# Pesticides used in Europe and autism spectrum disorder risk: can novel exposure hypotheses be formulated beyond organophosphates, organochlorines, pyrethroids and carbamates? - A systematic review

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**Background**

A growing body of evidences suggests an association between early exposure to organophosphates (OPs), organochlorines (OCs), pyrethroids or carbamates and autism spectrum disorder (ASD). However, there are limited data about the other pesticide groups, especially in Europe.

**Objectives**

Based on a systematic review, we aimed to assess the influence of neuro- and thyrotoxic agricultural and domestic pesticides (other than OPs, OCs, pyrethroids and carbamates) authorized in Europe on risk of ASD in children or ASD behavioral phenotypes in rodents.

**Methods**

Pesticides were initially identified in the Hazardous Substances Data Bank. 20 currently used (10 pesticide groups) were retained based on the higher exposure potential. Epidemiological (children) and *in vivo* (rodents) studies were identified through PubMed, Web of Science and TOXLINE, without restriction of publication date or country (last update: November 2019). The risk of bias and level of evidence were also assessed. This systematic review is registered at the International Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42019145384).

**Results**

In total, two epidemiological and 15 *in vivo* studies were retained, focusing on the azole, neonicotinoid, phenylpyrazole and phosphonoglycine pesticide groups. No study was conducted in Europe. Glyphosate, imidacloprid, clothianidin, myclobutanil, acetamiprid, tebuconazole, thiabendazole and fipronil, globally reported an association with an increased risk of ASD in children and/or ASD behavioral phenotypes in rodents. In children, glyphosate and myclobutanil showed a “moderate level of evidence” in their association with ASD, whereas imidacloprid showed an “inadequate level of evidence”. In rodents, clothianidin, imidacloprid and glyphosate showed a “high level of evidence” in their association with altered behavioral, learning and memory skills.

**Conclusion**

In the framework of environmental risk factors of ASD, novel hypotheses can be formulated about early exposure to eight pesticides. Glyphosate presented the most salient level of evidence. Given their neuro- and thyrotoxic properties, additional studies are needed for the 12 other pesticides not yet studied as potential ASD risk factors according to our inclusion criteria.

**Key words:** pesticides, environmental exposure, neurobehavior, autism spectrum disorder, Europe.

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## I-Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting approximately 1% of the general worldwide population (Baxter et al., 2015) and characterized by persistent deficits in communication and social interactions associated to restricted interests and repetitive behaviors (American Psychiatric Association, 2013). These core symptoms of ASD typically manifest around the age of two years and are often associated with a wide range of clinical symptoms, such as anxiety, cognitive impairment, hyperactivity, hyper/hypo-responsiveness to sensory stimuli (Dellapiazza et al., 2018; Abdallah et al., 2011; Matson et al., 2008; Matson and Nebel-Schwalm, 2007; Leyfer et al., 2006). The burden of the core and associated symptoms, along with the fact that they begin very early in life and are long-lasting, make ASD a major cause of disability. ASD is a multifactorial condition with a demonstrated strong heritability (Sandin et al., 2017). More recently, the growing body of evidences that the developing human brain is particularly vulnerable to toxic chemicals (Grandjean and Landrigan, 2006) has spawned investigations of potential environmental risk factors of ASD (Landrigan, 2012).

Among the toxic chemicals that the population is ubiquitously exposed to, synthetic pesticides are of particular concern (Bölte et al., 2019; Lyall et al., 2017; Modabbernia et al., 2017; Kalkbrenner et al., 2014; Lyall et al., 2014; Rossignol et al., 2014). Even though approximately 500 active compounds are currently authorized for use in the European Union (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN, visited on the 22 May 2019), only a few have thus far been investigated as potential ASD risk factors. Most epidemiological studies conducted between 2006 and 2018 about ASD were mainly focused on a few pesticide groups, probably because they have been the most widely used since the mid-XIX century. These groups comprised of the organochlorines (OCs), organophosphates (OPs), pyrethroids and carbamates (Roberts et al., 2019). Almost all were case-control studies conducted in California (USA). The first case-control study, carried out in 465 ASD children and 6975 typically developing children, reported the risk of ASD to be substantially higher in children whose mothers lived within 500 m of agricultural parcels in which OC was applied than those of mothers not exposed to these applications (odds ratio (OR) = 6.1, 95%CI: 2.4, 15.3) (Roberts et al., 2007). Similarly, within the Childhood Autism Risks from Genetics and Environment (CHARGE) study (486 cases of ASD and 316 controls), proximity to OP application during pregnancy was associated with a 60% increase in ASD risk (Shelton et al., 2014). Similar results were observed for pyrethroid insecticide exposure immediately prior to

conception or during third trimester of pregnancy (Shelton et al., 2014). These risks were amplified in children whose mothers had low folic acid intake (< 800µg) during the first trimester of pregnancy (Schmidt et al., 2017). Conflicting results have been reported in studies assessing prenatal OC exposure in serum. A case control study in California (545 cases of ASD and 418 controls) did not observe any significant relationship between OC metabolite levels in maternal serum and ASD risk (Lyall et al., 2017), whereas a prospective cohort study conducted in 778 matched case-control pairs in Finland showed higher levels of OC in maternal serum than in the Californian study and a significant relationship between high levels of exposure to OC during pregnancy and the risk of ASD (Brown et al., 2018). Another prospective study showed elevated maternal urinary levels of OP metabolites during pregnancy were also associated with an increased risk of ASD in girls, but not in boys (Philippat et al., 2018). Finally, numerous prospective birth-cohort studies conducted in the general population showed significant relationships between early exposure to pesticides and the onset of autistic behaviors or ASD-associated symptoms in healthy children (Eskenazi et al., 2007; Furlong et al., 2014; Brown et al., 2018; Sagiv et al., 2018). According to Roberts et al. (2019), the results from the whole literature provided enough evidences that OCs, OPs, carbamates and pyrethroids may play a role in the ASD onset.

Some of the biological effects of these pesticide groups, namely dysregulation of the excitation/inhibition neurotransmitter systems (OCs, OPs, carbamates and pyrethroids), oxidative stress (OPs, OCs), neuroinflammation (OPs, pyrethroids), and maternal thyroid function disruption during pregnancy (OCs, OPs), appear to also contribute to the pathophysiology of ASD. Consequently, these four common mechanisms have been proposed to explain how these pesticide groups may be biologically plausible contributors to ASD onset (Shelton et al., 2012; Heyer and Meredith, 2017).

The general population is actually exposed to a much wider range of pesticide groups other than the only OCs, OPs, carbamates and pyrethroids (Beranger et al., 2018; Ramos et al., 2017; Dereumeaux et al., 2016; Černá et al. 2012; Becker et al., 2008; Schroijen et al., 2008,). It is likely that among them, some pesticide groups, even though less used and/or newly marketed, share the same neurotoxic mechanisms as OCs, OPs, pyrethroids, and carbamates. Identifying pesticides with such toxicity mechanisms and determining the level of evidence that can already be drawn from the emerging literature about their potential ASD risk would be of great interest for future researches aiming to pinpoint modifiable ASD environmental risk factors.

Considering the lack of interest on pesticide families other than OCs, OPs, pyrethroids and carbamates in the framework of ASD environmental risk factors, the aims of the present work are to (1) identify these other pesticides of interest that may influence the risk of ASD among those currently used in Europe, based on their toxicity profile and probability of exposure in the general population; Then to (2) apply a systematic review of studies on the association between the identified pesticides and risk of ASD in children or ASD behavioral phenotypes in rodents.

## II-METHODS

### 1- Review framework

Firstly, we drew up an exhaustive list of neuro- and thyrotoxic pesticides other than OCs, OPs, pyrethroids and carbamates to which the European population is exposed.

Secondly, we systematically reviewed existing studies about these pesticides and their association with risk of ASD in children and ASD behavioral phenotypes in rodents. ASD behavioral phenotypes in rodents (Pasciuto et al., 2015) were gathered in psychomotor, cognitive functions and behavioral groups. The systematic review was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, Moher et al., 2009) and those of the National Toxicology Program Office of Health Assessment and Translation's (NTP/OHAT) Handbook for Conducting a Literature-Based Health Assessment (NTP/OHAT, 2019). The NTP/OHAT guidelines provide a standardized methodology to implement the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to environmental health assessments.

We developed a review protocol registered at the International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42019145384 on November 7, 2019.

Screening, data extraction, data synthesis, assessment of risk of bias and the rating of confidence in the body of evidence were jointly conducted by JSO and MM. A consensus was reached between the two authors when necessary and in case of disagreement, a third author (AB) was required.

### 2- Study question

The search question was: “Does the current human epidemiological and *in vivo* evidence support a potential role of neuro- and thyrotoxic pesticides authorized in Europe other than those widely studied (i.e. OCs, OPs, pyrethroids and carbamates) in the risk of ASD in children or ASD behavioral phenotypes in rodents?”

## 3- Selection of neuro- and thyrotoxic pesticides to which the European population is exposed

We used (1) the Hazardous Substances Data Bank (HSDB) of the U.S. National Library of Medicine (https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm) from May 6-23, 2019. Based on mechanisms than can potentially lead to ASD, the following terms were successively entered in the database: ("pesticide*" AND ("neuro*" OR "nervous"), then "pesticide*" AND "oxidative stress" and finally "pesticide*" AND ("thyroid*" OR "thyrotoxic*")). A total of 1646 chemicals were obtained among which 649 pesticides had adverse neuro- and/or thyrotoxic effects reported in humans and/or animals. (2) The next step consisted in excluding OP, OC, pyrethroid and carbamate groups. In addition, pesticides for which the reported effects were only limited to “headache”, “dizziness” or “weakness” were excluded (192 pesticides remaining). After deleting duplicates, we obtained 149 pesticides.

### 3.1- Selection of domestic pesticides authorized in Europe

For domestic pesticides, we used the European Chemical Agency (ECHA) database (https://echa.europa.eu) and checked for pesticide use status “approved” or “initial approval in progress” on May 24, 2019. Among the 149 pre-selected pesticides, we only retained 24 domestic pesticides.

### 3.2- Selection of agricultural pesticides authorized in Europe

We checked the status for use of the 149 pre-selected pesticides in the EU pesticides database according to Plant Protection Directive 91-414-EEC (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN). We retained those with status “Approved” or “Pending” on May 24, 2019. When a pesticide was not found in the EU pesticides database, we used a synonym proposed in the open chemistry database of the National Institutes of Health, PubChem (https://pubchem.ncbi.nlm.nih.gov/ ). The remaining “not found” pesticides were excluded. Finally, 85 agricultural pesticides were retained.

