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A systems biology approach to predictive developmental neurotoxicity of a larvicide used in the prevention of Zika virus transmission

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Abstract

The need to prevent developmental brain disorders has led to an increased interest in efficient neurotoxicity testing. When an epidemic of microcephaly occurred in Brazil, Zika virus infection was soon identified as the likely culprit. However, the pathogenesis appeared to be complex, and a larvicide used to control mosquitoes responsible for transmission of the virus was soon suggested as an important causative factor. Yet, it is challenging to identify relevant and efficient tests that are also in line with ethical research defined by the 3Rs rule (Replacement, Reduction and Refinement). Especially in an acute situation like the microcephaly epidemic, where little toxicity documentation is available, new and innovative alternative methods, whether *in vitro* or *in silico*, must be considered. We have developed a network-based model using an integrative systems biology approach to explore the potential developmental neurotoxicity, and we applied this method to examine the larvicide pyriproxyfen widely used in the prevention of Zika virus transmission. Our computational model covered a wide range of possible pathways providing mechanistic hypotheses between pyriproxyfen and neurological disorders via protein complexes, thus adding to the plausibility of pyriproxyfen neurotoxicity. Although providing only tentative evidence and comparisons with retinoic acid, our computational systems biology approach is rapid and inexpensive. The case study of pyriproxyfen illustrates its usefulness as an initial or screening step in the assessment of toxicity potentials of chemicals with incompletely known toxic properties.

Keywords

computational biology; developmental neurotoxicity; pesticide; pyriproxyfen; predictive toxicology; systems biology; toxicity testing

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1. Introduction

Neurodevelopmental deficits affect a large proportion of births (Adams et al., 2013), and prevalence rates of learning disabilities, behavioral disorders, and other adverse outcomes appear to be increasing (Landrigan et al., 2012). Even subclinical decrements in brain function can have severe consequences (Bellinger, 2009), as they diminish quality of life, reduce academic achievement, and disturb behaviors, with profound detriments for the welfare and productivity of entire societies (Gould, 2009). While the etiologies of developmental neurotoxicity (DNT) etiologies are poorly known, recent studies have pointed at pesticides as the largest group of industrial chemicals suspected of causing DNT (Grandjean and Landrigan, 2006)(Grandjean and Landrigan, 2014). A more vigilant attitude toward DNT testing is justified by the developing human brain being exquisitely vulnerable to toxic chemical exposures, with major windows of developmental vulnerability *in utero* and during infancy and early childhood (Rice and Barone, 2000).

Because many pesticides are designed to cause adverse effects in the nervous system of pest species, these chemicals are suspected of contributing to neurobehavioral effects in humans (Bjørning-Poulsen et al., 2008). The most recently updated list from 2014 included two pesticides, DDT/DDE and chlorpyrifos, as known human developmental neurotoxicants (Grandjean and Landrigan, 2014). Since then, convincing evidence has been published that warrants inclusion of another pesticide, kepone (Boucher et al., 2013). Increasing evidence on, e.g., pyrethroids (Viel et al., 2015) and thyroid disrupting pesticides (Freire et al., 2013), suggests that available epidemiological evidence tends to underestimate the risks to brain development due to pesticide exposure.

When an epidemic of microcephaly occurred in Brazil, Zika virus (ZKV) was soon identified as the likely culprit. Infection in early pregnancy appeared to damage the brain development in the fetus. However, ZKV infection could not be detected in all microcephaly cases, and the infection did not uniformly result in the congenital malformation (Albuquerque et al., 2016; Evans, 2016). Thus, the pathogenesis appeared to be complex (Rao et al., 2017). The evidence may be of more general relevance, as increasing prevalence of microcephaly has also been observed in Europe (Morris et al., 2016), although the causes are unknown. In the affected areas in Brazil, pyriproxyfen (PPF) larvicide is widely used to control mosquitoes responsible for transmission of the virus (Vazquez, 2016). Given that PPF is sprayed on water bodies and reservoirs to kill mosquito larvae, human exposures are suspected.

Available test data provided little evidence on possible DNT risks from PPF, but some expert evaluations concluded that little if any risk was present (European Food Safety Authority, 2014; World Health Organization, 2016). Other experts recommended further toxicology studies to fill the substantial gaps in evidence on teratogenic risks (Swedish Toxicology Sciences Research Center, 2016).

