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Comparative Toxicity of Endosulfan and Fipronil Insecticides: Utilizing *In Vivo* and *In Vitro* Data

Marilyn H Silva and Svetlana Koshlukova

Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA, USA 95812, USA

Corresponding author: Marilyn H Silva

Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA, USA 95812, USA.

Marilyn.silva@cdpr.ca.gov

Tel: 916 324 3482

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Abstract

Background: Endosulfan, an organochlorine compound, and fipronil, a phenylpyrazole, are insecticides with a common mechanism of toxicity. They interfere with Cl^- influx by binding to the gamma-aminobutyric acid receptor ($GABA_AR$) and blocking the inhibitory actions of $GABA_A$. *In vivo* they cause neurotoxicity, hepatotoxicity, developmental toxicity, and can alter endocrine and immune systems. The thyroid is a target of fipronil toxicity. Human exposure occurs via food residues, skin contact and/or air dispersion. They are environmentally persistent and bioaccumulate in food chains.

Method: Compare *in vivo* data with *in vitro* results from the U.S. Environmental Protection Agency (USEPA) Toxicology Forecaster (ToxCast) high-throughput screening assays and zebrafish models to assess their usefulness in predicting toxicity.

Results: Fipronil's *in vivo* toxicity occurred at lower doses than endosulfan for similar effects. ToxCast was a weak predictor of liver toxicity and estrogen receptor interaction. Missing is evidence of "true actives" for fipronil ToxCast assays with the thyroid receptor and for either compound with $GABA_AR$ or androgen receptors. Zebrafish models were good predictors of endosulfan and fipronil neurotoxicity in mammalian *in vivo* studies.

Conclusion: ToxCast assays do not provide support for *in vivo* neurotoxicity or endocrine disruption where zebrafishs are good predictors of both parameters.

Key Words: Endosulfan; Fipronil; Organochlorine; GABA Inhibitors; Toxcast; Zebrafish; Carcinogens

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Introduction

Endosulfan (ES: 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5α, 6, 9, 9α-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide; [isomers: α-; β-]) and fipronil (FP: (RS)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4- (trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile) represent the first and second generations, respectively, of chloride channel blocker insecticides whose primary target is the central nervous system [1, 2]. High potential for human exposure is indicated for both pesticides. Exposure to general public results from ingesting residues in food, inhaling vapors, skin contact. Workers' exposures are mainly dermal and inhalation from handling or re-entering treated fields.A large number of poisonings are reported from fipronil's use in veterinary products for flea and tick control. The exposure is likely higher for general public living near hazardous waste sites where endosulfan has been detected [3, 4]. As noncompetitive antagonists of the $GABA_A$-receptor, they block passage of Cl^- ions and thereby the actions of the inhibitory neurotransmitter GABA [2]. In mammals, seizures, vomiting and convulsions are symptoms of excessive nerve stimulation associated with $GABA_A$ antagonism [5]. Their action in the brain is very complex due to biotransformation to toxic metabolites that can act on $GABA_AR$ (e.g., FP sulfone [6]). Each is detoxified by P450s (CYP2B6, CYP3A4-5: ES; hCYP2C9: FP) [7, 8] but neither requires activation to be toxic[8]. ES and FP are associated with endocrine disruption. FP has induced thyroid and liver cancer in animal models (http://www.cdpr.ca.gov/

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docs/risk/toxsums/toxsumlist.htm; accessed 9/2015) [9]. These well characterized *in vivo* effects provide a basis for comparing toxicities.

Another means of comparison is the U.S. Environmental Protection Agency (USEPA) Toxicology Forecaster (ToxCast) *in vitro* high-throughput screening program; profiling more than 1,000 chemicals (~ 800 assays http://actor.epa.gov/dashboard2/; accessed 9/2015). In addition, zebrafish models are a rapid *in vivo* method for assessing developmental effects [10-13]. The goal of this brief communication is to examine if ToxCast and zebrafish results were predictive of targets and activities relevant to *in vivo* ES and FP endpoints.

Method

In vivo studies

In vivo studies in **Table 1** summarize relevant neurotoxic or endocrine disrupting effects for both chemicals. No-Observed-Effect-Levels (NOEL) and Lowest-Observed-Effect-Levels (LOEL) were established by the California Department of Pesticide Regulation from guideline studies required for pesticide registration (http://www.cdpr.ca.gov/docs/risk/rcd.htm) and from open literature.