### 3.3- Final selection of domestic and agricultural pesticides

For agricultural pesticides, to limit the final selection to those that the European population is the most likely to be exposed, we first used data from “The 2016 European Union report on pesticide residues in food” published by the European Food Safety Authority (EFSA) in 2018. In this EFSA’s report, 791 agricultural pesticide residues were measured in 84657 food

samples of the EU member states, Iceland and Norway in 2016 (EFSA, 2018). We then crossed the 85 agricultural pesticides with those found by the EFSA and which were prioritized by the European Union Control Program (EUCP); We retained those that were common to the two lists and 35 pesticides remained. In the last step, we crossed the 35 agricultural pesticides with the 165 indexed in the chronic Cumulative Assessment Groups (CAGs) for neurotoxicity (64 pesticides) and thyrotoxicity (101 pesticides) established by the EFSA (EFSA, 2013). Finally, we retained 20 agricultural pesticides.

We finally crossed the 24 domestic and 20 agricultural pesticides with those which have reached at least the level of detection in biological specimens considered in the framework of pesticide biomonitoring studies in Europe (Beranger et al., 2018; Dereumeaux et al., 2016) to select pesticides that could be detected in the European population. After removing duplicates, a total of 20 domestic and/or agricultural pesticides sorted into 10 groups was obtained. A detailed flowchart of the pesticide selection process is presented in Figure 1.

## 4- Literature review about the relationship between early exposure to the selected pesticides and ASD risk

### 4.1- Search strategy

We used PubMed electronic database without any limitation on language, publication date or geographical location of the study. For each pesticide group retained, a literature search was conducted, with a last update on November 2019, to identify relevant studies for this review.

Search terms covered early periods of exposure of the selected pesticides and ASD risk, ASD behavioral phenotypes in rodents. We chose these windows of exposure in humans because during these periods, the developing brain is particularly vulnerable to toxic chemicals (Grandjean and Landrigan, 2006).

The following terms were entered in PubMed: **(1)** for study population, ("offspring" OR "neonatal" OR "in utero" OR "development*" OR "pregnan*" OR "gestational" OR "newborn" OR "prenatal" OR "perinatal" OR "fetus" OR "fetal"), **(2)** for the outcome, ("neuro*" OR "neurobehavior*" OR "neurodevelopment*" OR "developmental neurotoxicity" OR "motor" OR "cogniti*" OR "behavior*" OR "central nervous system" OR "brain" OR "autis*" OR "autism spectrum disorder*), **(3)** for the exposure, ("name of pesticide group" OR "pesticide active substance 1" OR "pesticide active substance 2" OR …), e.g.: ("neonicotinoids" OR "imidacloprid" OR "acetamiprid" OR "clothianidin" OR "thiacloprid" OR "dinotefuran" OR "thiamethoxam"). We also used the Toxicology Literature Online (TOXLINE) database of the U.S. National Library of Medicine

(https://toxnet.nlm.nih.gov/newtoxnet/toxline.htm) as well as the Web of science database (http://apps.webofknowledge.com), last update on November 2019.

### 4.2- Study eligibility criteria

Eligibility criteria for the key PECO elements (population, exposure, comparators and outcomes) are defined and summarized in the PECO statement (Table 1).

**Table 1. PECO Statement.**

| Populations | Exposure | Comparators | Outcomes |
|---|---|---|---|
| Population included were:<br>**For epidemiological studies**: children (from *in utero* till early childhood)<br>**For *in vivo* studies**: rats and mice | Exposure to all neuro/thyrotoxic pesticides other than OPs, OCs, pyrethroids and carbamates authorized in Europe and derivatives on administered dose or concentrations, direct (biomonitoring) or indirect measures (environmental monitoring, e.g. air, soil, dust, questionnaires or Geographic Information System) | Reference groups of population not exposed or exposed at lower levels of targeted pesticides than the rest of population groups. | **Primary outcome**:<br>***in children**: ASD<br>***in rodents**: ASD behavioral phenotypes gathered in **psychomotor** (hyperactivity), **cognitive functions** (learning and memory) or **behavioral** (restricted interests, aggression, anxiety, depression) groups<br>**Secondary outcome**: not considered. |

Original articles available in full text in English, (2) prospective, case-control and cross-sectional epidemiological and *in vivo* studies were considered in this review. For *in vivo* studies we only focused on those conducted in rodents (rats or mice) with an oral route of exposure because, according to the United States Environmental Protection Agency (US EPA), the oral route in rodents is the most relevant to evaluate the suitability to support human health risk assessment (US EPA, 1998 and 2012). Exclusion criteria accounted for: (1) *in vitro* studies, (2) studies that reported pesticide levels in biological specimens without any ascertainment of neurodevelopmental outcomes, (3) studies on pesticide mixtures and (3) studies related to exposure to high doses of pesticides occurring during accidental or intentional poisoning.

### 4.3- Study selection

The article selection process was made according to guidelines reported by The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The selection began by reading article titles followed by reading abstracts when titles were unspecific. Finally, full-text articles eligible for this review were read.

### 4.4-Data extraction

The following information were extracted from eligible articles: (1) the name of the pesticide active substance, (2) author(s), publication year and country, (3) study design, population, and statistical methods, (4) exposure assessment, (5) outcomes and outcome assessments, and (6) main results.

### 4.5- Risk of bias assessment and confidence in the body of evidence

#### 4.5.1- Risk of bias assessment

We used the NTP/OHAT Risk of Bias Rating Tool for Human and Animal Studies' criteria (NTP/OHAT, 2019) to assess the risk of bias of each study included in this systematic review. This classification is based on a tiered approach (more details are provided in Supplemental Material, Section 2.1) which classifies studies in:

**Tier 1**: a study must be rated as “definitely low” or “probably low” risk of bias for key criteria **AND** “definitely low” or “probably low” risk of bias for most other applicable criteria.

**Tier 2**: study meets neither the criteria for tiers 1 nor 2.

**Tier 3**: a study must be rated as “definitely high” or “probably high” risk of bias for key criteria **AND** “definitely high” or “probably high” risk of bias for most other applicable criteria.

#### 4.5.2- Confidence in the body of evidence and level of evidence

We assessed the confidence in the body of evidence and related level of evidence using the NTP/OHAT framework (NTP/OHAT, 2019) which describes criteria for assessing the strength of evidences consistent with the Hill’s criteria for causation (Hill, 1965). The confidence in the body of evidence rating was assessed considering the initial rating of confidence based on the study design, factors that may downgrade and upgrade the initial confidence rating (more details are provided in Supplemental Material, Section 2.2). The final rate of evidence was classified in four descriptors: “high”, “moderate”, “low” or “very low” confidence and then translated into level of evidence in “high,” “moderate”, “low”, “evidence of no health effect,” and “inadequate evidence”. Details are presented in Table S5.

## III- RESULTS

### 1- Selected Pesticides

We selected 20 pesticide active substances neuro- and/or thyrotoxic (other than OPs, OCs, pyrethroids and carbamates) authorized in Europe. We gathered them into 10 pesticide groups: aniline-pyrimidine, azoles, carboxamides, neonicotinoids, oxadiazines, phenylpyrazoles, phosphonoglycine, strobilurins, triazines and urea herbicides (Table 2). These pesticides were mainly agricultural or had a mixed use (*i.e.* agricultural and domestic) and were mainly represented by fungicides, insecticide/acaricides or material preservatives.

### 2- Relationship between early exposure to the selected pesticides and risk of ASD in children or ASD behavioral phenotypes in rodents

#### 2.1- Article selection

A total of 1330 citations were obtained using the PubMed database, 13 additional from the TOXLINE database, and no additional from Web of science. There were 536 citations for phosphonoglycine, 517 for neonicotinoids, 121 for phenylpyrazoles, 73 for azoles, 27 for urea herbicides, 21 for triazine herbicides, 18 for oxadiazines, 19 for strobilurins, six for carboxines, and five for anilino-pyrimidines.

Among them, 691 were excluded due to the study design (ecotoxicity studies n=479, in vitro studies n=89, reviews n=45, reports/commentaries/article replies n=11, exposure/risk assessment, environmental monitoring n=55, molecular/genetic n=12), 38 on the basis of the exposure (non-targeted pesticides n=25, pesticide mixtures n=8, not a pesticide n=2, occupational exposure n=3), 388 on the basis of the study population (not children n=1 or not rodents n=387) and 165 on the basis of the outcomes (*i.e.* not ASD or ASD behavioral phenotypes).

Of the 61 remaining articles, after reading full texts, we excluded seven due to the study design (*in vitro* study n=1, exposure/risk assessment, environmental monitoring n=6), 13 due to the exposure (pesticide mixtures n=8, non-targeted pesticides n=5), 20 due to the outcomes (i.e. not ASD or ASD behavioral phenotypes) and three due to a non-targeted route of exposure in experimental studies (intravenous n=2, intraperitoneal n=1). We decided to exclude a last epidemiological study, which met inclusion criteria because in this study, analyses were not possible due to the limited number of ASD (2 cases).

Finally, according to the PRISMA statement guidelines (Moher et al. 2009), a total of 17 studies were retained in this review (Figure 2) and are summarized in Table 3.

#### 2.2- Study characteristics

The 17 studies were published between 2001 and 2019. Among them, two were epidemiological, all conducted in the USA, and 15 were in *vivo* studies. The studies exclusively involved four pesticide groups (azoles, neonicotinoids, phenylpyrazole, and phosphonoglycine) of the 10 obtained.

**Epidemiological studies**

The two epidemiological studies were population-based case-control ones (Keil et al., 2014; Von Ehrenstein et al., 2019) and investigated the effects of an early exposure to azoles, neonicotinoids and phosphonoglycine on the ASD risk in children.

Regarding exposure assessment, only indirect estimates were available. Exposures were assessed through parental interviews (Keil et al., 2014) or using methods that estimated the amount of agricultural pesticides used in an *a priori* defined distance from the residence (Von Ehrenstein et al., 2019). These last methods relied on public data from local agricultural application records, land use survey providing the location of specific crops and on geographical information systems (GIS) that allow projection of these data in a defined area around each residential address. The resulting exposure variables were all dichotomized (exposed *vs.* unexposed).

The time windows of exposure and adjustments in the statistical analyses were homogeneous between the two studies. Both investigated the effects of exposure during the prenatal period (Von Ehrenstein et al., 2019; Keil et al., 2014). The period just before conception (Keil et al., 2014) and the first years of life were also considered in the two studies (Von Ehrenstein et al., 2019; Keil et al., 2014). Overall, sex and age of the children, maternal education (Von Ehrenstein et al., 2019; Keil et al., 2014), as well as maternal age (Von Ehrenstein et al., 2019) were considered as potential confounding factors in the relationship between pesticide exposure and ASD risk.

