Abstract # 2748

# Imidacloprid & Fipronil Insecticides: Comparison of *In Vivo* Toxicity Endpoints & ToxCast Profiles


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## INTRODUCTION

- **US EPA's Toxcast program produces high throughput screening results (HTS & zebrafish assays) to profile more than 1,000 chemicals in ~ 800 assays.**
- **We examined whether the well-established *in vivo* endpoints for two pesticides (imidacloprid and fipronil) would coincide with results from biochemical pathway-specific ToxCast HTS assays by these methods:**
  1. **Evaluating *in vivo* studies, designed to identify adverse effects such as neurotoxicity, endocrine disruption or cancer, in order to establish critical endpoints for risk assessment.**
  2. **Examining ToxCast data for use in pesticide risk assessments, hazard identification, and prioritization.**
- **The pesticides imidacloprid and fipronil were selected because they have (1) a *war in vivo* and *in vitro* database with well-defined major endpoints; (2) several *in vivo* endpoints in common; (3) numerous active ToxCast assays including those associated with neurotoxicity, thyroid and liver cancer, metastasis, immune function, endocrine disruption and developmental/neurobehavioral toxicity (zebrafish).**

## Mode of Action for IMIDACLOPRID AND FIPRONIL

***IMIDACLOPRID (Class: Meonicotinoid):***

- **Nicotinic acetylcholine receptor (nAChR) agonist at the neuronal and neuromuscular junctions leads to accumulation of acetylcholine (ACh), an important neurotransmitter, resulting in paralysis, and death.**

***FIPRONIL (Class: Pyrazole):***

- **Blocks γ-aminobutyric acid (GABA)-gated Cl- channels causing build up of excessive levels of ACh at neuromuscular junctions and in the central nervous system which results in hyperactivity, tremors and death.**

## DATA HANDLING

- ***In vivo* No-Observed-Effect-Levels *(NOEL)* and Lowest-Observed-Effect-Levels *(LOEL)* for significant endpoints were obtained from CDPR Risk Characterization Documents (RCD), Toxicology Summaries, USEPA evaluations and the open literature.**
- **Focus of the ToxCast investigation was on all *active HTS assays* (Table 1).**
- ***Z-scores*, indicators of chemical/endpoint specificity and potency (≥ 3 true active), were determined by the USEPA (**http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2014-0614-0003**)**
- ***In Vitro* to *In Vivo* Extrapolation (IVIVE) was used to convert the ToxCast and zebrafish $AC_{50}$ values (50% maximal activity) into a rat *oral equivalent dose (OED)*, by using estimated pharmacokinetic data derived from linear regression and published *in vitro* hepatic metabolism and protein binding from rat data.**
- **Comparisons were made between *in vivo* LOELs and the OEDs derived from ToxCast and zebrafish data.**
- ***ToxPi analyses*: ToxPi is a *dimensionless index score (ToxPi Score)* Reif et al., 2010 and 2013) that is calculated, in our case, as a *weighted combination of active ToxCast assays* among defined components for imidacloprid, fipronil and 2 pesticides that have similar *in vivo* activities to imidacloprid and fipronil:**
  - ***Endosulfan* (organochlorine: blocks $GABA_A$-gated chloride channels; *reference compound* to which the others are compared.**
  - ***Methidathion* (organophosphate-AChE inhibitor)**

  **The input data were $AC_{50}$ values for active ToxCast assays and the scaling type –log10(x)+6 was used for all ToxPi figures shown. ToxPi was also used to compare *activity between fipronil (reference compound) and endosulfan* with the *ER, AR and TR*, since both have endocrine disrupting activity and are active for those receptors.**
- **Data from *ZF embryos* with the *chorion intact* (NHEERL; Padilla et al. 2012) as well as *dechorionated embryos* (Oregon State University; Tanguay et al. 2013; Truong et al., 2014) were examined for developmental toxicity. The OED for zebrafish were compared to *in vivo* LOELs.**