PPF acts as a hormone analogue of insect juvenile hormone (Harmon et al., 1995; World Health Organization, 2004). Furthermore, interaction with the mammalian retinoid X receptor and interference with retinoic acid (RA) metabolism have been reported (European

Food Safety Authority, 2009). PPF has been tested mostly on developmental toxicity in rats and rabbits (World Health Organization, 2004). According to the producer, PPF tests showed no effects on the reproductive system or nervous system in mammals (Evans, 2016). However, a 1988 test report notes one case of arhinencephaly and decreased brain weights in male pups at a dose of 300 mg/kg of body weight per day, although brain weight changes were not significant in 10 pups at 1,000 mg/kg, the highest dose tested (Saegusa, 1988; Evans, 2016). Further, some of PPF's terpenoid analogues bind to the mammalian RXR and appear to cause developmental toxicity (Harmon et al., 1995) including microcephaly (Benke, 1984)(Kam et al., 2012), which has also been reported after excess intake of retinoid acid (RA) (Rothman et al., 1995). Accordingly, PPF could conceivably act as a co-factor in the microcephaly epidemic and this possibility would deserve further exploration.

Although there is a desire to understand the possible DNT effects of PPF, it is challenging to identify reliable and efficient tests in line with the EU-promoted 3Rs rule (Replacement, Reduction and Refinement), i.e., a guideline for promoting ethical research and testing of animals. This is also true in a wider perspective, given the overall magnitude of the public health problem that brain disorders represent. This challenge is of particular relevance in an acute situation like the microcephaly epidemic, where insufficient toxicity documentation is available on a widely-used pesticide. In response to the calls for further testing, so far a zebrafish assay has been carried out, without revealing signs of DNT (Dzieciolowska et al., 2017).

In this perspective, alternative methods such as *in vitro* and *in silico* tests would be highly attractive within an adverse outcome pathway (AOP) framework (Crofton et al., 2014) (Tohyama, 2016). A recent approach illustrates how *in vitro* and *in silico* data can be rapidly applied to predict potential human health risks (Sipes et al., 2017), thus allowing rapid estimation of plausible biological interactions.

Computational predictive approaches are highly attractive alternative, as they can handle and integrate massive amounts and diverse types of information. In its report on Toxicity Testing in the 21st Century, the National Research Council already 10 years ago called for the development of new approaches to human health risk assessment that would rely, in part, on computer-based models, rather than animal testing and epidemiology (Krewski et al., 2010). The U.S. EPA (Knudsen et al., 2013) reiterated the recommendation that *in silico* approaches be included in future assessments of toxicity. Still, progress has been slow, although *in vitro* screening programs have been initiated, e.g., the U.S. EPA ToxCast high-throughput screening assays program, where so far 2,000 chemicals have been examined in at least 700 assays (Silva et al., 2015). Likewise, while the 'Tox21 robot' project (Tice et al., 2013) has screened nearly 10,000 chemicals on selected assays. Unfortunately, while systematic DNT testing has been called for (Bjørning-Poulsen et al., 2008), ToxCast and other databases are still limited in this regard, as a primary focus is on endocrine endpoints and reproductive functions.

To the advantage of computational methods, toxicological and chemical databases have expanded substantially during recent years, thereby adding to the reliability of *in silico* approaches to toxicity assessment (Knudsen et al., 2013)(Kongsbak et al., 2014). Through

international efforts, publicly available databases can now be combined and fine-tuned to provide linked information on chemical name, synonym, chemical structure, hazard, chemical exposure and potential risk to human health within different categories. The categories include: acute, developmental toxicity, reproductive toxicity and carcinogenic potential (Judson et al., 2008). For example, ChemProt, a disease chemical biology database, provides information on chemical links to proteins along with their name, structure and related disease in addition to other adverse outcomes (Kim Kjærulff et al., 2013). All these data resources can now be used to develop computational models for the purpose of predicting toxicological endpoints and possible biological mechanisms related to neurotoxicity, e.g., associated with pesticides.

