應用基準劑量技術評估 4 種疑似內分泌干擾農藥之風險

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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。

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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.

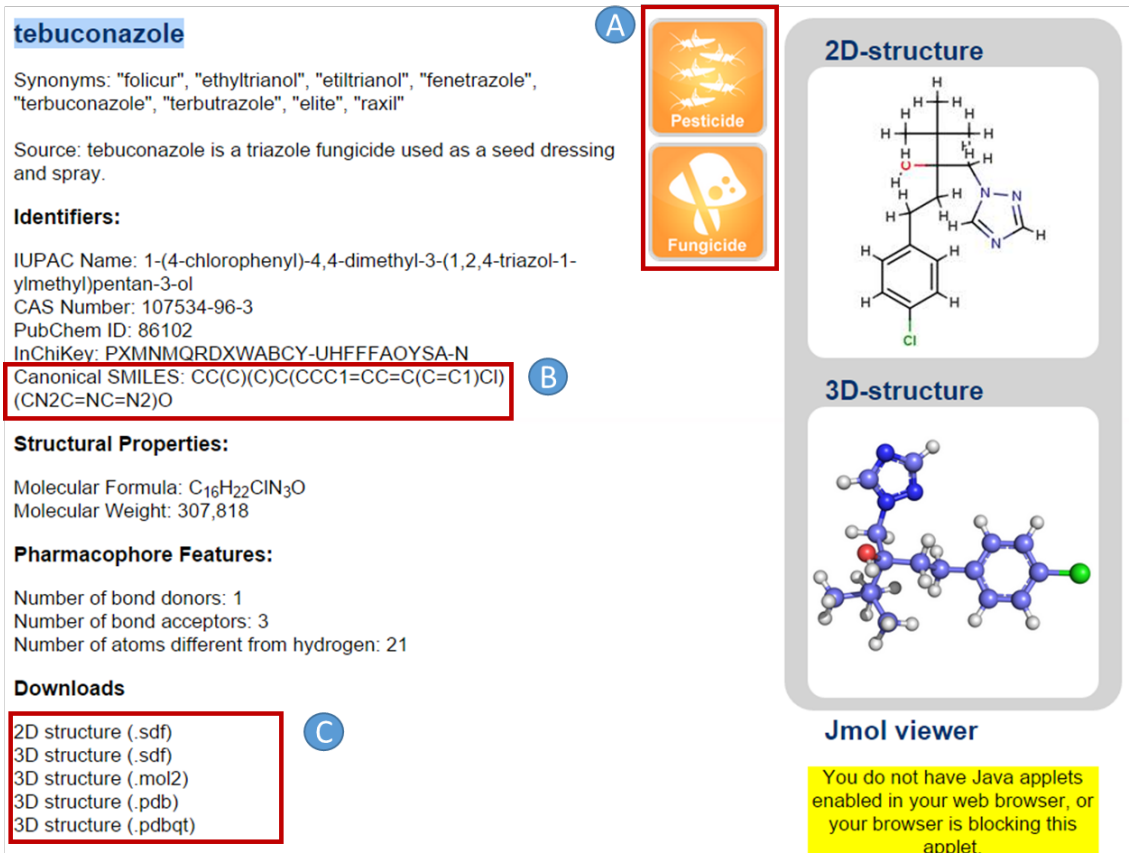


Figure 1. Tebuconazole in the EDCs DataBank. A) Categories of tebuconazole. B) SMILES is a string representation of the chemical structure of tebuconazole. C) 2D and 3D structures can be downloaded as input for target prediction tools