**Table 1. ToxCast ERα and AR Vendors and Assay Descriptions[a]**

| *Vendor* | *Tissue* | *Cell Line/ Receptor* | *Assay Format* | *Biological Process Target* | *Detection Technology* |
|---|---|---|---|---|---|
| APR | Human liver | HEPG2 | Cell-based | Reporters: Indicators of cell health | Fluorescence |
| ATG | Human liver | HepG2 | mRNA induction | Regulation of TF activity via nuclear receptor | Inducible reporter (mRNA) |
| BSK | Human | Primary Cells | Cell-based | Binding Reporter | ELISA |
| NVS | Cell-free | Nuclear receptor | Receptor binding | Inhibition (activation) of enzyme activity; inhibition of ligand binding | Radioligand/ fluorescent receptor binding; enzyme-substrate intensity/mobility shift |
| OT | Human (cervix, kidney) Hamster | HeLa HEK293T CHO-1 | Protein fragment complementation | Binding Reporter Dimerization (cofactor complex) | Luciferase or Fluorescent protein induction |
| Tox21 | Human (kidney, ovarian) | HEK293T, BGI | Reporter gene protein product; transcription suppression | Steroid-nuclear receptor interaction | BLA or fluorescent protein induction |
| ZF | *Danio rerio* | +/- Intact embryo | Embryonic Development | Neuro-, behavioral-, developmental toxicity | Visual |

**VENDORS: Apredica (APR); Attagene (ATG), Bioseek (BSK); Novascreen (NVS), OdyseyThera (USEPA 2013); NIH Chemical Genomics Center (Tox21) Zebrafish (ZF: *Danio rerio*) HTS *in vivo* assays for ToxCast screening: NEERL ZF (intact embryos; Padilla et al. 2012) and embryos with the chorion removed (Tanguay et al. 2013).**

## RESULTS

**IMIDACLOPRID and FIPRONIL *IN VIVO* TOXICITY (Table 2):**

- **Primary effects occurred at the lowest LOELs: neurotoxicity; liver and thyroid pathology/tumors**
  **Secondary effects (Higher LOELs): developmental toxicity, reproductive and endocrine effects**
- **Fipronil had lower LOELs than imidacloprid in all studies.**