Such computational systems and toxicology approaches therefore offer promising guidance for future research on the etiology and pathogenesis of preventable brain diseases and dysfunctions. In particular, *in silico* methods can serve as part of a tiered screening system before targeted DNT *in vivo* tests are carried out. In this way, a computational integrative strategy may contribute to the reduction of the use of animals in chemical testing regimens. Many computational models developed so far are chemical structure-based. In these models, chemicals are grouped together according to their structural features or those of their metabolites for possible linkage to a particular toxicity endpoint (e.g., ToxMatch (Patlewicz et al., 2008), LHASA). In some of these adaptations, quantitative structure activity relationship approaches (QSAR) are used as CAESAR (Cassano et al., 2010)(Nicolotti et al., 2014)(Benfenati et al., 2013) or read-across. In parallel, systems biology modeling has emerged (Audouze et al., 2010) (Audouze et al., 2013) (Kongsbak et al., 2014).

As a proof-of-concept, such integrative approaches have been applied to the persistent organic pesticide, DDT and its metabolites to identify the main culprits responsible for disease associations. Additionally, a network of complex diseases has been developed to integrate molecular pathways associated to both genetic and environmental factors (Gohlke et al., 2009). Recently a human environmental disease network allowed exploration of common mechanisms of diseases associated with chemical exposure (Taboureau and Audouze, 2017). One of the strengths of the systems biology approach is that the focus can be directed exclusively towards human effects, avoiding the challenge of inter-species extrapolation.

Given this background and the paucity of experimental data on PPF, a computational systems biology study was carried out to explore chemical affinities that could reveal associations with relevant diseases. This predictive toxicology approach is based on existing technologies and biological data integration (including the updated ToxCast database) (Richard et al., 2016) that have recently been exploited to predict potential pesticide toxicities, as outlined below (Fig. 1) (Audouze et al., 2013)(Kongsbak et al., 2014). We have previously highlighted the feasibility of this approach, allowing for its limitations, and we now apply the methodology to the apparent uncertainty regarding microcephaly pathogenesis in the presence of ZKV transmission and the concomitant insecticide interventions.

2. Materials and methods

Due to the known adverse effects of RA and the suspected interaction of PPF with the human retinoic acid receptor, we performed a computational systems toxicological study to explore potential toxic effects of PPF, as compared to RA. The approach is illustrated in Fig. 1. In the first step we used existing knowledge of high throughput chemical screening data compiled in the ToxCast database (ToxCast) (Richard et al., 2016). Specific information regarding human RA-proteins and human PPF-proteins was extracted for the purposes of our study. In addition, only the proteins defined as “active” by ToxCast were kept. The protein lists were compared, and proteins common to both substances were further explored to decipher potential similar modes of action between RA and PPF by integrating protein-protein interactions and protein-pathway annotations. Subsequently, protein-disease annotations were integrated within the common protein lists to rank statistically the RA and PPF connections to neurological disorders.

2.1 Chemical-protein associations

Human proteins interacting with both PPF and RA were extracted from the ToxCast chemical library, which includes the U.S. EPA’s test results and other contributions to the Tox21 database (as of the May, 2017 version)(Richard et al., 2016). As of today, the ToxCast database contains information of high throughput assays with the aim to inform chemical safety decisions. The version used for the proposed study has information on over 9,000 chemicals and data on 1,081 different bioassay endpoint components.

2.2 Protein-protein interactions

Since proteins tend to function in complexes, an important step was to verify that among the selected proteins, some of them could directly interact, and then form protein complexes that can be considered for brain disorders enrichment analysis. Considering the common list of proteins and using the STRING database (version 10.5) (Szklarczyk et al., 2017), which is a high-confidence protein-protein association-based network source of information, we showed that the proteins mimic the true biology and therefore could be considered as a protein complex. A clustering analysis was performed using MCL algorithm to group the proteins within the network (using an inflation parameters of 10) based on the distance matrix obtained from the String database (the string global scores). The protein complex was then tested for significant pathway associations and disease annotations related to brain disorders by biological enrichment. A similar enrichment analysis was performed with gene ontology terms, i.e. biological process, molecular function and cellular component, in order to reveal significant related functions of gene sets.

2.3 Pathways and disease enrichment analysis

To identify pathways potentially related to RA and PPF, we integrated protein-pathways information from the KEGG database (as of June, 2016) (Kanehisa et al., 2017). Three major sources of protein-disease information were also integrated independently into the protein complex (all accessed as of June, 2016): the GeneCards database (Safran et al., 2010), the DisGeNET database(Piñero et al., 2015), and the Comparative Toxicogenomics Database (CTD)(Davis et al., 2016).