In vitro studies

We accessed the recently updated ToxCast database (http://actor.epa.gov/dashboard2/) for active assays that could inform effects manifested in overt toxicity in *in vivo* studies.

Zebrafish studies

Data from two published methods included embryos treated with chorion intact [10, 11] or with chorion removed [12, 13].

In vivo to *in vitro* extrapolation (IVIVE)

IVIVE was used to convert zebrafish NOELs expressed as µM concentrations into oral equivalent doses (OED: mg/kg/d). Estimated pharmacokinetic data derived from linear regression, published *in vitro* hepatic metabolism and protein binding with **rat data** are used for the conversion [14].

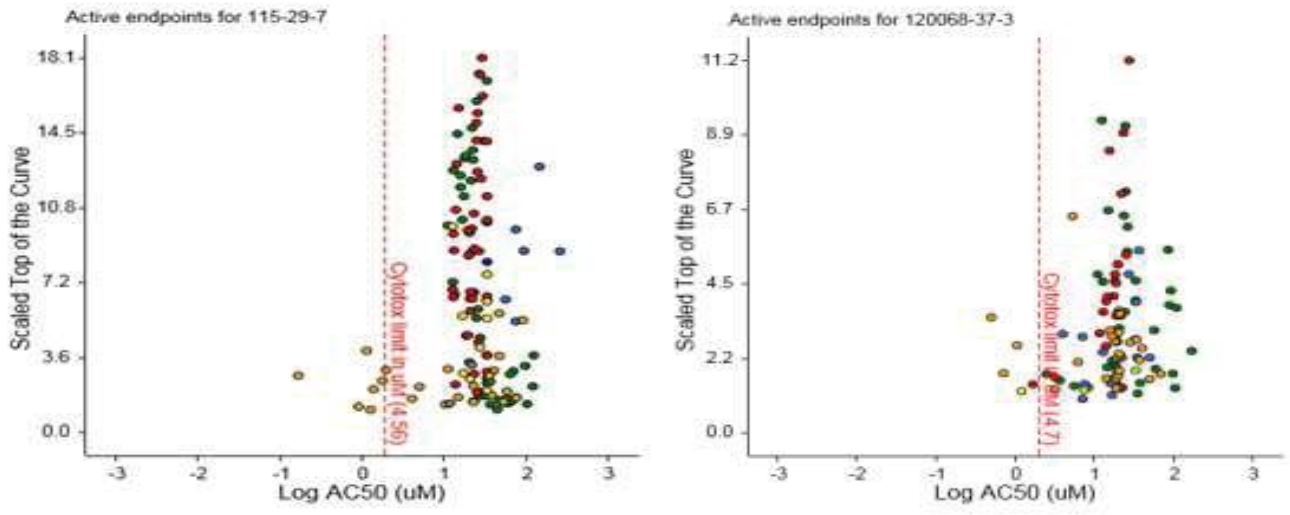
Results

In vivo

Both insecticides were neurotoxic [15, 16] with LOELs of 2-2.5 mg/kg/d in rats and dogs (**Table 1**). ES developmental effects in rat pups and fetuses occurred at up to 7-fold higher doses than FP. Potential endocrine disruption was shown in both compounds by developmental delays and skeletal variations. Liver effects in rats and mice at LOELs of 3.95 mg/kg/d (ES) and 0.13 mg/kg/d (FP) included increased liver weights and hepatocellular carcinomas in mice (FP [17]). FP thyroid toxicity was evident by altered thyroid function and follicular cell adenomas in rats (LOEL 0.06 mg/kg/d) [18].