**Table 2. Toxicity Reported in CDPR RCD, USEPA Reports and Registrant-Submitted Studies[a]**

| Toxicity Endpoint | Imidacloprid | NOEL/LOEL[b] | Fipronil | NOEL/LOEL[c] range mg/kg/d |
|---|---|---|---|---|
| Neurotoxicity | **Dog[A]: tremors, ataxia, vomiting**<br>**Rat: ↓motor activity, ↓behavioral abnormalities; tremors, ataxia, piloerection**<br>**Mouse: labored breathing, decreased motility, staggering gait, trembling** | **Dog: 8/24 [1]**<br>**Rat: 9/42[2]**<br>**Mouse: 10/?[3]** | **Human: headache, nausea, seizures; neurotoxicity**<br>**Rat: Exaggerated startle & tail-pinch response; convulsions, tremors, ↓vision; ↓ hindleg landing splay, ↓ rearing**<br>**Rabbit: hyperactivity, diarrhea**<br>**Dog: convulsions, tremors, ataxia, muscle twitching, abnormal reflexes, ↓vision, limb jerk, constricted pupils, ↓body temperature** | **Human: NA**<br>**Rat: 0.2/2.5[11]**<br>**Rabbit: 5/10[12]**<br>**Dog: 2/10[13]** |
| DNT[c] | **Rat pup: ↓motor activity, ↓brain size** | **Rat: 19.4/54.7[4]** | **Rat pup: ↑Startle response; ↓ability to swim (unable to stay afloat, swim in straight line, or keep heads out of water)** | 0.05/0.9[14] |
| Thyroid Pathology/tumors | **Dog: follicular atrophy**<br>**Rat: thyroid follicular atrophy; Mineralized particles** | **Dog: 7.3/31[5]**<br>**Rat: 5.7/17[6]** | **Rat: follicular cell hypertrophy & hyperplasia; altered thyroid function (↓T4, ↑TSH); ↑thyroid weight; ↑follicular cell adenomas & carcinomas** | 0.02/0.06 [15, 20] |
| Liver Pathology/ tumors | **Dog[A]: Hepatocellular atrophy; metabolic changes**<br>**Rat: necrosis & hypertrophy of hepatocytes; liver enzymes effects; adenoma, cholangiocellular carcinoma** | **Dog: 7.3/31[5]**<br>**Rat: 5.7/17[7]** | **Rat: ↑liver weight, clinical chemistry change; fatty change**<br>**Mouse: ↑liver weight; periacinar vacuolation; hepatocarcinomas** | **Rat: 0.3/3.4[16]**<br>**Mouse: 0.06/0.13[17]** |
| Cyp-Related | **Dog: p450 induction** | **Dog: 15/41[5]** | **Rat and Human: CYP induction *in vitro***<br>**Rat: CYP induction and ↑T4 clearance** | **Rat/human: 0.1-25 μM[18]**<br>**Rat: 0.06-1.49[19, 20]** |
| Developmental/ Reproductive | **Rat: ↓ pup body weights (2 gen); ↑ wavy ribs; high number of male fetuses (59% > control)**<br>**Rabbit: ↑post-implantation loss; ↓fetal wt** | **Rat: 30/100[8]**<br>**Rabbit: 24/72[10]** | **Rat: Alterations in the ↑progesterone, ↓estradiol levels; lengthening of the estrous cycle ; ↓litter survival & pup viability; delayed pinna attachment, incisor eruption, vaginal patency & preputial separation** | **Rat: 0.05/0.9[14, 21]** |

a- Oral administration unless specified otherwise
b- No-Observed-Effect-Level (NOEL); Lowest-Observed-Effect-Level (LOEL)
c- Developmental Neurotoxicity


References: 1. Ruf, 1990; 2. Bomann, 1989a, b; 3. Bomann, 1989c; 4. Sheets, 2001; 5. Block, 1987; 6. Eiben and Kaliner, 1991; 7. Eiben, 1991; 8. Allen et al., 1989; 9.. Becker et al., 1992; 10. Becker and Biedermann, 1992; 11. Gill, 1993; Hughes, 1997; Driscoll and Hurley, 1993; 12. Hermansky and Wagner, 1993; 13. Holmes, 1991; 14. Mandella, 1995; 15 Peters, 1996; Holms, 1991; King, 1992; 16. Peters, 1996; Holms, 1991; 17. Broadmeadow, 1991; 18. Tang et al., 2004, Das et al., 2006; 19. Roques et al., 2012; 20. Aughton, 1993; 21. Ohi et al., 2004


***IMIDACLOPRID and FIPRONIL ToxCast Active Assays (Table 3):***

- **Active assays for imidacloprid and fipronil are divided into categories associated with *in vivo* endpoints.**

***IMIDACLOPRID:***

- **Active in ion channel assay (NVS_LGIC_hNNR_NBungSens, NVS_LGIC_rNNR_NBungSens), CYP-related (ATG_PXRE_CIS_up, ATG_PXR_CIS_up) and thyroid/ metastasis-related assays (ATG_NRF2_ARE_CIS_up, NVS_rab12C) (Kleinstreuer et al., 2013, 2014).**
- **The Mean OED for assays associated with neurotoxicity, CYP-induction and thyroid pathology/metastasis *correlated well* with LOEL values from in vivo studies reporting those endpoints (Table 3).**