***In vivo* studies**

The *in vivo* studies performed behavioral tests that are used to detect ASD behavioral phenotypes in genetic rodent models (Pasciuto et al., 2015). Social interaction was evaluated by observing aggressive behaviors in the resident-intruder test (Sano et al., 2016). Restricted interests were assessed using the open field test (OFT) (Ait Bali et al., 2018; Ait Bali et al., 2017; Khalil et al., 2017; Cattani et al., 2017; Gallegos et al., 2016; Godinho et al., 2016; Moser et al., 2001). This test consists in placing mice in a large and brightly light box. The

time spent in the more aversive center zone and time spent in the peripheral zone are quantified. Restricted interests can induce a diminution of the exploratory behavior. The OFT was also commonly used to test hyperactivity and anxiety-like behavior. Other tests, such as the elevated plus maze (Ait Bali et al., 2018; Ait Bali et al., 2017; Gallegos et al., 2016) or the light dark exploration (Sano et al., 2016) based on rodents' innate preference for small, dark and enclosed spaces, were also used to reveal anxiety-like behavior. Depression-like behavior was assessed using the forced swim (Khalil et al., 2017; Cattani et al. 2017) and the tail suspension tests (Ait Bali et al., 2018; Ait Bali et al., 2017). Learning and memory deficits were mainly explored by observing the rodents' behavior in mazes (Morris water maze test and the probe trial, water T-maze) (Bhaskar et al., 2017; Kara et al., 2015; Ozdemir et al., 2014; Tanaka, 2012a, 2012b; Moser et al. 2001), whereas one study used the new object recognition task (Godinho et al., 2016). Finally, some of the *in vivo* studies explored general neurological functioning by observing the sensorimotor development of rodents (Khalil et al., 2017; Gallegos et al., 2016; Udo et al., 2014; Tanaka, 2012a, 2012b; Moser et al., 2001).

Most of the *in vivo* studies exposed female rodents during the gestational and lactation periods and performed the behavioral tests in the offspring early in life (from the first postnatal days to just after weaning) (Bhaskar et al., 2017; Sano et al., 2016; Gallegos et al., 2016; Godinho et al., 2016; Udo et al., 2014; Tanaka, 2012b) or slightly later, after the period of exposure in the offspring (Cattani et al., 2017; Tanaka, 2012a; Moser et al., 2001; Tanaka, 2001). Some studies exclusively investigated the effects of postnatal exposure to pesticides in young rodents (Godinho et al., 2016; Kara et al., 2015; Ozdemir et al., 2014), with periods of exposure ranging from 15 days to three months. For imidacloprid, in particular, a compound from the neonicotinoids group, the population study was limited to male rodents (Bhaskar et al., 2017; Khalil et al., 2017; Kara et al., 2015). The doses selected varied between studies. Some chose to investigate the effects of very low exposure doses. In this design, experimental groups were exposed to doses inferior to the non-observable adverse effect level (NOAEL) as defined for each compound on TOXNET (https://toxnet.nlm.nih.gov/newtoxnet/toxline.htm) (Bhaskar et al., 2017; Khalil et al., 2017; Cattani et al., 2017); Others studies focused on the effects of low (doses similar to the NOAEL) (Ait Bali et al., 2018; Ait Bali et al., 2017; Gallegos et al., 2016; Sano et al., 2016; Kara et al., 2015; Moser et al., 2001) or high doses (doses superior to the NOAEL) (Godinho et al., 2016; Udo et al., 2014; Tanaka, 2012a, 2012b; Tanaka, 2001).

### 2.3- Epidemiological and *in vivo* studies reviewed for relevance

### 2.3.1- Azole group

- **Myclobutanil**

**Epidemiological evidences**

We found only one study, a case-control, conducted in California (USA) that enrolled 2961 children with ASD and 35370 controls (Von Ehrenstein et al., 2019). ASD cases were selected from the registry of the California Department of Developmental Services. The ASD diagnosis was based on the DSM-IV-R criteria (American Psychiatric Association, 2000). Children were considered to be exposed if myclobutanyl was applied in agricultural parcels within a 200m radius of their residence during the prenatal period and first year of life. A non-significant association was found between prenatal exposure to myclobutanil and ASD risk (OR: 1.04, 95% CI: 0.96, 1.12). This association became significant when ASD was associated with intellectual disability (ID) with an OR of 1.32, 95% CI: 1.09, 1.60 (Von Ehrenstein et al., 2019).

***In vivo* evidences**

No *in vivo* study met our inclusion criteria.

- **Tebuconazole**

**Epidemiological evidences**

No epidemiological study met our inclusion criteria.

***In vivo* evidences**

Moser et al. (2001) investigated the effect of prenatal and postnatal exposure to tebuconazole on neurological integrity, motor activity, sensorimotor responses, and learning and memory in rats. The results showed that prenatal exposure followed by early postnatal exposure to doses equivalent to the NOAEL (20-60 mg/kg/day) may induce alterations in spatial learning and working memory in rats (Moser et al., 2001).

- **Thiabendazole**

**Epidemiological evidences**

No epidemiological study met our inclusion criteria.

***In vivo* evidences**

One study investigated the effect of prenatal and postnatal exposures to thiabendazole on sensorimotor development, exploratory behavior and learning in mice (Tanaka, 2001). In comparison to control groups, mice pre- and postnatally exposed to a dose equivalent to 700±

1800 mg/kg bw/day of thiabendazole (> NOAEL) showed a delay in sensorimotor development and a decrease in emotionality during the exploratory behavior test.

❖ **Cyproconazole and triadimenol**

No epidemiological nor *in vivo* study met our inclusion criteria.

### 2.3.2- Neonicotinoids group

❖ **Acetamiprid**

**Epidemiological evidences**

No epidemiological study met our inclusion criteria.

***In vivo* evidences**

Male mice exposed to 1.0 mg/kg of acetamiprid (< NOAEL) during the prenatal and lactation periods presented a reduction of anxiety level, a hyperactivity and an increase of aggressive behaviors but no differences in spatial learning ability, behavioral flexibility and impulsivity during adulthood in comparison to the control group (Sano et al., 2016).

❖ **Clothianidin**

**Epidemiological evidences**

No epidemiological study met our inclusion criteria.

***In vivo* evidences**

Tanaka conducted two experimental studies in mice in 2012 to investigate the effects of an early exposure to doses equivalent to 4-33 mg/kg bw/day of clothianidin (similar to NOAEL) on the sensorimotor development and exploratory behavior. The period of exposure lasted from early gestation to the end of the lactation period in the first study (Tanaka, 2012b), while the second study pursued the exposure in the offspring until the 11$^{th}$ week of life (Tanaka, 2012a). Both studies observed an acceleration of the sensorimotor development in the early postnatal days in the exposed groups, as well as an increase in motor activity during the exploratory behavior when mice were three weeks old. Such increased activity was still observed at eight weeks of age when exposure ended at weaning (*i.e.* 21 days of age) (Tanaka, 2012b). In contrast, in the other experiment, mice still being exposed to clothianidin were less active than controls (Tanaka, 2012a). No influence of clothianidin was observed on spatial learning in mice (Tanaka, 2012a, 2012b).

This last observation was reproduced in another *in vivo* study conducted in juvenile male rats exposed to similar doses of clothianidin (24 mg/kg) as those in Tanaka's studies administrated from the early postnatal period to three months of age (Ozdemir et al., 2014). In contrast to

the studies of Tanaka, this study showed that the consolidation of memory was adversely affected in the exposed juvenile rats compared to the control group (Ozdemir et al., 2014).

❖ **Imidacloprid**

**Epidemiological evidences**

Two case-control studies investigated the effects of early exposure to imidacloprid on the ASD risk. The first one, already described in the section on myclobutanyl (Von Ehrenstein et al., 2019), did not reveal any significant association between early exposure to imidacloprid used on agricultural parcels nearby maternal residence and the ASD risk, with an estimated OR close to 1. The other case control study was interested in maternal use of imidacloprid in the domestic context during the prenatal period and the three first years of life, estimated through maternal interviews performed at the time of the study, *i.e.* several years after pregnancy. Also conducted in California (US), this study was conducted on a smaller sample than the previous one, 407 ASD children from the CHARGE study and 262 controls. The results showed a non-statistically significant increase of 30% in the risk of ASD among the children of women using imidacloprid in the domestic context during pregnancy (OR =1.3, 95%CI: 0.78, 2.2) (Keil et al., 2014).

***In vivo* evidences**

Maternal exposure to very low doses of imidacloprid, 0.65 mg/kg/day (< NOAEL) during the lactation period in mice (approximately one month) did not alter working memory performances in the offspring (Bashkar et al., 2017). In contrast, a chronic postnatal exposure to very low doses of imidacloprid (0.5 and 1.0 mg/kg bw/day) induced anxiety- and depression-like behaviors in young rats (Khalil et al., 2017), and low doses, 2 and 8 mg/kg (similar to the NOAEL), significantly decreased learning skills and memory consolidation in young rats (Kara et al., 2015).

❖ **Dinotefuran, thiacloprid and thiamethoxam**

No epidemiological nor *in vivo* study met our inclusion criteria.

### 2.3.3- Phenylpyrazole group

❖ **Fipronil**

**Epidemiological evidences**

No epidemiological evidence met our inclusion criteria.

***In vivo* evidences**

The two *in vivo* studies retained were conducted in Brazil and used a commercial formulation of fipronil (Regent®800WG). In the first study, prenatal exposure of rats to low doses of fipronil (0.1 and 1 mg/kg/day) delayed sensorimotor development in the offspring (Udo et al. 2014). In the second study, high doses, 10 and 30 mg/kg (>NOAEL), of Regent® 800WG were given to young rats for 15 days to investigate the effects of such postnatal exposure on memory skills and locomotor activity. The results showed memory alterations and decreased locomotor activity in exposed rats compared to controls (Godinho et al., 2016).

#### 2.3.4- Phosphonoglycine group

- **Glyphosate**

**Epidemiological evidences**

We found one study, conducted in the USA, already been described in the section on myclobutanil (Von Ehreinstein et al., 2019). The results showed an increased risk of ASD, with or without ID, in children prenatally exposed to agricultural glyphosate application near their mother's residence (OR: 1.33, 95%CI: 1.05, 1.69; and OR: 1.16, 95% CI: 1.06, 1.27, respectively) (Von Ehreinstein et al., 2019).

***In vivo* evidences**

Effects of glyphosate on motor activity were investigated in four studies using the open field test. Prenatal and postnatal exposures to 100-500 mg/kg/day of glyphosate were associated with hypoactivity in rats and mice (Ait Bali et al., 2018; Ait Bali et al., 2017; Gallegos et al., 2016), whereas no effect was observed in rat offspring exposed to very low doses (70 mg /kg/day) during the prenatal period (Cattani et al., 2017).

Rodents exposed to very low (Cattani et al., 2017) and low doses (Ait Bali et al., 2018; Ait Bali et al., 2017) of glyphosate during the pre- and postnatal periods also showed depression-like behaviors compared to the control groups.