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

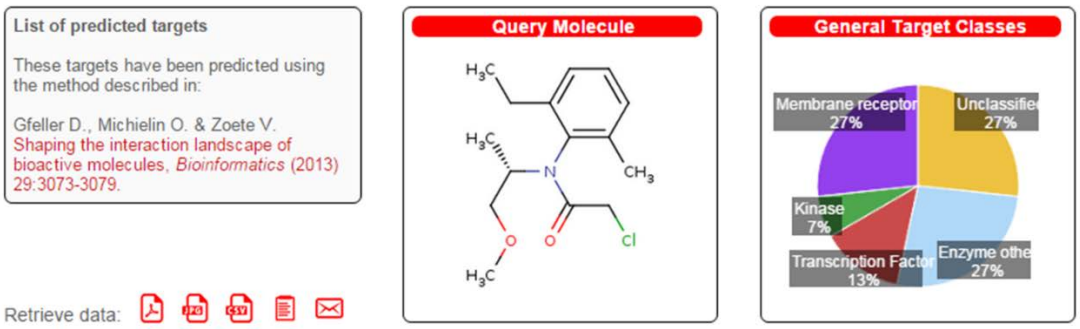
Target prediction tools

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.

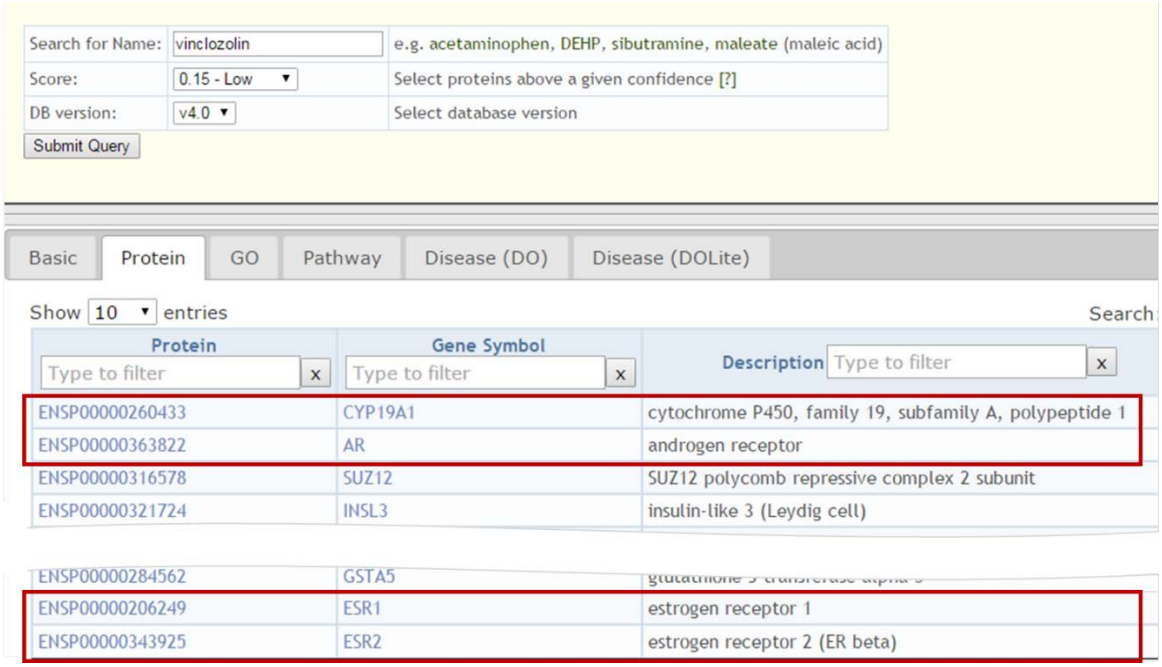


Figure 3. The interacting proteins of vinclozolin from ChemDIS. Marked records are endocrine disruption related proteins including CYP19A1, AR, ESR1 and ESR2.

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.