ToxCast data

Results are reported as AC_{50}s (½ maximal activity concentrations: Dashboard: http://actor.epa.gov/dashboard/2.All active assays appear in **Figure 1** (dots) to the right and left of the cytotoxicity limit; however true actives have AC_{50}s below the cytotoxicity limits (e.g. $\leq$ 4.56 uM: ES; $\leq$ 4.7 uM FP). Active assays beyond the cytotoxicity limit may not be specific to a chemical-receptor interaction but represent a burst of cellular responses indicative of cytotoxicity. ES true actives were assays having to do with xenobiotic metabolism (human constitutive androstane receptor: hCAR; immune response: DR5; pregnane-x: affecting hCYP2B6 genes; hCYP3A4 xenobiotic response element: PXRE) and the estrogen receptor (ERα; estrogen response element: ERE). True actives for FP were related to cell adhesion molecules (kinases), hCYP2C9, xenobiotic metabolism (hCAR) and ERE. **Figure 2** shows



(A) Endosulfan (B) Fipronil

The red line indicates the region where cytotoxicity begins. True actives are to the left of that line; to the right cytotoxicity increases with increasing Log AC_{50}s. AC_{50} = activity at ½ maximal concentration.

Figure 1 ES (A) and FP (B) active ToxCast assays.

active versus inactive assays and associated components for each compound (abbreviations at: http://www.epa.gov/comptox/toxcast/data.html).

Zebrafish data

Zebrafish had an AC_{50} of 1 µM after exposure to ES (chorion intact) [10]; with a Toxicity Score (all malformations) of 40 (highest possible score) at peak concentrations (4 µM). When embryo chorions were removed, ES was highly toxic to larval development at ≥ 0.0064 µM [12]. ESα-treated embryos (chorion intact) showed neurobehavioral effects (abnormal swim behavior, disorientation, abnormal touch response) at ≥ 1.0 µM (NOEL 0.5 µM) [22].

FP-treated zebrafish (chorion intact) had an AC_{50} of 15.5 µM with a "Toxicity Score" of 40 [10]. Without the chorion, however, FP had no effects on development [10]. Zebrafish (chorion removed) treated with FP at ≥ 0.23 µM had irreversible effects on body length, notochord degeneration, abnormal axial muscle morphology, muscle fiber degeneration [11].

IVIVE for ES neurotoxicity in zebrafish (chorion removed) [13] estimated an OED of 0.019 mg/kg/d (0.5 µM NOEL) [9]; 25-fold lower than the neurotoxicity NOEL in dogs (0.5 mg/kg/d) (**Table 1**) [15]. For FP the zebrafish OED was 0.37 mg/kg/d based on neurotoxicity at 23 µM [13]. This OED is equivalent to the *in vivo* NOEL (0.2 mg/kg/d) from an acute neurotoxicity rat study [16].

Discussion/Conclusion

Data indicate that *in vivo*, FP's toxicity occurred at lower doses than ES (**Table 1**) for parameters examined. It is classified as a "possible human carcinogen" based on thyroid tumors in rat. ToxCast true actives for CYPs (ES: hCYP2B6 & hCYP3A4; FP: hCYP2C9) correlated well with *in vivo* toxicity [7, 8]. Other true activities for ES and FP were for generalized liver enzyme activity, which could be predictive of liver toxicity observed *in vivo*.

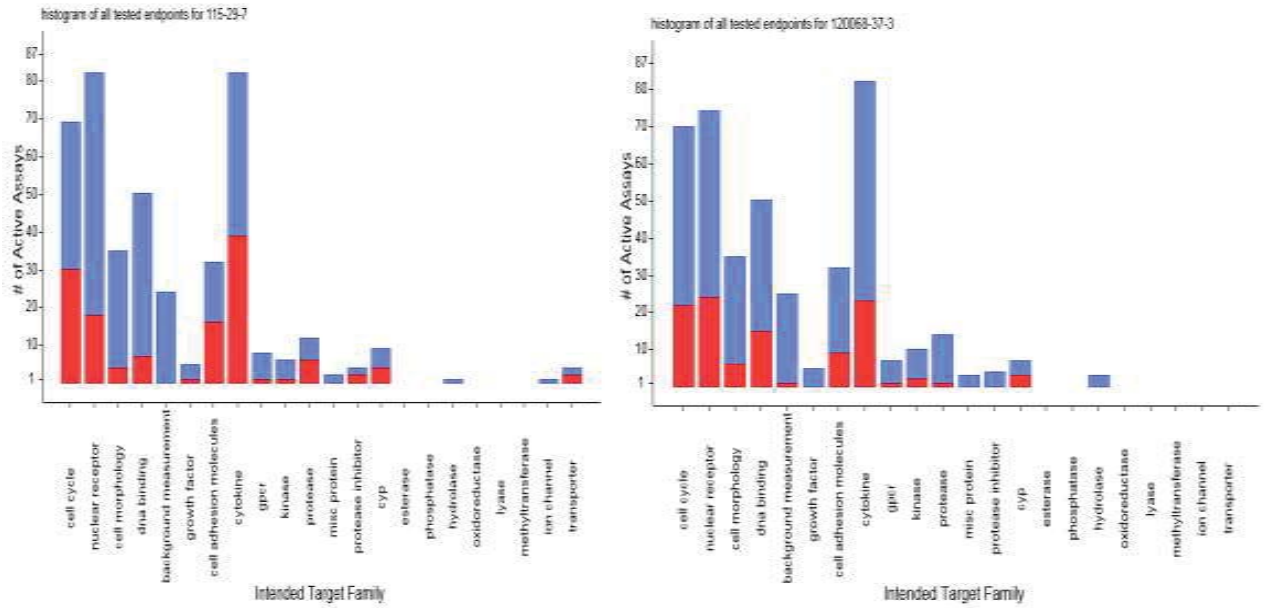
Missing were true actives for the $GABA_AR$ ToxCast assays (0/5 total) for either compound. Since neither compound needs metabolic activation to be toxic, the inactivity could be due to a lack of adequate assay design.