Inconsistent results were observed regarding the effects of glyphosate on anxiety-like behavior. Although undergoing similar behavioral tests to assess anxiety, rats exposed to very low doses of glyphosate during the prenatal period did not behaved differently than the control group (Gallegos et al., 2016), whereas mice exposed to slightly higher doses during a long postnatal period showed an anxiety-like behavior, unlike the control group (Ait Bali et al., 2018; Ait Bali et al. 2017).

#### 2.3.5- Anilino-pyrimidine, carboxamide, oxadiazine, strobilurins, triazine and urea herbicide groups

No epidemiological or *in vivo* evidence according to our inclusion criteria was found for anilino-pyrimidine (pyrimethanil), carboxamides (boscalid), oxadiazine (indoxacarb), strobilurins (azoxystrobin), triazine (terbuthylazine), or urea herbicides (diuron and isoproturon).

### 2.4- Risk of bias assessment

The results of the risk of bias assessment is presented in Table 4 and the rationale of each article's evaluation is presented in Supplemental Material Table S6 - S22.

**Epidemiological study**

For the key bias domains, the study of Keil et al. (2014) presented a "probably high" risk of bias for the exposure characterization and was classified in Tier 2 for risk of bias. The study of Von Ehreinstein (2019) well reported all key bias domains and was classified as tier 1 for a risk of bias.

***In vivo* studies**

The studies of Sano et al. (2016), Kara et al. (2015), and Ozdemir et al. (2014) presented a "definitely high" risk of bias for exposure characterization, which is one of the key bias domains. Approximately half of the studies (Bhaskar et al., 2017; Khalil et al., 2017; Godinho et al., 2016; Ozdemir et al., 2014; Tanaka, 2012a; Moser et al., 2001 and Tanaka, 2001) did not mention potential conflicts of interest.

Globally, the *in vivo* studies were mainly classified as tier 1 (Ait Bali et al., 2018; Cattani et al., 2017; Ait Bali et al., 2017; Bhaskar et al., 2017; Khalil et al., 2017; Gallegos et al., 2016; Godinho et al., 2016; Udo et al., 2014; Tanaka, 2012a, 2012b; Moser et al., 2001 and Tanaka, 2001) or tier 2 (Sano et al., 2016; Kara et al., 2015; Ozdemir et al., 2014) for the risk of bias.

### 2.5- Confidence in the body of evidence and level of evidence

**Epidemiological studies**

For the epidemiological studies, when a single study investigated the ASD risk for a pesticide active substance, we adapted the criteria to this single study to rate the confidence in its findings.

The evaluation showed there was: i) an "inadequate level of evidence" in the body of evidence for an association between early exposure to imidacloprid and an increased ASD risk; ii) a "moderate level of evidence" in the body of evidence for an association between early exposure to myclobutanyl and an increased ASD risk, and iii) a "moderate level of evidence" in the body of evidence for an association between early exposure to glyphosate

and an increased ASD risk, as shown in Table 5 (individual rating details are shown in tables S23 to S25).

***In vivo* studies**

The diversity of the ASD behavioral phenotypes in rodents made the construction of the body of evidence challenging for *in vivo* studies. We gathered studies investigated the same ASD behavioral phenotypes, namely behavioral development impairment for clothianidin, learning and memory deficit for imidacloprid, and depression-like behaviors and anxiety for glyphosate.

We found a "high level of evidence" in rodents for an association between i) exposure to clothianidin and behavioral development impairment, ii) exposure to imidacloprid and learning and memory deficits, and iii) exposure to glyphosate and the presence of depression-like symptoms and anxiety, as presented in Table 5 (individual rating details are shown in tables S26 to S28).

## IV-DISCUSSION

### 1- Summary of evidences

We identified 20 pesticide active substances, divided into 10 pesticide groups, which are in current use in Europe for which the levels are detectable in food and European individuals and which share similar toxicity mechanisms (neurotoxicity and/or thyrotoxicity, Table 1) with pesticides already considered to be probable ASD environmental risk factors (OPs, OCs, pyrethroids and carbamates). Among them, nine have both agricultural and domestic uses (diuron, cyproconazole, tebuconazole, thiabendazole, azoxystrobin, acetamiprid, imidacloprid, thiacloprid, and indoxacarb), six are exclusively agricultural (pyrimethanil, triadimenol, boscalid, myclobutanil, glyphosate, terbuthylazine) and five, exclusively domestics (isoproturon, fipronil, thiametoxam, dinotefuran, clothianidin). Among these pesticides, the European legislation distinguishes between plant protection products (used for crops and gardens) and biocides (for domestic use, except gardens), which explains why some are authorized for certain uses and not for others.

The 17 studies selected concerned eight pesticides (glyphosate, imidacloprid, clothianidin, myclobutanil, acetamiprid, tebuconazole, thiabendazole and fipronil) from four groups (azoles, neonicotinoids, phenylpyrazoles, and phosphonoglycines). The risk of bias was rated as "definitively low" or "probably low" for all studies. For the eligible pesticide, glyphosate and myclobutanil presented "moderate level of evidence" in their association with ASD risk in children and imidacloprid, an "inadequate level of evidence". In rodents, clothianidin,

imidacloprid and glyphosate showed a "high level of evidence" in their associations with altered behavior and learning and memory skills.

### 2- Levels of exposure in the European population

Overall, epidemiological studies retained in this systematic review did not permit to appreciate the exposure frequency and intensity of our pesticides of interest in the general population. However, some of them have been reported in biological and/or environmental monitoring studies in Europe. The recent study from Beranger et al. (2018) measured a panel of pesticides in hair samples of 311 French pregnant women. The azole, carboxamide, neonicotinoid, phenylpyrazole, strobilurins and urea herbicide groups were the most detected among our pesticide of interest. Thiabendazole (azole fungicide) and fipronil (phenylpyrazole insecticide) presented the highest percentage of detection with respectively 90% (median concentration of 0.68 pg/mg) and 87% with a median concentration of 1.62 pg/mg (Beranger et al., 2018).

Still in pregnant women (n=1036) coming from various locations in France, the frequency of glyphosate detection in urine samples was <1%, whereas a detection rate of 43% was observed in 546 women in Brittany (Dereumeaux et al., 2016). In Germany, glyphosate was detected in 40% of the 319 urine samples of young adults in 2015 (Conrad et al., 2017). While the levels of glyphosate were <1µg/L in these studies in the general population (Dereumeaux et al., 2016 and Conrad et al., 2017), occupational studies reported higher glyphosate levels in agricultural workers and their family. A Finnish study in forest workers using Roundup (glyphosate) reported glyphosate urine concentrations reaching 85 µg/L (Jauhiainen et al.,1991). In France, Mesnage et al. (2012) found in farmers, glyphosate urine concentrations of 9.5 µg/L after spraying and 2 µg/L in some children's farmer two days after spraying. In Ireland, two studies conducted in Amenity horticulturalists revealed glyphosate urine mean concentrations varying between 0.71 (Connolly et al., 2017) and 1.08 µg/L (Connolly et al., 2018) before glyphosate spraying and 1.35 (Connolly et al., 2017) and 1.72 µg/L (Connolly et al., 2018) post-spraying.

Regarding environmental monitoring studies, a French study found fipronil in indoor dust from households located in different agricultural (<1000m from vineyards, cereals, or orchards crops; 28-33% of detection) and urban areas (>2000m from crops, 8% of detection), at median concentrations varying from 1750 to 61536 ng/m$^2$ (Beranger et al., 2019).

Although there is a lack of data on exposure levels of many pesticides we identified as potential risk factors of ASD in this systematic review, those for which we found data

highlight that these pesticides are widespread, and populations are exposed to them at a non-negligible frequency.

### 3- Epidemiological studies

Based on our inclusion criteria, no conclusion could be drawn from the existing literature about the relationships between early exposure to imidacloprid (neonicotinoid insecticide) and ASD risk in children. In fact, the level of evidence in the body of evidence was considered "inadequate" (Von Ehrenstein et al., 2019; Keil et al., 2014) meaning an insufficient number of evidence to draw a conclusion in favor or against this association. This finding highlights the need for more studies to better appreciate whether or not imidacloprid could increase ASD risk in children.

This review also showed that early exposure to myclobutanil (azole fungicide) and glyphosate (phosphonoglycine herbicide) increased ASD risk in children and level of evidence was "moderate". This level of evidence was based on the results of a well-conducted study with a "probably low risk of bias" (Von Ehrenstein et al., 2019). The magnitude of the association was limited to 6 to 27% increase in the risk of ASD in children from the general population environmentally exposed during the prenatal period compared to non-exposed children (Von Ehrenstein et al., 2019). Although well conducted, this study needs to be compared in future to others ones to see if the finding is confirmed or not.

Globally, this review showed a lack of epidemiological studies on our pesticides of interest and ASD risk in children. Researchers should be encouraged to study in addition to OPs, OCs, pyrethroids and carbamates, at least imidacloprid, myclobutanil and glyphosate for a beginning in the framework of environmental risk factors of ASD in children.

### 4- *In vivo* studies

In rodents, imidacloprid appears to alter the spatial learning and consolidation of memory skills. The level of evidence in studies (Kara et al., 2015; Bhaskar et al., 2017) was evaluated as "high", confirming the robustness of this finding. A study (Ozdemir et al., 2014) with "probably low risk of bias" showed that clothianidin, another neonicotinoid insecticide, altered spatial learning and memory in rodents. Even we could not draw a level of evidence for this single study on clothianidin, its suggests a potential risk which should be verified by further studies. Contrarily with the three previous studies, another neonicotinoid insecticide, acetamiprid did not alter spatial learning ability in rodents (Sano et al. 2016). However, this result should be taken with caution because it was classified in Tier 2 in the bias risk assessment meaning plausible bias that raises some doubt about the results. This finding was

similar to another study with "probably low risk of bias" conducted by Bhaskar et al. (2017) which found a non-significant alteration of working memory after exposed mice to imidacloprid. Godinho et al. (2016) demonstrated in a "probably low risk of bias" study, a loss of memory in rats exposed to fipronil (phenylpyrazole insecticide) suggesting the role plaid by this pesticide in the onset of a usual ASD phenotype (Pasciutto et al., 2015). Tebuconazole (azole fungicide) also seemed to alter memory in rodents (Moser et al. 2001); This study presented a "probably low risk of bias".

Studies on clothianidin (Tanaka, 2012a; 2012b), reported behavioral developmental impairments in rodents. The level of evidences in the set of studies was "high" supporting the robustness of the findings. Another pesticide, thiabendazole (azole fungicide) appeared to induce an impairment in behavioral developmental in rodents (Tanaka, 2001) in a "probably low risk of bias" study. An increase of aggressive behaviors and hyperactivity were also observed when mice received acetamiprid (Sano et al. 2016). A last study (Udo et al. 2014) suggested that fipronil delayed reflexes in rodents.