The GeneCards database(Safran et al., 2010) contains manually curated information about drugs and environmental chemicals and their associations to genes and proteins. The current version has information on 34,216 curated genes/proteins-diseases associations.

The DisGenNET database(Piñero et al., 2015) is a discovery platform integrating information on gene-disease associations from several public data sources and the literature. The current version (DisGeNET v4.0) contains 429,036 associations between 17,381 genes and 15,093 diseases.

The CTD(Davis et al., 2016) is a database providing manually curated information about chemical-gene/protein (1,518,440 unique associations for all organisms covered) and disease-gene/protein information (34,216 curated associations).

All diseases were kept for the analysis, but only central nervous system disorders outcomes were considered for the interpretation. All three sources of disease information provided complementary information, and the proteins associated with particular brain disorders were only partially overlapping.

To assess the relevance of protein-pathway and disease relationships, each data source was individually studied for statistical significance based on the hypergeometric distribution. A significance level of 0.05 after Bonferroni correction for multiple testing of the p values was used to select the most relevant associations. To explore unexpected RA- and PPF-neurological associations (via pathways and diseases that are likely affected by incomplete data), we also report non-significant associations. A flow chart of the pipeline applied in the analysis is represented in Figure 2.

3. Results

3.1 Protein lists

From the ToxCast database, PPF and RA are active on 72 and 145 assays, respectively. Filtering on human proteins, PPF and RA were active on 43 and 75 human proteins, respectively, of which 28 proteins showed affinities at the micromolar scale for both compounds (Table 1, Table S1 and Fig. 3). Thus, PPF and RA may both affect the activity of several important proteins, including nuclear receptors (Table S2), which, at different levels of evidence, are linked to teratogenicity, developmental toxicity as well as changes in neuroprotective functions (Leung et al., 2016). Among notable proteins identified, CD40 is a member of the tumor necrosis factor receptor superfamily (TNFRSF) that modulates neuronal differentiation (Hou et al., 2008).

3.2 Protein-protein interactions (PPIs)

The protein list was used to generate a PPIs network by determining PPI first-order partners for each of the 28 proteins as well as the connections between the 28 proteins themselves. All evidence was taken into consideration, i.e., from text mining, experimental results, databases, co-expression, gene-fusion and co-occurrence using as a minimum a median confidence score of 0.4 for the interaction and a maximum of 20 interactors (Szkarczyk et al., 2017). A protein complex of 48 nodes was obtained, with 296 edges. The input list was

therefore enriched with 20 proteins. Nevertheless, it appears that the 28 proteins have more interactions among themselves than typically expected for a random set of proteins, thus indicating that these proteins as a group are at least partially biologically connected.

We then investigated the protein complex in term of biological relevance for each of the Gene Ontology categories (GO): Biological Process, Cellular Component and Molecular Function (Fig. 4). The results show that 32 of the 48 proteins are part of developmental processes in the biological process category.

3.3 Translation into pathways associations

Pathway enrichment analyses using the KEGG database (Kanehisa et al., 2017) reveal that some of the proteins are highly associated with pathways relevant to brain development as well as other signaling pathways (Table S3). For example, among the most significant enrichments, the thyroid hormone signaling pathway (hsa04919) appears ($p = 2.31\text{e-}06$).

Other interesting pathways appear on top of the list as the cell adhesion molecules pathway (CAMs) (hsa04024) ($p = 3.31\text{e-}04$) and the PPAR signaling pathway (hsa03320) ($p = 8.15\text{e-}04$). The CAMs pathway is involved in neuronal cell adhesion and dysfunction and is related to neurodevelopmental brain disorders (O'Dushlaine et al., 2011). The PPAR signaling pathway is known to be expressed in placental development and to be involved in brain functions and neurodegenerative diseases (Torres-Espinola et al., 2015).

3.4 Translation into disease associations

Disease enrichment analysis was then performed using three sources of information, each of them providing different levels of details. From the GeneCards database, atherosclerosis was the most significant disease (even if not related to brain disorders (corrected $p = 4.3\text{e-}06$). When looking at related brain disorders, some were identified as viral encephalitis and spinal stenosis, but were not statistically significant. Other proteins were uniquely, though less clearly associated with brain diseases, thus arguing against synergistic effects of PPF and RA (Table S4).