ES had 2/18 total true estrogen-receptor actives and FP had 1/18. There were no true actives for the androgen receptor (0/11 total) and (especially for FP) the thyroid receptor (0/4 total). There was activity for ER, AR and TR (http://actor.epa.gov/dashboard/2) but only above the cytotoxicity limit). There was poor correlation between *in vivo* endocrine disruption from both chemicals and true actives with ER, AR and TR in ToxCast.

Zebrafish assays were useful for predicting developmental neurotoxicity. The OEDs reasonably correlated with NOELs observed *in vivo*: (ES: 25 fold difference; FP equivalent). With the continued rapid development of the ToxCast and zebrafish assays, they could be used to support modes of action and adverse outcome pathways for new chemicals.

Disclaimer

The 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. The authors state that their design and interpretation is not compromised by any sponsor as a condition of review and publication.



(A) Endosulfan (A) Endosulfan

Red indicates assays considered active and blue is for inactives.

Figure 2 Proportion of active to inactive ToxCast assays by parameter measured.

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Table 1 Toxicity Reported in California Department of Pesticide Regulation Risk Assessment Documents and the Open Literature[a] [15-23].

Toxicity Endpoint	Endosulfan[a]	NOEL/LOEL[b] mg/kg/d	Fipronil[a]	NOEL/LOEL[b] mg/kg/d	Fold Difference Endo:Fipronil[c]
Neurotoxicity[d]	**Dog:** Jerky/tonic muscle contractions, convulsive movements, noise sensitivity, frightened reactions to optical stimuli, impaired reflex excitability	**Dog:** 0.5/2.0[d] [15]	**Rat adult:** Exaggerated startle & tail-pinch response; convulsions, tremors, ↓vision; ↓ hindleg landing splay, ↓ rearing	**Rat:** 0.2/2.5[16]	↓1.0
DNT[e] **i.p.**	**Rat pup:** ↓ neurotransmitter binding; ↑foot-shock fighting behavior	**Rat pup:** 1.0/4 [19]	**Rat pup:** ↓Startle response; ↓ability to swim (unable to stay afloat, swim in straight line, or keep heads out of water)	**Rat pup:** 0.05/0.9[22]	↑4.0
Thyroid Pathology/ tumors[d]	**No effects in any study**	**Not Applicable**	**Rat adult:** follicular cell hypertrophy & hyperplasia; altered thyroid function (↓T4, ↑TSH); ↑thyroid weight; ↑follicular cell adenomas and carcinomas	**Rat:** 0.02/0.06[17]	**Not Applicable**
Liver Pathology / tumors[d]	**Rat adult:** ↑liver weight	**Rat adult:** 1.92/3.95[20]	**Mouse adult:** ↑liver weight; periacinar vacuolation; hepato carcinomas	**Mouse:** 0.06/0.13[18]	↑30
Developmental[d]	**Rat fetus:** ↓bodyweight, ↓ %live fetuses & length; ↑growth retardation, skeletal anomalies, % resorptions	**Rat adult:** 2.0/6.0[21]	**Rat fetus:** ↓litter survival, pup viability; delayed pinna attachment, incisor eruption, vaginal patency & preputial separation	**Rat:** 0.05/0.9[23]	↑7.0