***FIPRONIL:***

- **Active assays for fipronil are related to *cytotoxicity/compromised* cell health (Table 3; Figure 1). Others are predictive of *inflammation, tissue remodeling, vascular biology or immune response potentially predictive of liver and thyroid pathology* which could be related to carcinogenesis and metastasis (Kleinstreuer et al., 2013, 2014).**
- **Figure 1 lists active assays *directly related* to endpoints in a *carcinogenesis pathway*.**
- ***Developmental effects observed in vivo* could result from perturbations in pathways related to activity observed in *ToxCast assays for AR, ER and TR*.**
- **Mean OEDs for assays associated with CYP induction, liver pathology, thyroid pathology/metastasis, cancer and neurodevelopment *correlated well* with LOEL values from studies reporting those endpoints.**
- ***Z-scores for all assays were > 3*, indicating that both imidacloprid and fipronil had specificity for the assay endpoints tested.**

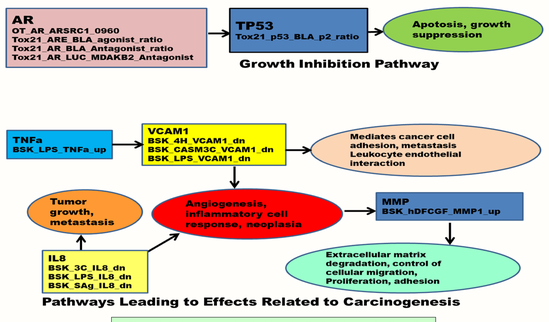


**Figure 1. Pathways associated with active ToxCast Assays for Fipronil**

**Table 3. ToxCast Assay AC50, Z-scores and OEDs**

| Effect | ASSAY | AC50 μM | Z-score[a] | OED mg/kg/d | OED mg/kg/d mean (range) *in vivo* LOEL[b] |
|---|---|---|---|---|---|
| ion channels | *NVS_LGIC_hNNR_NBungSens* | *9.03* | *7.86* | 14.9 | *Imidacloprid: 18 (15-22); LOEL 24-71* |
| | *NVS_LGIC_rNNR_BungSens* | *13.3* | *7.22* | 21.9 | |
| CYP Related | *ATG_PXRE_CIS up* | *20.7* | *8.08* | 44.0 | *Imidacloprid: 89.5 (44-133); LOEL 41* |
| | *ATG_PXR_TRANS up* | *81.8* | *4.18* | 135 | |
| | ATG_PBREM_CIS up | 18.9 | 12.90 | 0.45 | Fipronil: 0.41 (0.012-0.86); LOEL 1.49 |
| | NVS_ADME_hCYP2C19 | 0.698 | 18.41 | 0.012 | |
| | NVS_ADME_hCYP2C9 | 1.04 | 17.74 | 0.017 | |
| | ATG_PXRE_CIS up | 5.2 | 15.05 | 0.12 | |
| | ATG_PXR_TRANS_up | 27.5 | 12.27 | 0.65 | |
| | OT_FXR_FXRSRC1_0480 | 36.1 | 11.82 | 0.86 | |
| | Tox21_FXR_BLA_antagonist_ratio | 33.4 | 11.95 | 0.79 | |
| Acute Inflammation | BSK_3C_IL8_down | 21.1 | 12.71 | 0.50 | Fipronil: 0.40 (0.05-0.78); No LOEL Reported |
| | BSK_LPS_IL8_down | 11.4 | 13.74 | 0.27 | |
| | BSK_SAg_IL8_down | 12.7 | 13.56 | 0.30 | |
| | BSK_CASM3C_MCSF_down | 19.3 | 12.86 | 0.46 | |
| | BSK_LPS_MCSF_down | 19.3 | 12.86 | 0.46 | |
| | BSK_LPS_TNFa_up | 3.17 | 15.88 | 0.05 | |
| | BSK_3C_Eselectin_down | 20.2 | 12.79 | 0.48 | |
| | BSK_LPS_Eselectin_down | 32.7 | 11.98 | 0.78 | |
| | BSK_SAg_Eselectin_down | 14.7 | 13.32 | 0.35 | |
| Chronic Inflammation | BSK_3C_MCP1_down | 17.3 | 13.05 | 0.41 | Fipronil: 0.40 (0.028-0.64); No LOEL Reported |
| | BSK_4H_MCP1_down | 18.7 | 12.92 | 0.44 | |
| | BSK_LPS_MCP1_down | 24.6 | 12.48 | 0.58 | |