A series of *in vivo* studies (Ait Bali et al., 2018; Ait Bali et al., 2017; Cattani et al., 2017; Gallegos et al., 2016) suggested that exposure to glyphosate (phosphonoglycine herbicide) induces in rodents, mood disorders, in particular depressive behaviors and this finding presented a "high level of confidence".

Globally, azole fungicides, neonicotinoid and phenylpyrazole insecticides and phosphonoglycine herbicide appeared to alter variously core symptoms frequently found in the context of ASD in humans (Pasciutto et al., 2015). These findings should arouse the interest of researchers in investigating the effect of these pesticides in the ASD human context.

### 5- Pesticide groups without any evidence founded according to our inclusion criteria

In this systematic review, we found no epidemiological nor *in vivo* study based on our inclusion criteria for any of the six pesticide groups, anilino-pyrimidines (pyrimethanil), carboxamides (boscalid), oxadiazines (indoxacarb), strobilurins (azoxystrobin), triazines (terbuthylazine), or urea herbicides (diuron and isoproturon). Some of them share the same neurotoxicological mechanisms with the most studied pesticides OPs, OCs, pyrethroids and carbamates (Abreu-Villaça et al., 2016; Shelton et al., 2012), for which the association with ASD in the literature have been sufficiently demonstrated (Roberts et al., 2019; Schmidt et al., 2017; Brown et al., 2018; Keil et al., 2014; Shelton et al., 2014; Eskenazi et al., 2007; Roberts

et al., 2007). However, lack of evidence does not exclude the potential harmfulness of these pesticides. It simply highlights the fact that much remains to be done for these pesticides in the context of ASD, at least experimentally for a start.

## 6- Strength and limitations of this systematic review

### 6.1- Strengths

One of the main strengths of this systematic review is the thorough selection process of pesticides through an exhaustive search within the two main European pesticide/chemical databases, the EU pesticides database (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN) and the ECHA database (https://echa.europa.eu). Secondly, we focused on both agricultural and domestic pesticides, helping to provide a global overview of environmental exposure to pesticides. Thirdly, many data crossings were performed with reports of an official European agency (EFSA) and findings of recent well-conducted European studies (Beranger et al., 2018; Dereumeaux et al., 2016). In contrast to many previous systematic reviews on pesticides, we also included *in vivo* studies in rodents, which permitted us to have more evidences and suggestions on the effects of our pesticides of interest on the onset of ASD behavioral phenotypes commonly founded in the context of ASD in human (Pasciutto et al., 2015). Moreover, these ASD behavioral phenotypes in rodents are well-established prognostic factors of a non-favorable clinical evolution of ASD in children (Maski et al., 2011). Finally, for pesticides for which we rated the body of evidence, we were able to have a relevant assessment of the level of confidence to grant to selected articles.

### 6.2- Limitations

Some limitations in this review should be considered. The first is inherent to this systematic review, because even we focused on pesticides authorized in Europe, all included studies were exclusively carried out in the USA, more specifically in California. There is thus a true need to conduct researches in Europe because the possibility of extrapolating the American findings to the European context is limited. Firstly, pesticide exposure patterns may be different, independently of the pesticide group. Globally, exposure levels appear to be higher in the USA (Philippat et al., 2018; Millenson et al., 2017; Wagner-Schuman et al., 2015; Furlong et al., 2014; Eskenazi et al., 2007) than in Europe (Dereumeaux et al., 2018; Llop et al., 2017; Debost-Legrand et al., 2016). Secondly, some factors associated with a reduced ASD risk, such as preconceptional/pregnancy folic acid supplementation appears to be more

prevalent in Europe than in the USA (Toivonen et al., 2018; National Center for Health Statistics, 2012). Finally, there may be a possible regional specificity in gene-environment interactions, leading to potential regional genetic vulnerability in favor of ASD onset during environmental exposure to pesticides (D'Amelio et al., 2005). So, more studies in Europe are needed to compare findings of American studies.

This review is related to the periodicity of pesticide use. We did not include banned pesticides even though some may be persistent in the environment and the population may still be exposed to them. We made this choice because additional precautionary measures are limited for these banned pesticides and we then preferred to focus on pesticides in current use that might still be modifiable risk factors of ASD.

Finally, *in vivo* studies were more common than epidemiological ones in this review and we cannot rule out that human exposure levels may be far different from and not comparable to those used for experimentation in rodents. However, studies focused on an oral route of exposure in rodents are the most relevant to support health risk in human (US EPA, 1998 and 2012).

## V- CONCLUSION AND FUTURE DIRECTIONS

To our knowledge, this is the first systematic review on the relationship between exposure to anilino-pyrimidines, azoles, carboxamides, neonicotinoids, oxadiazines, phenylpyrazoles, phosphonoglycine, strobilurins, triazines and urea herbicides and ASD in children or ASD behavioral phenotypes in rodents. We found articles for only eight pesticides (glyphosate, imidacloprid, clothianidin, myclobutanil, acetamiprid, tebuconazole, thiabendazole and fipronil) gathered in four groups (azoles, neonicotinoids, phenylpyrazoles and phosphonoglycine) of the 10 selected. It then appears that novel hypotheses can be formulated about early exposure to these eight pesticides and ASD risk. The current level of evidence suggests that these compounds may be good candidates as environmental risk factors of ASD. Like OPs, OCs, pyrethroids and carbamates, these pesticides should systematically be included in the panel of chemicals that should be tested in future European studies investigating the question of environmental risk factors of ASD. In such future studies, a particular attention should be paid to glyphosate, for which the level of evidence for an association with the risk of ASD in children and/or ASD behavioral phenotypes in rodents was the most salient.

Other pesticide active substances from various groups (anilino-pyrimidines, carboxamides, oxadiazines, strobilurins, triazines and urea herbicides) were also identified as potential risk

factors of ASD based on their toxicity mechanisms, but there is currently no evidence according to our inclusion criteria. Experimental *in vivo* studies are then needed first to determine whether these substances should also be investigated in future epidemiological studies carried out in the framework of the search of environmental risk factors of ASD.

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**CRediT authorship contribution statement**

**Jeanne Ongono**: Conceptualization, Methodology, Formal analysis, Validation Investigation, Writing - original draft, Writing - review & editing, Visualization. **Remi Béranger**: Methodology, Writing - review & editing, Visualization. **Amaria Baghdadli**: Conceptualization, Methodology, Validation, Writing - review & editing, Visualization, Supervision. **Marion Mortamais**: Conceptualization, Methodology, Formal analysis, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision.

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**CRediT authorship contribution statement**

**Jeanne Ongono**: Conceptualization, Methodology, Formal analysis, Validation Investigation, Writing - original draft, Writing - review & editing, Visualization. **Remi Béranger**: Methodology, Writing - review & editing, Visualization. **Amaria Baghdadli**: Conceptualization, Methodology, Validation, Writing - review & editing, Visualization, Supervision. **Marion Mortamais**: Conceptualization, Methodology, Formal analysis, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision.

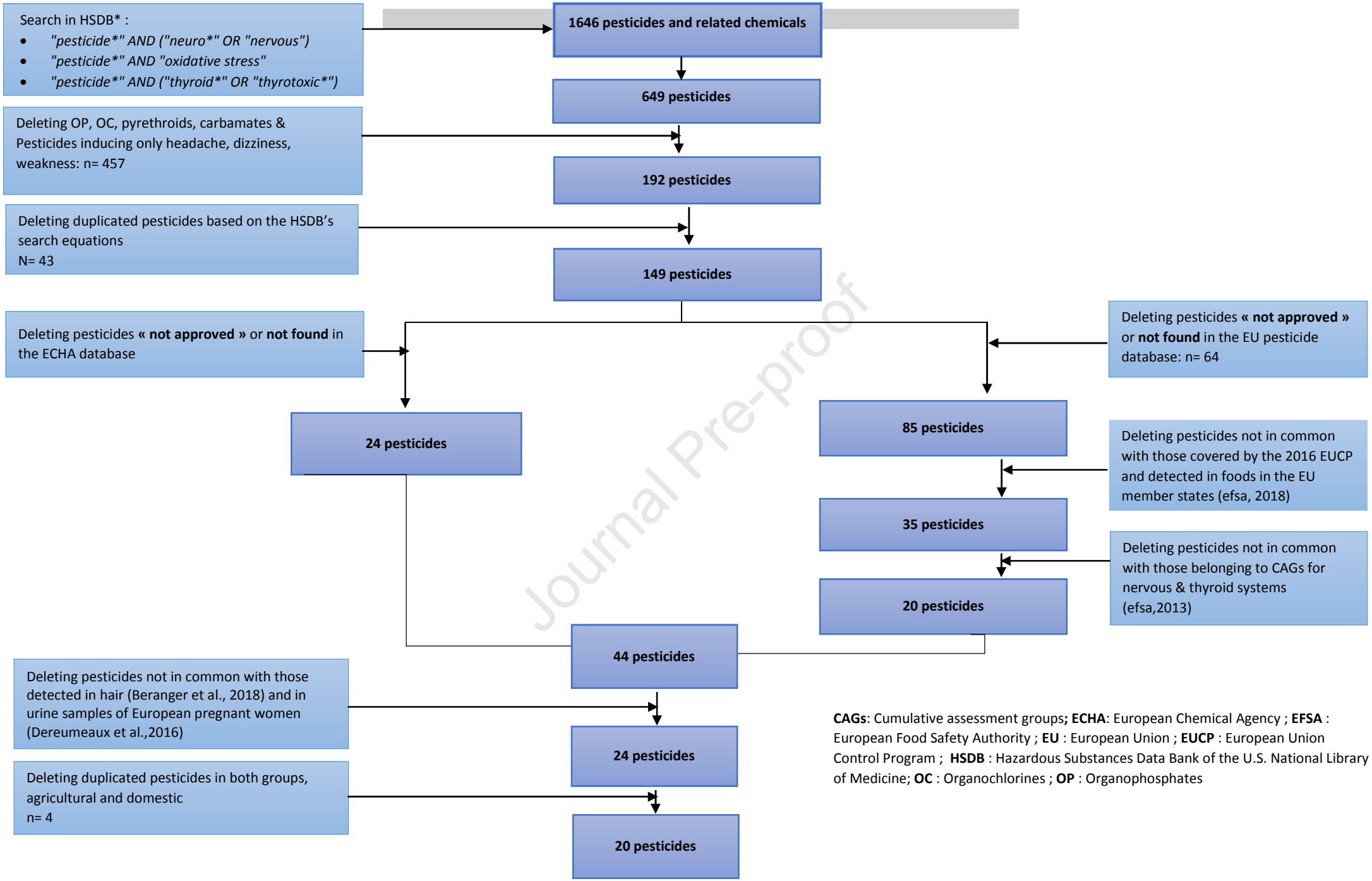




**Figure 1. Selection of pesticides neuro- and/or thyrotoxic (other than organochlorines, organophosphates, pyrethroids, carbamates) to which the European population is exposed.**