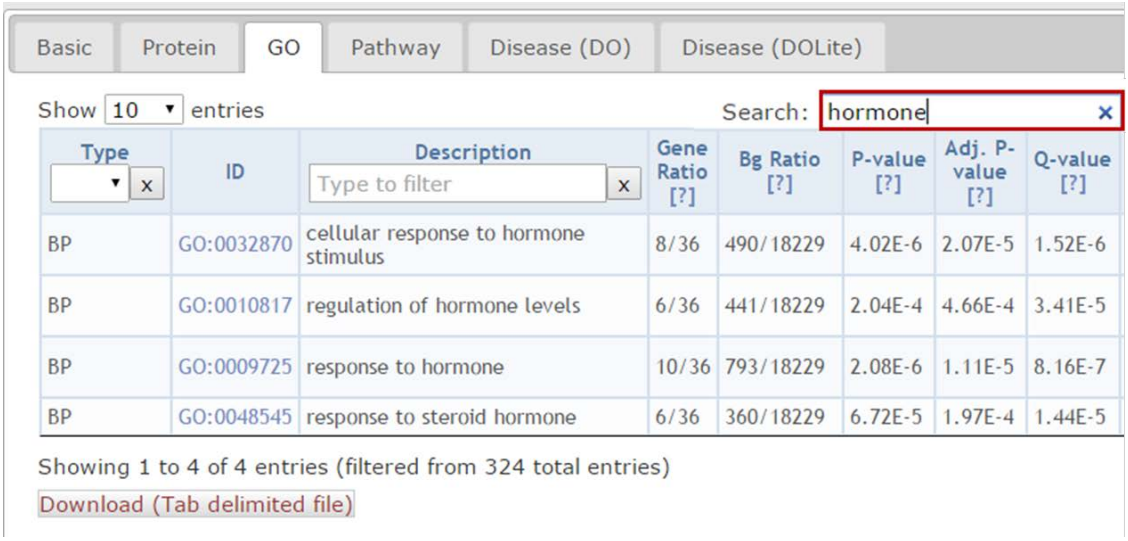


Figure 4. The hormone-related functions potentially affected by vinclozolin from ChemDIS.

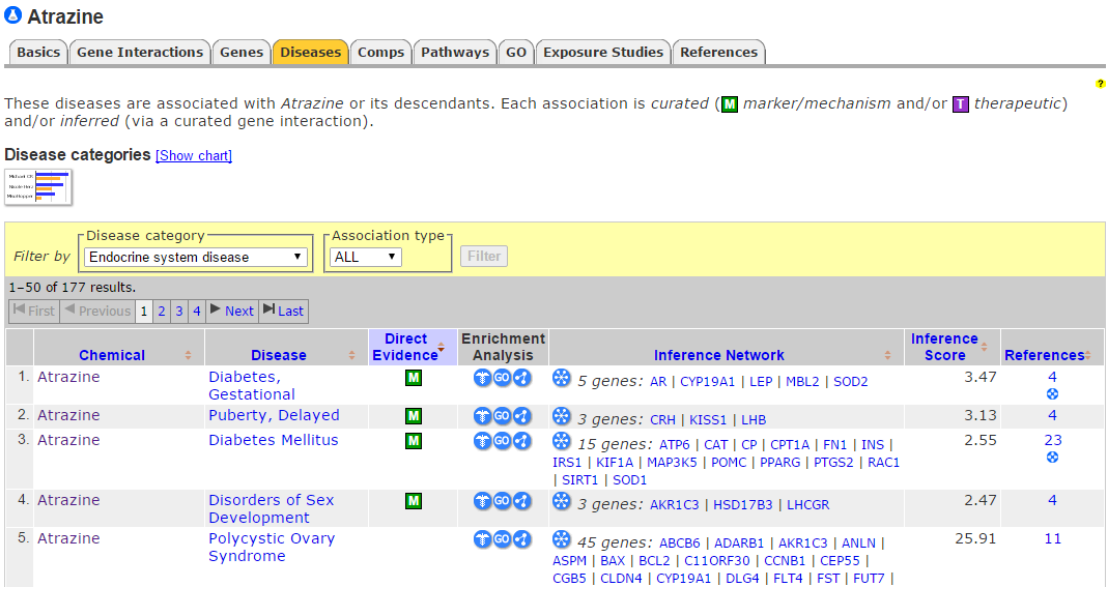


Figure 5. The inferred endocrine system diseases associated with atrazine from CTD.

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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