| | BSK_SAg_MCP1_down | 15.2 | 13.26 | 0.36 | |
| | BSK_4H_Rantes3_down | 17.9 | 12.99 | 0.43 | |
| | BSK_4H_VCAM1_down | 17.8 | 13.00 | 0.42 | |
| | BSK_CASM3C_VCAM1_down | 18.3 | 12.95 | 0.43 | |
| | BSK_LPS_VCAM1_down | 23.8 | 12.51 | 0.57 | |
| | BSK_BE3C_IP10_down | 17.9 | 12.99 | 0.43 | |
| | BSK_hDFCGF_IP10_down | 1.64 | 16.98 | 0.03 | |
| | BSK_KF3CT_IP10_down | 22 | 12.64 | 0.52 | |
| | BSK_BE3C_MIG_down | 21.2 | 12.71 | 0.50 | |
| | BSK_SAg_MIG_down | 27 | 12.30 | 0.64 | |
| Immune Response | ATG_AP_1_CIS up[c,c] | 7.4 | 14.46 | 0.18 | Fipronil: 0.51 (0.14-1.60); No LOEL Reported |
| | ATG_DRE_CIS up | 6.06 | 14.80 | 0.14 | |
| | ATG_HSE_CIS up | 32.7 | 11.98 | 0.78 | |
| | ATG_NF_kB_CIS up | 32.6 | 11.99 | 0.77 | |
| | ATG_Oct_MLP_CIS up | 12.6 | 13.58 | 0.30 | |
| | BSK_SAg_CD38_down | 22.9 | 6.31 | 0.54 | |
| | BSK_LPS_CD40_down | 13.8 | 13.42 | 0.33 | |
| | BSK_SAg_CD40_down | 21.4 | 6.42 | 0.51 | |
| | BSK_SAg_CD69_down | 19.4 | 12.85 | 0.46 | |
| | BSK_3C_HLADR_down | 10.8 | 13.83 | 0.26 | |
| | BSK_BE3C_HLADR_down | 12.6 | 13.58 | 0.30 | |
| | BSK_4H_Pselectin_down | 19.5 | 12.85 | 0.46 | |
| | Tox21_GR_BLA_Antagonist_ratio | 67.3 | 10.78 | 1.60 | |
| Tissue Remodeling | BSK_BE3C_PAI1_down | 13.8 | 13.42 | 0.33 | Fipronil: 0.74 (0.02-0.81); No LOEL Reported |
| | BSK_hDFCGF_EGFR_up | 1.18 | 17.53 | 0.02 | |
| | ATG_RARa_TRANS up | 18.4 | 12.94 | 0.44 | |
| | ATG_C_EBP_CIS up | 18.8 | 12.91 | 0.45 | |
| | ATG_EGR_CIS up | 34 | 11.92 | 0.81 | |
| | ATG_Sp1_CIS up | 16.4 | 13.14 | 0.39 | |
| | BSK_BE3C_uPA_down | 7.26 | 14.50 | 0.17 | |
| Vascular Biology | BSK_CASM3C_Thrombomodulin_up | 17.9 | 12.99 | 0.43 | Fipronil: 0.42 (0.34-0.78); No LOEL Reported |
| | BSK_4H_VEGFRI_down | 32.7 | 11.98 | 0.78 | |
| | BSK_3C_SRB_down | 24.4 | 12.47 | 0.58 | |
| | BSK_4H_SRB_down | 25.5 | 12.40 | 0.61 | |
| | BSK_BE3C_SRB_down | 14.3 | 13.36 | 0.34 | |
| | BSK_SAg_SRB_down | 25.7 | 12.38 | 0.61 | |
| | BSK_CASM3C_SRB_down | 19.4 | 12.85 | 0.46 | |
| | BSK_LPS_SRB_down | 23.2 | 12.56 | 0.55 | |
| | BSK_3C_uPAR_down | 14.5 | 13.34 | 0.34 | |
| | BSK_4H_uPAR_down | 18.4 | 12.94 | 0.44 | |
| Liver Pathology | Tox21_p53_BLA_p2_ratio | 6.96 | 14.57 | 0.17 | Fipronil: 0.26 (0.008-0.47); LOEL 0.13-3.4 |
| | ATG_CMV_CIS up | 8.21 | 14.29 | 0.20 | |
| | ATG_LXRa_TRANS up | 19.6 | 12.84 | 0.47 | |
| | ATG_LXRb_TRANS up | 13.8 | 13.42 | 0.33 | |
| | ATG_Xbp1_CIS up | 15.8 | 13.20 | 0.38 | |
| | NVS_NR_hCAR_Antagonist | 0.491 | 19.00 | 0.008 | |
| Thyroid Pathology Metastasis | *ATG_NRF2_ARE_CIS up* | *23.4* | *6.27* | *36.56* | *Imidacloprid: 28.26 (17.96-38.56); LOEL 17-31* |
| | *NVS_rab12C* | *10.9* | *7.55* | *17.96* | |