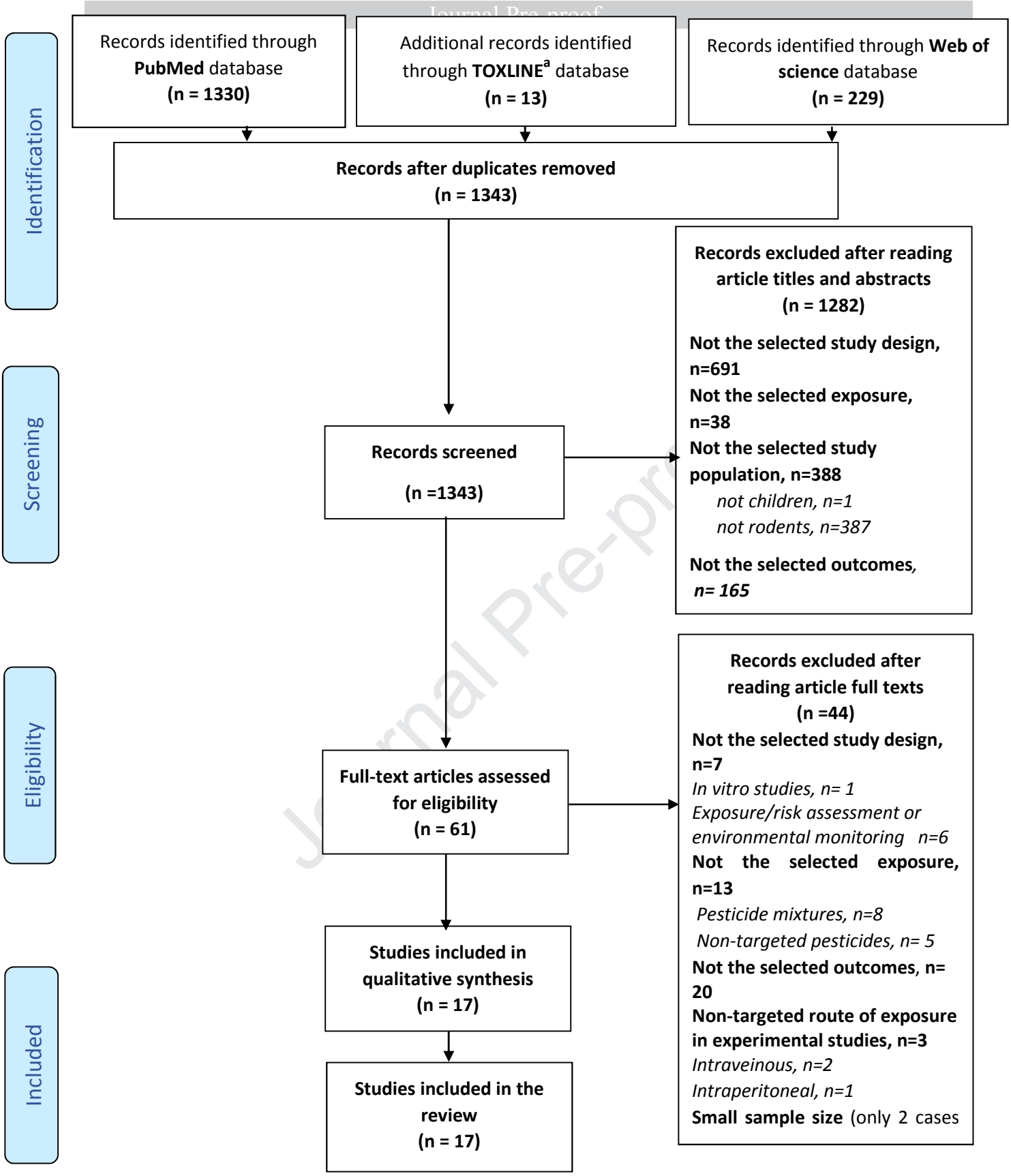


**Figure 2. The PRISMA flow diagram (Moher et al., 2009) of the systematic review search strategy about early exposure to pesticides (other than organochlorines, organophosphates, pyrethroids, carbamates) and risk of ASD in children or ASD behavioral phenotypes in rodents.**

***Note**: [a]TOXLINE: Toxicology Literature Online is a database of the U.S. National Library of Medicine*

**Table 2. Pesticides (other than organochlorines, organophosphates, pyrethroids, carbamates) to which the European population is exposed and underlying mechanisms related to autism spectrum disorder.**

| Pesticides Groups | CAS registry number | NOAEL[a] for neurotoxicity in rodents (mg/kg bw/day) | Agricultural pesticides | Household pesticides | Use | Neurotoxicity mechanisms related to ASD | |
|---|---|---|---|---|---|---|---|
| | | | | | | Direct neurotoxicity | Indirect neurotoxicity |
| **Anilino-Pyrimidine** | | | | | | | |
| **Pyrimethanil** | 53112-28-0 | - | x | | Fungicide | - | Serum thyroid hormone disruption: decrease of T4, increase of TSH serum levels *(Hurley, 1998)* |
| **Azoles** | | | | | | | |
| **Cyproconazole** | 94361-06-5 | - | x | x | Fungicide materials Preservatives | - | Serum thyroid hormone disruption via a presumed hepatic enzyme induction *(EFSA, 2013)* |
| **Myclobutanil** | 88671-89-0 | - | x | | Fungicide | - | Thyroid toxicity: unknown mechanism *(EFSA, 2013)* |
| **Tebuconazole** | 107534-96-3 | - | x | x | Fungicide Materials Preservatives | - | Thyroid toxicity: unknown mechanism *(EFSA, 2013)* |
| **Thiabendazole** | 148-79-8 | - | x | x | Fungicide Materials Preservatives | - | Serum thyroid hormone disruption via a presumed hepatic enzyme induction *(EFSA, 2013)* |
| **Triadimenol** | 55219-65-3 | **NA** | x | | Fungicide | Unknown mechanism: probable increase dopamine turnover in nigrostriatal and mesolimbic brain dopamine | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | pathways *(EFSA, 2013)* | |
| **Carboxamides** | | | | | | | |
| **Boscalid** | 188425-85-6 | - | x | | Fungicide | - | Probable serum thyroid hormone disruption via a presumed hepatic enzyme induction *(Montoya et al., 2014)* |
| **Neonicotinoids** | | | | | | | |
| **Acetamiprid** | 135410-20-7 | Subchronic neurotoxicity - **14.8** / **16.3** (Male/Female) | x | x | Insecticides acaricides | Nicotinic acetylcholine receptor (nAChR) agonist *(EFSA, 2013)* | Probable serum thyroid hormone disruption: increase of T3, T4 serum levels *(Sekeroglu et al., 2012)* |
| **Clothianidin** | 210880-92-5 | Subchronic neurotoxicity **60.0** / **71.0** (male/female) | | x | Insecticides Acaricides Materials preservatives | nAChR agonist *(EFSA, 2013)* | - |
| **Dinotefuran** | 165252-70-0 | Subchronic Neurotoxicity: **33** / **40** (male/female) | | x | Insecticides Acaricides | nAChR agonist *(EFSA, 2013)* | - |
| **Imidacloprid** | 138261-41-3 | Subchronic Neurotoxicity: **9** | x | x | Insecticides Acaricides | nAChR agonist *(EFSA, 2013)* Oxidative stress in brain *(Abd-Elhakim et al., 2018)* | - |
| **Thiacloprid** | 111988-49-9 | Subchronic neurotoxicity **24.2** / **27.9** (male/female) | x | x | Insecticide Materials preservatives | nAChR agonist *(EFSA, 2013)* | - |
| **Thiamethoxam** | 153719-23-4 | Subchronic neurotoxicity **NOAEL = 34.5 mg/kg/day** | | x | Insecticides Acaricides Materials | nAChR agonist *(EFSA, 2013)* | Probable serum thyroid hormone disruption via a presumed hepatic enzyme induction *(EFSA, 2013)* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | Preservatives | | |
| **Oxadiazine** | | | | | | | |
| **Indoxacarb** | 173584-44-6 | Subchronic neurotoxicity: **0.57** / **0.68** (male/female) | x | x | Insecticide | Presumed blocking of sodium channels in insects *(EFSA, 2013)* | - |
| **Phenylpyrazoles** | | | | | | | |
| **Fipronil** | 120068-37-3 | for neurotoxicity and systemic effects: **0.3** | | x | Insecticides<br>Acaricides | Blocking the passage of chloride ions through the GABA receptor *(EFSA, 2013)* | Serum thyroid hormone disruption: decreased of T3 and/or T4 serum levels *(EFSA, 2013)* |
| **Phosphonoglycine** | | | | | | | |
| **Glyphosate** | *1071-83-6* | Subchronic neurotoxicity: **1546.5** / **1630.6** (male/female) | x | | Herbicide | Glutamate excitotoxicity *(Cattani et al., 2014)*<br>Oxidative stress *(Cattani et al., 2014)* | Serum thyroid hormone disruption: decrease of TSH serum levels *(de Souza et al., 2017)* |
| **Strobilurins** | | | | | | | |
| **Azoxystrobin** | 131860-33-8 | Subchronic Neurotoxicity:<br>**NOAEL: NA** but **NOEL=38.5** (male/female) | x | x | Fungicide<br>Materials preservatives | - | Thyrotoxicity: unknown mechanism |
| **Triazine** | | | | | | | |
| **Terbuthylazine** | 5915-41-3 | Sub-chronic neurotoxicity: **7** / **8** (male/female) *(Moxon 2003)* | x | | Herbicide | Oxidative stress *(Tariba Lovaković et al., 2017)* | Serum thyroid hormone disruption: Increase of the thyroid hormone dependent GH3 cell growth *(Ghisari et al., 2015)* |
| **Urea herbicides** | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Diuron** | 330-54-1 | Sub-chronic neurotoxicity: **NA** | x | x | Herbicide<br>Materials Preservatives | Oxidative stress *(TOXNET, https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~xB8YXb:1 )* | - |
| **Isoproturon** | 34123-59-6 | Sub-chronic neurotoxicity: **NA** | | x | Materials Preservatives | Oxidative stress *(TOXNET,https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~xB8YXb:5)* | - |

**Notes:**

***NA**: not available*

***NOAEL** (No Observable Adverse Effect Level) is the highest dose level/concentration of a substance that under defined conditions of exposure causes no observable/detectable adverse effect (alteration) on morphology, functional capacity, growth, development, or life span of the test animals.*