From the DisGeNET database, six proteins (AhR, EGR1, CXCR3, PLAUR, PPARA, PPARG) were highly correlated with inflammation ($p = 1.7\text{e-}05$). Maternal inflammation has recently been reported to affect placental functions leading to an increased risk of neurodevelopmental disorders in the offspring (Goeden et al., 2016). Other outcomes, such as peripheral nervous system diseases, Alzheimer's disease, and autistic disorders were also identified (Table S4).

From the Comparative Toxicogenomics Database (CTD), the disease ontology is less specific, while linking more proteins to the diseases. Among nervous system diseases, several are significantly associated to proteins, e.g., demyelinating autoimmune disease (corrected $p = 1.46\text{e-}10$), brain diseases ($2.51\text{e-}12$) and neurodegenerative diseases ($7.81\text{e-}07$), with a total of 7, 7 and 10 proteins, respectively (Table 2). Among the predicted diseases at high statistical significance, demyelinating diseases include acute disseminated encephalomyelitis, an immune and inflammatory-mediated CNS disorder with predilection to early childhood and multiple sclerosis. In addition, predisposition to viral infections can

also lead to demyelinating diseases, although they appear not to be directly related to PPF and RA in this analysis.

As a complement, we then tested the complex of 48 proteins for associations with the disease 'atherosclerosis' using the GeneCards database. A total of 59 out of the 9,048 proteins in GeneCards were linked to the disease, and among the 48 proteins associated with RA and PPF, 45 were retrieved in GeneCards and 8 were associated with the disease. When considering all diseases in the GeneCards database, these findings were associated with a p value of $3.57\text{e-}10$ or, after Bonferroni adjustment, $4.3\text{e-}06$.

Relying on information from the combined data sources, the integrative systems biology analysis shows that proteins from the protein complex linked to both PPF and RA are associated directly or indirectly with brain disorders, thereby supporting the plausibility of a hypothesis of PPF neurotoxicity, given that RA has been reported to possess such potentials (Fig. 2). The findings obtained from the CTD database are more comprehensive than those from the other two data sources. The p values are lower, although the connection to disease etiologies is less specific. In the GeneCards and DisGenNET databases, the p values are weaker, most likely due to incomplete affinity information.

Although the findings of the present study are tentative only, and do not provide firm evidence of definite interaction of PPF with mechanisms involved in the generation of microcephaly, the results from three different data sets suggest clear parallels with RA as a known nervous system teratogen.

Finally, as ZKV infection of human cortical neural precursor cells results in gene expression changes (Tang, 2016), we were able to compare our list of 48 proteins affected by PPF and retinoic acid with the genes shown to be deregulated by ZKV, thus making it possible to identify similarities that might suggest potentially common mechanisms of action that could be involved in the generation of microcephaly. Based on the RNA seq data derived from the ZKV-infected human cells (Wang and Ma'ayan, 2016), we found nine proteins (EP300 and CREBBP, CCND1, MMP2, PLA2, VCAM1, JUN, FOS, EGR1), all of which are perturbed by PPF, retinoic acid as well as ZKV. The proteins derived from these genes are associated with nervous system development and brain morphology and may therefore point to common molecular processes associated with the microcephaly phenotype.

4. Discussion

The acute situation with widespread increases in microcephaly incidence and the possible causative role of a pesticide highlighted the need for a rapid response to elucidate this possibility. Although neurotoxicity due to PPF was dismissed by some authorities, the fact that ZKV infection appeared not to explain the full extent of the microcephaly epidemic malformation (Albuquerque et al., 2016; Evans, 2016). suggested that the possibility of PPF toxicity needed to be explored. We chose to apply an *in silico* approach to elucidate the DNT potential. The findings reported in this article appear highly relevant to the risk of microcephaly linked to ZKV infection during pregnancy but also potentially associated with concomitant or prior larvicide exposure (Bar-Yam, 2016). An increased incidence of this

particular malformation by itself would be unlikely to be caused by a teratogenic chemical, where the dose would be expected to affect the severity of the outcome. However, recent data on congenital outcomes associated with intrauterine ZKV infection suggest a more complex picture with a variety of neurobehavioral deficits as well as more pervasive adverse effects (Costello et al., 2016). Accordingly, the emerging clinical appearance of the neonates with possible intrauterine ZKV infection now seems to better resemble a range of adverse effects that could plausibly be produced by or aggravated by exposures to toxicants, such as pesticides (Rizzati et al., 2016). Still, in the absence of a characteristic clinical picture or of pathognomonic signs, the attribution to a particular cause is problematic.