| | BSK_SAg_MIG_dn | 27 | 12.30 | 0.64 | Fipronil: 0.51 (0.16-0.86); LOEL 0.06 |
| | ATG_VDRE_CIS up | 16.4 | 13.14 | 0.39 | |
| | ATG_MRE_CIS up | 36 | 11.82 | 0.86 | |
| | ATG_NRF2_ARE_CIS up | 6.94 | 14.57 | 0.16 | |
| Androgen Receptor | OT_AR_ARSRC1_0960 | 20.1 | 12.80 | 0.48 | Fipronil: 0.44 (0.07-0.49); No LOEL Reported |
| | Tox21_AR_BLA_Antagonist_ratio | 15.5 | 13.23 | 0.37 | |
| | Tox21_AR_LUC_MDAKB2_Antagonist | 20.8 | 12.74 | 0.49 | |
| | Tox21_ARE_BLA_agonist_ratio | 3.91 | 15.53 | 0.070 | |
| Estrogen Receptor | ATG_ERa_TRANS up | 19.5 | 12.85 | 0.46 | Fipronil: 0.37 (0.053-0.48); No LOEL Reported |
| | ATG_ERE_CIS up | 3.14 | 15.90 | 0.053 | |
| | Tox21_ERa_BLA_Antagonist_ratio | 20.2 | 12.79 | 0.48 | |
| | Tox21_ERa_LUC_BG1_Antagonist | 20 | 12.80 | 0.48 | |
| Thyroid Receptor | ATG_TRHR1_TRANS up | 49.1 | 11.30 | 1.17 | Fipronil: 0.83 (0.49-1.17); No LOEL Reported |
| | Tox21_TR_LUC_GH3_Antagonist | 20.6 | 12.75 | 0.49 | |
| Endocrine Effects | Tox21_Aromatase_Inhibition | 20.2 | 12.79 | 0.48 | Fipronil: 0.48; No LOEL Reported |
| Cancer | ATG_BRE_CIS up | 26.7 | 12.32 | 0.63 | Fipronil: 0.67 (0.44-0.93); LOEL for Thyroid carcinomas: 0.06; liver carcinomas: 0.13 |
| | ATG_PPARg_TRANS up | 39.3 | 11.68 | 0.93 | |
| | ATG_RORE_CIS up | 18.6 | 12.92 | 0.44 | |
| Neuro-development | ATG_CRE_CIS up | 19 | 12.89 | 0.45 | Fipronil: 0.81 (0.45-1.17); LOEL 0.9 |
| | ATG_PAre_CIS up | 49.1 | 11.30 | 1.17 | |
| Cytotoxicity Cell Health | APR_HepG2_CellCycleArrest_24h_dn | 83.8 | 10.41 | 1.99 | Fipronil 1.84 (0.04-3.94) |
| | APR_HepG2_CellCycleArrest_72h_dn | 19 | 12.89 | 0.45 | |
| | APR_HepG2_CellLoss_24h_dn | 95.5 | 10.28 | 2.15 | |
| | APR_HepG2_CellLoss_72h_dn | 16.5 | 13.13 | 0.39 | |
| | APR_HepG2_MicrotubuleCSK_24h_up | 86.6 | 10.36 | 2.06 | |
| | APR_HepG2_MicrotubuleCSK_72h_up | 166 | 9.27 | 3.94 | |
| | APR_HepG2_MicrotubuleCSK_72h_up | 14.6 | 13.33 | 0.35 | |
| | APR_HepG2_MitoMembPot_1h_dn | 56.1 | 11.08 | 1.33 | |
| | APR_HepG2_MitoticArrest_24h_up | 96.2 | 10.18 | 2.29 | |
| | APR_HepG2_OxidativeStress_24h_up | 103 | 10.07 | 2.45 | |
| | APR_HepG2_OxidativeStress_72h_up | 37 | 11.78 | 0.88 | |
| | APR_HepG2_StressKinase_72h_dn | 107 | 10.00 | 2.54 | |
| | BSK_CASM3C_Proliferation_down | 17.8 | 13.00 | 0.42 | |
| | BSK_SAg_Proliferation_down | 12.2 | 13.63 | 0.29 | |
| | BSK_3C_Proliferation_down | 24.1 | 12.49 | 0.57 | |
| | BSK_3C_Vis_down | 32.7 | 11.98 | 0.78 | |
| | BSK_SAg_PBMCCytotoxicity_up | 3.59 | 15.67 | 0.06 | |
| | Tox21_PPARd_BLA_Agonist_viability | 34.6 | 11.89 | 0.82 | |
| | Tox21_AR_BLA_Antagonist_viability | 18.4 | 12.94 | 0.44 | |
| | Tox21_MitochondrialToxicity_ratio | 2.44 | 16.32 | 0.04 | |