***NOEL** (No Observable Effect Level) is the highest dose or exposure level of a substance or material that produces no noticeable (observable) toxic effect on tested animals.*

[a] **NOAELs** and **NOEL** *of this table derived from fact sheets of the United States Environmental Protection Agency (US EPA) available on https://www.epa.gov/*

**Table 3. Evidences of epidemiological and *in vivo* studies about early exposure to pesticides (other than organochlorines, organophosphates, pyrethroids and carbamates) and ASD in children or ASD behavioral phenotypes in rodents.**

| Pesticide active substance | Authors, year (Country) | Study design, population, and statistical methods | Exposure assessment | Outcomes & Outcomes assessment | Main results |
|---|---|---|---|---|---|
| **Azoles** | | | | | |
| **1°) Epidemiological studies** | | | | | |
| **Myclobutanil** | Von Ehrenstein et al. 2019 (USA) | Case-control study<br>2961 ASD cases [including 445 with intellectual disability (ID)],<br>Controls derived from birth records<br>Matching 10 controls for 1 case, by sex and birth year.<br>**Adjusting variables:** Sex, year of birth, maternal age, maternal race/ethnicity and education, NOx | **Exposure variable:** Residential proximity to agricultural pesticides use vs. none within a 2000 m radius of each residential address.<br>**Exposure assessment method:** GIS with data from local agricultural application records (CA-PUR reports), combined with the location of specific crops.<br>**Windows of exposure** : prenatal period, and the first year of life. | ASD, as reported on the registry of the California Department of Developmental Services, diagnosis based on the DSM-IV-R criteria | Prenatal exposure and all cases of ASD: OR (95%CI)=1.04 (0.96- 1.12)<br>First year of life exposure and all cases of ASD: OR (95%CI)= 1.01 (0.93 to 1.09)<br>Prenatal exposure and ASD with ID: OR(95%CI)= 1.32, (1.09 - 1.60)<br>First year of life exposure and ASD with ID: OR (95%CI)= 1.27 (1.05 - 1.54) |
| **2°) In vivo studies** | | | | | |
| **Tebuconazole** | Moser et al. 2001 (USA) | Experimental study in rats.<br>F0: Pregnant Sprague-Dawley rats (GD 4-5): n ≥ 15/dose<br>F1 (Males and females): 10 rats/dose/sex and 8 rats/sex at the high dose | Tebuconazole (purity grade: 97.4%) was given to pregnant F0 (from GD14 to PND 7), then to their offsprings F1 (until PND42) at:<br>-0 mg/kg/day in control group and<br>- 6, 20, 60 mg/kg/day in treated groups | **Neurological integrity** using a functional observational battery (FOB).<br>**Motor activity** using an automated chamber shaped like a figure-eight and the open-field test.<br>**Sensorimotor responses** using a variety of stimuli in the | In comparison to control group, in F1, learning of the position of the platform at a slower rate in the Morris water maze, for males and females. Other neurological and behavioral endpoints were not significantly different. |

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| | | | | framework of the open field test.<br>**Learning and memory task** using a passive avoidance test on PND56–69<br>**Spatial learning and working memory** using a Morris water maze (spatial training and probe trial) beginning on PND74 | |
| **Thiabendazole** | Tanaka 2001 (Japan) | Experimental study in mice.<br>F0: Male and female mice (4 weeks of age) purchased from Charles River Japan Inc.<br>F1 (Males and females):<br>Experimental groups: 60 mice (10/sex/group)<br>Control group: 20 mice (10/sex) | Thiabendazole (purity grade: > 99%) was given, from 5 weeks of age of the F0 generation to 9 weeks of age of the F1 generation, at dietary concentrations of:<br>- 0% in control group and<br>-0.031% in low-dose group<br>- 0.125% in mid-dose group<br>- 0.5% in high-dose group | **Behavioral developmental parameters**:<br>-Surface righting on post-natal day (PND) 4 and 7<br>-Negative geotaxis on PND 4 and 7<br>-Cliff avoidance on PND 7<br>-Swimming behavior on PND 4 and 14<br>-Olfactory orientation on PND 14<br>**Exploratory behaviour** using an animal movement analysing system (ANIMATE AT-420) at 8 weeks of age in F0 and at 3 and 8 weeks of age in F1.<br>**Spatial learning** using a Biel-type multiple water T-maze at 7 weeks of age in F1 | In comparison to control group, mice in the high dose (equivalent to 700± 1800 mg/kg bw/day) group showed:<br>- Differences in behavioral developmental parameters: in both sexe, surface righting was significantly delayed at PND7 and swimming limb movement and olfactory orientation were significantly depressed at PND14.<br>- Differences in exploratory behavior: at 3 weeks of age in F1 generation, vertical time and number of defecations were significantly decreased, suggesting a decreased emotionality.<br>- No adverse effects of thiabendazole on multiple water T-maze performance. |
| **Neonicotinoids** | | | | | |
| **1°) Epidemiological studies** | | | | | |

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| **Imidacloprid** | Keil et al. 2014 (USA) | Case-control study<br>407 children with ASD & 262 controls from the general population<br>Pairing on age, sex, region of birth<br>CHARGE Study<br>**Adjusting variables:** Maternal education, race/ethnicity, parity, pet ownership during pregnancy, child's sex, age, region of birth | **Exposure variables:** Maternal use of household imidacloprid (flea and tick medication containing IMI (≈ 9 unity)): exposed (any household usage) vs unexposed<br>OR<br>- occasional users (imidacloprid use during some, but not all months of prenatal period); consistent users (imidacloprid use during all months of prenatal period) vs unexposed<br>**Exposure assessment method:** data collected through maternal phone interview.<br>**Windows of exposure**: from 3 months before conception through 3 years of age. | ASD cases were recruited from an administrative database of the California Department of Developmental Services. Confirmation of ASD status using the ADI-R and ADOS. | Prenatal exposure and ASD risk (exposed vs non exposed): OR (95%Credible Interval [CrI])= 1.3 ( 0.78, 2.2).<br>OR increased to 2.0 (95% CrI: 1.0, 3.9) when examining consistent users vs unexposed.<br>OR was higher for exposures during the prenatal period than during the preconception period and the first three years of life. |
| **Imidacloprid** | Von Ehrenstein et al. 2019 (USA) | Case-control study<br>2961 ASD cases [including 445 with intellectual disability (ID)],<br>Controls derived from birth records<br>Matching 10 controls for 1 case, by sex and birth year.<br>**Adjusting variables**: Sex, year of birth, maternal age, maternal race/ethnicity and education, NOx | **Exposure variable:** Residential proximity to agricultural pesticides use vs. none within a 2000 m radius of each residential address.<br>**Exposure assessment method:** GIS with data from local agricultural application records (CA-PUR reports), combined with the location of specific crops.<br>**Windows of exposure :** prenatal period, and the first year of life | **ASD**, as reported on the registry of the California Department of Developmental Services, diagnosis based on the DSM-IV-R criteria | Prenatal period and all cases of ASD: OR (95%CI)= 0.93 (0.86 to 1.00)<br>First year of life and all cases of ASD: OR (95%CI)= 0.95 (0.88 to 1.02)<br>Results for both periods were similar within the subgroup of ASD with ID. |

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| | | **2°) In vivo studies** | | | |
| **Acetamiprid** | Sano et al. 2016 (Japan) | Experimental study in mice<br>Male and female mice<br>Mice/group:<br>18 in the control group, 19 in the low-dose one and 20 in the high-dose one for the assessment of male aggressive behavior and emotional behavior.<br>13 in the control group, 11 in the low-dose one, 13 in the high-dose one for the assessment of behavioral flexibility. | Acetamiprid (purity grade: NR) was given from gestational day 6 to lactation day 21 at doses of:<br>-0 mg/kg in control group and<br>-1.0 mg/kg in low-dose group<br>- 10.0 mg/kg high-dose group | -**Male aggressive behavior** *(chasing, boxing, wrestling, biting, tail rattling, and offensive lateral attack)* using the resident-intruder test<br>-**Emotional behaviors**: using the light-dark transition test (LDT) test for both male and female mice<br>-**Spatial learning ability**, **behavioral flexibility and impulsivity** (for both male and female mice) using the IntelliCage | In comparison to control group, mice exposed to acetamiprid showed:<br>- significantly increased male aggressive behaviors in the low-dose group only.<br>- for males only, longer time spent in the light compartment in the LDT test reflecting not only decreased anxiety, but also hyperactivity under specific stressful conditions.<br>-no differences in spatial learning ability, behavioral flexibility and impulsivity during adulthood in both sex |
| **Clothianidin** | Tanaka 2012a (Japan) | Experimental study in mice<br>Male and female mice (4 weeks of age)<br>Experimental group: 60 mice (10/sex/group)<br>Control group (20 mice: 10/sex) | Clothianidin (purity grade: >99.0%) was given, from 5 weeks of age of the F0 generation to 11 weeks of age of the F1 generation), at dietary concentrations of:<br>-0% in control group and<br>-0.003%<br>-0.006% (equivalent to 9–33 mg/kg bw/day)<br>-0.012%, in treated groups | **Behavioral developmental parameters**:<br>- Surface righting on postnatal day (PND) 4 and 7<br>- Negative geotaxis on PND 4 and 7<br>- Cliff avoidance on PND 7<br>- Swimming behavior on PNDs 7 and 14<br>- Olfactory orientation on PND 14<br>**Exploratory behavior** using an animal movement analysis system SCANET CV-40 at 8 weeks of age in the F0 generation, and at 3 and 8 weeks | **In the F0 generation**<br>- a significant increase of average time of movement, number of rearing, and rearing time in adult males in exploratory behavior.<br>**In the F1 generation**<br>- **behavioral developmental parameters in females:** accelerated surface righting in the low-dose group, accelerated negative geotaxis (indicative of the development of the motor coordination) with an effect-dose related, accelerated development of swimming head angle (indicative of the sense of equilibrium) in the low- and middle-dose groups.<br>- **behavioral developmental parameters in males:** delayed |