The parallels identified between PPF and RA are supported by experimental studies that show similarities in biochemical effects associated with several terpenoids. Unfortunately, the majority of assays where PPF and RA have been tested in ToxCast are based on hepatocytes or kidney cell lines and not neuronal cells. Thus, the assumption had to be made that the bioactivity gathered from ToxCast would apply also to the central nervous system. Also, the cytotoxicity has not been considered, although PPF and RA could potentially be cytotoxic at concentrations showing activity for the protein targets.

Overall, the protein interactions suggest a potential role in brain diseases and nervous system dysfunctions. The notion that PPF could be potentially involved in brain disorders via adverse outcome pathways similar to those of RA (Kam et al., 2012) may be of particular relevance in a country like Brazil, where vitamin A deficiency is an important public health concern (Fernandes et al., 2014). Perhaps more importantly, the overlap in proteins affected by ZKV in human neural progenitor cells and those potentially influenced by PPF supports the existence of some parallels affecting neurodevelopment, although the involvement of the proteins in microcephaly formation is not clear.

The scant evidence on PPF toxicity illustrates a general complication that only a few pesticides have been properly tested for developmental neurotoxicity (Bjørling-Poulsen et al., 2008). Still, substantial epidemiological evidence suggests that some pesticides contribute to a silent pandemic of neurotoxicity (Grandjean and Landrigan, 2014). Thus, the general paucity of toxicological data hampers a much-needed health risk evaluation and the search for the causation, especially in cases such as the recent surge in microcephaly and associated adverse effects. Although the possible role of larvicides in this regard remains uncertain, the present study suggests that the greatly increased use of PPF and other larvicides in connection with ZKV and other arbovirus epidemics (Evans, 2016) should be accompanied by further DNT testing.

The present study further illustrates the usefulness of computational systems toxicological approaches in situations with an acute need for identifying potential causation and distinguishing between possible candidates. The *in silico* approach can be easily applied as a tool to identify specific adverse outcome pathways for suspected culprits that could then be explored further, e.g., by high through-put toxicological screening tests (Audouze et al., 2013) (McPartland et al., 2017).

In conclusion, the proposed approach of comparing protein affinities and disease associations between related substances with known and unknown toxicity appears promising as an initial screening step. While the validity of computational system toxicological methods depends on the availability of affinity data and the linkage to clinical outcomes, our findings more specifically support the need to adequately test PPF for developmental neurotoxicity. It would be unfortunate if mosquito eradication efforts to counteract ZKV transmission result in adverse effects similar to those that we aim to prevent. This conundrum is particularly relevant as some pesticide applications appear to have insufficient effect on mosquito populations (Bowman et al., 2016)(Haug et al., 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Workflow of the systems toxicology approach

Human proteins (P) interacting with both retinoic acid (RA) and pyriproxyfen (PPF) were extracted from the ToxCast database (arrow 1). The list of 28 proteins was enriched with protein-protein first order interactors to form a complex of 48 proteins. Biological enrichment was performed for diseases (GeneCards, CTD, DisGeNET) (arrow 2) and pathways (KEGG) (arrow 3) for the protein complex. The links between RA and PPF in regard to potential neurological phenotypic outcomes is ranked by their statistical significance.

Fig. 2. Flow chart of the approach considered for this study

1) Extraction of the active biological assays for the compounds pyriproxyfen and retinoic acid using the ToxCast database. 2) Selection of the unique proteins involved within these assays, for both compounds. 3) Integration of protein-protein interactions known to interact with the overlapping 28 proteins between both compounds using the data source STRING. 4) To investigate potential linkage(s) between these compounds and neurological outcomes, statistical analysis have been performed on the set of proteins and biological pathways, diseases, biological processes and functions.