**Italics and highlighted in yellow are active assays for imidacloprid and the rest are active values for fipronil. LOELs were included when the Tox-Cast assay and the in vivo endpoint were available & associated. from USEPA: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2014-0614-0003 Z-score (chemical, assay) = logAC50 (chemical, assay) – median [logAC50(chemical,cytotoxicity)] ÷ Global cytotoxicity MAD**

***ToxPi***

- ***ToxPi Scores* (Reif et al, 2010) were as follows (Figure 2): *Endosulfan reference (8.0); fipronil (6.47); methidathion (2.12) and imidacloprid (0.585)* based on relative contribution of each data domain or component (see Legend) represented by positive ToxCast assays.**
- ***Fipronil Activity:* components related to "*regulation of transcription factor activity*" (ATG assays) and "*protein stabilization*" (OT assays); assays related to components related to cell health: "*oxidative phosphorylation*," "*cell death*," "*mitochondrial depolarization*" (ATG assays)**
- ***Imidacloprid Activity:* components related to "*regulation of catalytic activity*" (NVS assays) and "*receptor binding*" (NVS)**

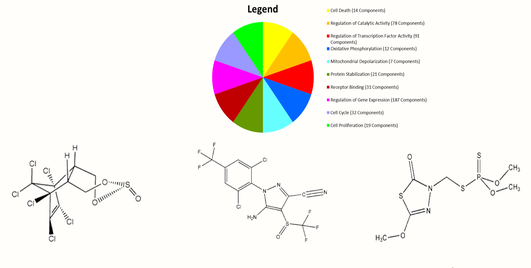

**Figure 2. Endosulfan: ToxPi Score 8.0 Fipronil: ToxPi Score 6.47 Methidathion ToxPi Score 2.123 Imidacloprid ToxPi Score 0.587**

