toxicology Resource. Toxicol. Appl. Pharmacol. 233: 7-13.
12. Judson R.S., Martin M.T., Egeghy P., Gangwal S., et al., 2012. Aggregating data for computational toxicology applications: The U.S. Environmental Protection Agency (EPA) Aggregated Computational Toxicology Resource (ACToR) System. Int. J. Mol. Sci. 13: 1805-1831.
13. Kanehisa M., Goto S., Sato Y., Kawashima M., et al., 2014. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 42: D199-205.
14. Kibbe W.A., Arze C., Felix V., Mitraka E., et al., 2014. Disease Ontology 2015 update: an expanded and updated database of human diseases for linking biomedical knowledge through disease data. Nucleic Acids Res.
15. Kuhn M., Szklarczyk D., Pletscher-Frankild S., Blicher T.H., et al., 2014. STITCH 4: integration of protein-chemical interactions with user data. Nucleic Acids Res. 42: D401-407.
16. Lin Y.C., Wang C.C., Tung C.W., 2014. An in silico toxicogenomics approach for inferring potential diseases associated with maleic acid. Chem. Biol. Interact. 223C: 38-44.
17. Liu X., Ouyang S., Yu B., Liu Y., et al., 2010. PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. Nucleic Acids Res. 38: W609-614.
18. Montes-Grajales D., Olivero-Verbel J., 2015. EDCs DataBank: 3D-Structure database of endocrine disrupting chemicals. Toxicology 327: 87-94.
19. Shen J., Xu L., Fang H., Richard A.M., et al., 2013. EADB: an estrogenic activity database for assessing potential endocrine activity. Toxicol. Sci. 135: 277-291.
20. Tomasulo P., 2002. ChemIDplus-super source for chemical and drug information. Med. Ref. Serv. Q. 21: 53-59.
21. Tung C.W., 2015. ChemDIS: a chemical-disease inference system based on chemical-protein interactions. J. Cheminform. 7: 25.
22. Vedani A., Dobler M., Hu Z., Smiesko M., 2015. OpenVirtualToxLab--a platform for generating and exchanging in silico toxicity data. Toxicol. Lett. 232: 519-532.
23. Wolber G., Langer T., 2005. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J. Chem. Inf. Model 45:

160-169.