Fig. 3. Human protein-disease network for retinoic acid (RA) and pyriproxyfen (PPF)
View of proteins (green) known to be associated with RA and PPF (grey), according to data extracted from the ToxCast database. Neurologically related diseases (blue) were identified from the biological enrichment of the protein complex using the Comparative Toxicogenomics Database.

Fig. 4. Representation of the three Gene Ontologies (GO) categories for the proteins common to RA and PPF

The height of the bar represents the number of genes present from the 48 genes. The categories are defined as followed: in red the biological processes, in blue the cellular component and in green the molecular function.

Table 1
List of overlapping proteins associated with pyriproxyfen (PPF) and retinoic acid (RA)

Data from ToxCast Database (as of May, 2017).

Gene Symbol	Gene Name
AHR	aryl hydrocarbon receptor
AR	androgen receptor
CD40	CD40 molecule, TNF receptor superfamily member 5
CREB	cAMP responsive element binding protein 1
CXCL9	chemokine (C-X-C motif) ligand 9
EGR1	early growth response 1
ESR1	estrogen receptor 1
HLA-DRA	major histocompatibility complex, class II, DR alpha
MTF1	metal-regulatory transcription factor 1
MMP	TIMP metalloproteinase inhibitor
NR1H2	nuclear receptor subfamily 1, group H, member 2
NR1H3	nuclear receptor subfamily 1, group H, member 3
NR1H4	nuclear receptor subfamily 1, group H, member 4
NR1I2	nuclear receptor subfamily 1, group I, member 2
NR1I3	nuclear receptor subfamily 1, group I, member 3
NR4A2	nuclear receptor subfamily 4, group A, member 2
PLAUR	plasminogen activator, urokinase receptor
POU2F1	POU class 2 homeobox 1
PPARA	peroxisome proliferator-activated receptor alpha
PPARD	peroxisome proliferator-activated receptor delta
PPARG	peroxisome proliferator-activated receptor gamma
PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa
RORA	RAR-related orphan receptor A
RORB	RAR-related orphan receptor B
RORC	RAR-related orphan receptor C
SELE	selectin E
VCAM1	vascular cell adhesion molecule 1
VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor

Table 2
Diseases, from the nervous systems category, extracted after disease enrichment for the complex of 48 proteins
 Disease enrichment was performed using the CTD database (as of May, 2017).

Disease name	Disease ID	P-value	Corrected p-value	Protein number
Nervous System Diseases	MESH:D009422	4,71E-20	3,17E-17	26
Central Nervous System Diseases	MESH:D002493	1,78E-18	1,20E-15	20
Brain Diseases	MESH:D001927	3,74E-15	2,51E-12	17
Demylinating Autoimmune Diseases, CNS	MESH:D020278	2,18E-13	1,46E-10	7
Autoimmune Diseases of the Nervous System	MESH:D020274	2,05E-12	1,37E-09	7
Demylinating Diseases	MESH:D003711	5,71E-12	3,84E-09	7
Multiple Sclerosis	MESH:D009103	8,78E-12	5,90E-09	6
Cerebrovascular Disorders	MESH:D002561	4,59E-11	3,09E-08	8
Neurodegenerative Diseases	MESH:D019636	1,16E-09	7,81E-07	10
Brain Ischemia	MESH:D002545	2,08E-09	1,39E-06	6
Neuromuscular Diseases	MESH:D009468	9,62E-09	6,47E-06	9
Chronobiology Disorders	MESH:D021081	4,86E-07	3,26E-04	3
Rubinstein-Taybi Syndrome	MESH:D012415	1,25E-06	8,37E-04	2
Stroke	MESH:D020521	1,38E-06	9,30E-04	4
Alzheimer Disease	MESH:D000544	2,57E-06	0,00173	4
Tauopathies	MESH:D024801	3,39E-06	0,00228	4
Motor Neuron Disease	MESH:D016472	8,67E-06	0,00583	4
Cerebral Infarction	MESH:D002544	1,50E-05	0,01007	3
Brain Infarction	MESH:D020520	2,11E-05	0,01416	3
Dementia	MESH:D003704	2,36E-05	0,01586	4
Epilepsy	MESH:D004827	3,80E-05	0,02555	5
Status Epilepticus	MESH:D013226	6,13E-05	0,04118	3
Spinal Cord Diseases	MESH:D013118	7,10E-05	0,0477	4