a-Lowest No-Observed-Effect-Level (NOEL) for each category.
b-NOEL/Lowest-Observed-Effect-Level (LOEL)
c-Fold difference between the lowest LOELs (where effects are observed at the lowest dose administered): Fold difference = endosulfan LOEL : fipronil LOEL
d-Endosulfan or fipronil administered orally
e- Developmental Neurotoxicity; i.p. = intraperitoneal administration of endosulfan; oral administration of fipronil

References

1 Meister R, Sine C (2014) MeisterPRO Crop Protection Handbook. MeisterMedia 100: 274.

2 Casida JE (1993) Insecticide action at the GABA-gated chloride channel: Recognition, progress and prospects. Arch Insect Biochem Physiol 22: 13-23.

3 Agency for Toxic Substances and Disease Registry (2015) Toxicological profile for Endosulfan. Public Health Service, U.S. Department Of Health And Human Services.

4 USEPA (2011) Fipronil: Review of Human Incidents. Office of Prevention, Pesticides And Toxic Substances. United States Environmental Protection Agency

5 Kamijima M, Casida JE (2000) Regional modification of [3H] ethynylbicycloorthobenzoate binding in mouse brain GABAAreceptor by endosulfan, fipronil and avermectin B1a. Toxicol Appl Pharm 163:188-194.

6 Hainzl D, Cole LM, Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem Res Toxicol 11:1529–1535.

7 Lee, HK, Moon JK, Chang CH, Choi H, Park HW, et al. (2006) Stereoselective metabolism of endosulfan by human liver microsomes and human cytochrome P450 isoforms. Drug Metab Dispos 34: 1090-1095.

8 Tang J, Usmani A, Hodgson E, Rose RL (2004) In vitro metabolism of fipronil by human and rat cytochrome P450 and its interactions with testosterone and diazepam. Chemico-Biological Interactions 147: 319–329.

9 Silva M, Pham N, Lewis C, Iyer S, kwok E, et al. (2015) A Comparison of ToxCast Test Results with In Vivo and Other In Vitro Endpoints for Neuro, Endocrine, and Developmental Toxicities: A Case Study Using Endosulfan and Methidathion. Birth Defects Res:B 104: 71–89.

10 Padilla S, Corum D, Padnos B, Hunter DL, Beam A, et al. (2012) Zebrafish developmental screening of the ToxCast Phase I chemical library. Reprod Toxicol 33: 174-187.

11 Stehr CM, Linbo TL, Incardona JP, Scho NL (2006) The Developmental Neurotoxicity of Fipronil: Notochord Degeneration and Locomotor Defects in Zebrafish Embryos and Larvae. Toxicol Sci 92:270–278.

12 Truong L, Reif DM, Mary LS, Geier MC, Truong HD, et al. (2014) Multidimensional in vivo hazard assessment using zebrafish. Toxicol Sci 137: 212-233.

13 Stanley KA, Curtis LR, Massey-Simonich SL, Tanguay RL (2009) Endosulfan I and endosulfan sulfate disrupts zebrafish embryonic development. Aquat Toxicol 95: 355-361.

14 Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, et al. (2012) Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol Sci 125: 157-174.

15 Testing for toxicity by repeated oral administration (1-year feeding study) to Beagle dogs (1989) CDPR.

16 Exposure Peroral (Gavage) Neurotoxicity Study in Sprague Dawley Rats (1993) CDPR.

17 Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks (1993) CDPR.

18 Oncogenicity study by dietary administration to CD-1 mice for 78 weeks (1993) CDPR.

19 Seth PK, Saidi NF, Agrawal AK, Anand M (1986) Neurotoxicity of endosulfan in young and adult rats. Neurotoxicology 7: 623-635.

20 13-Week toxicity study in rats followed by a 4-week withdrawal period (final report) (1985) CDPR.

21 Teratology study with FMC rats (1980) CDPR.

22 A Developmental Neurotoxicity Study of Fipronil in the Rat Via Dietary Administration (1995) CDPR.

23 Developmental toxicology study in the rat by gavage (1998) CDPR.

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