**The comparison between Fipronil (reference compound) and Endosulfan for activity with the ER, AR and TR showed similar ToxPi scores (Figure 3): Endosulfan = 2.1; fipronil =2.76.**

**Figure 3. Fipronil: ToxPi Score 2.76 Endosulfan: ToxPi Score 2.18**

***ZEBRAFISH***

***Imidacloprid*:**

- **Chorion Removed (Truong et al, 2014): malformations of the *trunk, caudal fin and axis at 64 μM*; *mortality within 24 hours at 6.4 μM* (LOEL= 6.4 μM). The *OED (10.5 mg/kg/d) correlated fairly well with the in vivo LOEL (54.7 mg/kg/d)* from a rat developmental neurotoxicity study.**
- **Chorion Intact (Padilla et al., 2012): *Terata Score = 0* (no malformations ≤ 80 μM; dose range: 0.001-80 μM). Non-hatching and mortality not reported.**

***Fipronil*:**

- **Chorion Removed: showed *no effects at up to 64 μM***
- **Chorion Intact: *Terata Score ~ 38 at ~ 15.6 μM (OED = 0.37 mg/kg/d)* and after a single dose of *80 uM the Terata Score = 40*. The *OED (0.37 mg/kg/d) correlated well with the in vivo LOEL (0.9 mg/kg/d)* from the developmental neurotoxicity study.**
- **Stehr et al (2006) showed neurobehavioral effects after embryos were exposed to fipronil at 0.7 μM (*notochord degeneration, rostral-caudal body axis shortened, ineffective tail flips and uncoordinated muscle contractions along the body axis in response to touch*). At the *LOEL of 0.23 μM (OED = 0.0038* mg/kg/d), animals had *shortened body length and decreased swimming response to touch*.**

## DISCUSSION AND CONCLUSIONS:

- **Fipronil had lower *in vivo* NOEL/LOELs than imidacloprid for all endpoints examined.**
- **The active ToxCast assays for both compounds were good indicators for known *in vivo* endpoints (developmental effects, thyroid and liver pathology/tumors). Imidacloprid was not active in as many ToxCast assays because it is not as toxic or because of the assay design.**
- **OEDs from ToxCast assays with toxicity categories informative to *in vivo* endpoints for both compounds correlated well with *in vivo* LOELs.**
- **Z-scores for both compounds were >3 for positive assays, indicating specificity and potency for the compounds to the active assays.**
- **ToxPi: Because there were so few active ToxCast assays for imidacloprid the visual comparison with fipronil alone was not informative. When used in conjunction with endosulfan and methidathion that have modes of action and endpoints in common with imidacloprid and fipronil and many active ToxCast assays, the comparison was relevant. ToxPi identified the relative strengths of various components, associated with Tox-Cast assays for a variety of endpoints.**
- **For the GABA-gated ion channel blockers, endosulfan had a higher ToxPi Score than fipronil, indicating that for the components tested, endosulfan was more active. For the AChE affectors, methidathion had the higher ToxPi Score, compared to imidacloprid.**
- **Few assays were active in the AR, ER and TR pathways, indicating that they may not be the primary mode of developmental and reproductive toxicity. The fipronil AR antagonist pathway was considered to have weak activity based on data compiled at: http://actor.epa.gov/edsp21/**
- **The OEDs for zebrafish by the method of Truong et al. (2014) and Padilla et al (2012) correlated well with the *in vivo* LOELs for imidacloprid and fipronil, respectively. However, Stehr tested for neurobehavioral effects with fipronil by the Padilla method and obtained a much lower OED. Hence, neurobehavioral effects may be the most sensitive endpoint with zebrafish for fipronil.**


These opinions and conclusions expressed in this paper are those of the authors and do not necessarily represent the views or opinions of the Department of Pesticide Regulation, or the California Environmental Protection Agency