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| | | | | of age in F1.<br>**Spatial learning** using a Biel-type multiple water T-maze at 7 weeks of age in F1. | development of swimming head angle (effect dose-related), accelerated time taken to olfaction orientation in the middle-dose group.<br>-**Exploratory behavior:** increased number of rearing of females and movement time in males in a dose-related manner.<br>-**Spatial learning:** no differences between the groups. |
| **Clothianidin** | Tanaka 2012b (Japan) | Experimental study in mice<br>Male and female mice (4 weeks of age)<br>Experimental group: 30 female mice (10/group)<br>Control group: 10 female mice | Clothianidin (purity grade: >99%) was given during the gestation and lactation periods in the F0 generation at dietary concentrations of:<br>-0% in control group and<br>- 0.002%, 0.006%, 0.018%, in treated groups | **Behavioral developmental parameters**:<br>- Surface righting on postnatal day (PND) 4 and 7<br>- Negative geotaxis on PND 4 and 7<br>- Cliff avoidance on PND 7<br>- Swimming behavior on PNDs 7 and 14<br>- Olfactory orientation on PND 14<br>**Outcome assessment :** Behavior of mice was measured in an animal movement analyzing system SCANET CV-40 from 9 weeks to 10 weeks of age in the F1 generation | In comparison to control group, mice in the high dose group showed:<br>- a significant accelerated surface righting at postnatal day(PND) 7 of female offspring<br>- an increase of movement activity of exploratory behavior at 3 weeks of age, average speed (cm/s) of male offspring |
| **Clothianidin** | Ozdemir et al. 2014 (Turkey) | Experimental study in albino Wistar rats<br>*** In the study of effects of CLO on juvenile male rat models (newborn)** | Clothianidin (purity grade: NR) was given, from the 7th day in the juvenile rat models and during a 3-months period in both adult and juvenile models, at dietary doses of:<br>- 0 mg/kg for control group and | -**Spatial Learning** using the Morris water maze learning performance test<br>-**Memory** using the Probe trial test | No change in learning ability in the adult and infant rats after CLO administration.<br>In the probe trial (consolidation of memory) test, the infant group given a |

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| | | Experimental group: 12 rats (4/group)<br>Control group: 4 rats<br>***In the study of effects of CLO on adult male rat models (8–9 week-old)**<br>Experimental group: 18 rats (6/group)<br>Control group : 6 rats | - 2, 8, 24 mg/kg in treated groups | | high dose of CLO was adversely affected compared to the control group. |
| **Imidacloprid** | Kara et al. 2015 (Turkey) | Experimental study in rat<br>Albino Wistar rat:<br>Experimental & control groups<br>-for infant model: newborn males (6/group)<br>-for adult model: 24 male rats 8 to 9-week-old (6/group) | Imidacloprid (purity grade: NR) was given daily for a period of 3 months in both infant and adult rat models at dietary doses of:<br>-0 mg/kg bw in control group and<br>-0.5, 2, 8 mg/kg bw, in treated groups | **Spatial learning and consolidation of memory** using The Morris water maze learning performance and probe trial tests. | **In infant rat model, compared to the control group:**<br>Significant decrease in learning skills at 2 and 8 mg/kg bw doses.<br>Significant decrease in consolidation of memory at 8 mg/kg bw doses.<br>**In adult rat model, compared to the control group:**<br>Significant decrease in learning skills at 2 mg/kg bw dose.<br>Significant decrease in consolidation of memory at 8 mg/kg bw doses. |
| **Imidacloprid (Tatamida®)** | Bhaskar et al. 2017 (India) | Experimental study in mice<br>Swiss albino mice (age: NA)<br>Experimental & control groups: 12 pregnant mice/group,<br>15-17 F1 mice/group | Exposure to imidacloprid (concentration: 17.8% wt/wt) through the diet of lactating female from PND1 to PND28 at the dose of:<br>- 0 mg/kg/day in control group and<br>- 0.65 mg/kg/day in treated group | **Working memory** using the T-maze test on PND 29 and 63 | Results were reported only in male pups.<br>In comparison to control group, there was no significant alteration of working memory. |
| **Imidacloprid** | Khalil et al. 2017 (Egypt) | Experimental study in rats<br>Experimental & control groups: 18 Adult (3 months | Imidaclorpid (purity grade: 100%) was given for a period of 60 days at dietary doses of: | **Anxiety-like behavior and locomotor activity** using the open field test. | In comparison to the control group, there was:<br>- a reduction in the frequency of entries and time spent in the central arena |

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| | | of age) male Sprague - Dawley rats (6/group) | -0 mg/kg bw/day in control group and<br>-0.5 mg/kg bw/day and 1.0 mg/kg bw/day, in treated group | **Depression-like behavior** using the forced swim test.<br>**Motor coordination and sense of equilibrium** by observing the mice's swimming performances<br>Tests were performed at the end of the experimental period. | (indicative of anxiety) in the open field test, as well as a substantial increase of grooming in the high-dose group.<br>-an increased in immobility time and decline in swimming time (indicative of depression-like behavior) in both exposed groups<br>-No differences in swimming performances between groups. |
| **Phenylpyrazoles** | | | | | |
| **In vivo studies** | | | | | |
| **Fipronil** (Regent®800WG) | Udo et al. 2014 (Brasil) | Experimental study in rat<br>Wistar rats: 15 males, 40 female (~90 day-old)<br>Rat pairing: 2-3 females for 1 male<br>Experimental & control groups:<br>pregnant female rats (10/group)<br>Offsprings: (20/group, 10 males and 10 females) | Fipronil (purity grade: 80%) was given daily to pregnant female rats from GD6 to GD20 at dietary doses of:<br>- 0 mg/kg/day in control group and<br>- 0.1, 1, 10 mg/kg/day in treated groups | **Maternal behavior at PND5**: active (latency for litter grouping, pup-licking, self-grooming, …) & reflexive parameters (latency for the first hovering, number of dams that expressed FMB)<br>Reflex development in offspring<br>*from PND2 to PND35:*<br>surface righting reflex<br>palmar grasp reflex<br>*From PND5 to PND35*<br>negative geotaxis reflex | In comparison to control group, there was:<br>**For maternal behaviors**<br>-an impairment of active and reflexive parameters in the 0.1 mg/kg/day group<br>- a stereotyped active response in the 10 mg/kg/day group<br>**For Offspring reflex development**<br>-A delayed negative geotaxis reflex in the 0.1 mg/kg/day group<br>- an early loss of palmar grasp in the 0.1 mg/kg/day & 1 mg/kg/day groups |
| **Fipronil** (Regent®800WG) | Godinho et al. 2016 (Brasil) | Experimental study in rat<br>Wistar rats: young males<br>Experimental & control groups:<br>10 rats /group) | Fipronil (purity grade: 80%) was given for a period of 15 days at doses of:<br>-0 mg/kg in control group and<br>-10, 30 mg/kg in treated group | **Memory and learning** using the "new object recognition task" and its recognition index (proportion of time that the animal spends investigating the novel object compared to the familiar object) & the "eight | In comparison to control group, there was in treated group:<br>**Memory**<br>-a decrease in the novel object recognition index for both short-term and long-term memory. |

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| | | | | radial arm maze task"<br>**Locomotor activity** using the open field test | - increase in the latency to find food, the number of arms visited incorrectly, and the number of arms revisited (demonstrating a loss of memory in animals exposed) in the eight radial arm maze task.<br>- No modification in **Locomotor activity** |
| | | | **Phosphonoglycine** | | |
| | | **1°)** | **Epidemiological studies** | | |
| **Glyphosate** | Von Ehrenstein et al. 2019 (USA) | Case-control study<br>2961 ASD cases [including 445 with intellectual disability (ID)],<br>Controls derived from birth records<br>Matching 10 controls for 1 case, by sex and birth year.<br>**Adjusting variables**:<br>Sex, year of birth, maternal age, maternal race/ethnicity and education, NOx | **Exposure variable:** Residential proximity to agricultural pesticides use vs. none within a 2000 m radius of each residential address.<br>**Exposure assessment method:**<br>GIS with data from local agricultural application records (CA-PUR reports), combined with the location of specific crops.<br>**Windows of exposure** : prenatal period, and the first year of life. | **ASD**, as reported on the registry of the California Department of Developmental Services, diagnosis based on the DSM-IV-R criteria | Prenatal exposure and all cases of ASD: OR (95%CI)=1.16 (1.06- 1.27)<br>First year of life exposure and all cases of ASD:<br>OR (95%CI)= 1.15 (1.05 to 1.26)<br>Prenatal exposure and ASD with ID:<br>OR(95%CI)= 1.33, (1.05 - 1.69)<br>First year of life exposure and ASD with ID:<br>OR (95%CI)= 1.51 (1.18 to 1.92) |
| | | **2°)** | **In vivo studies** | | |
| **Glyphosate** (Roundup®) | Cattani et al. 2017 (Brazil) | Experimental study in Wistar rats<br>Experimental & control groups:<br>≥6 rats/group for pregnant | Roundup herbicide Original® (Glyphosate concentration 360 g/L) was given from gestational day 5 to the end of the lactating period (PND21), at dietary doses of: | On PND60:<br>**Motor activity** using the open-field test<br>**Motor coordination and** | In comparison to control group, males exposed to glyphosate showed a prolonged immobility time and decreased time of climbing in the swimming forced test. |

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| | | rats<br>4/group for offsprings | -0% for control group<br>-0.36% (≈70 mg /Kg/day) for experimental group<br>Then, from PND22 to PND60, F1 males received glyphosate at:<br>-0% for control group<br>-1% for treated group | **balance** using the rotarod test<br>**Depression-like behavior** using the forced swimming test<br>**Anhedonia** using the sucrose consumption test | No differences between groups in the open field test, the rotarod test and the sucrose consumption test. |
| **Glyphosate** (Roundup®) | Ait Bali et al. 2017 (Morocco) | Experimental study in male Swiss mice (1 month old of age)<br>Experimental & control groups:<br>18 mice (6/group) into 3 groups (acute, subchronic and chronic) | Pesticide Roundup® (Glyphosate concentration 360 g/l in the form of Gly isopropylamine salt 486 g/L) was given at dietary doses of:<br>-0 mg/kg/day for control group and<br>- 250 mg/kg/day for acute group<br>-500 mg/kg/day for subchronic and chronic groups<br>Acute group: one administration<br>Subchronic and chronic: daily administration for 6 and 12 weeks, respectively | **Locomotor activity and emotional reactivity** using the open field test<br>**Anxiety-like behavior** using the elevated-plus-maze test<br>**Depression** using the tail suspension and the splash test | In comparison to control group, treated groups exhibited (especially the 500 mg/kg group) showed:<br>-significant decrease in total distance traveled, the velocity and the percentage<br>of the time spent in the central zone in the open field test:<br>-a significant increase in the immobility time and a decrease in grooming time in tail suspension and splash tests. |
| **Glyphosate (Roundup®)** | Ait Bali et al. 2018 (Morocco) | Experimental study in male Swiss mice (1 month old of age)<br>Experimental & control groups:<br>18 mice (6/group) into 3 groups (acute, subchronic and chronic) | Pesticide Roundup® (Glyphosate concentration 360 g/l in the form of Gly isopropylamine salt 486 g/L) was given at dietary doses of:<br>-0 mg/kg/day for control group and<br>- 250 mg/kg/day for acute group<br>-500 mg/kg/day for subchronic and chronic groups<br>Acute group: one administration<br>Subchronic and chronic: daily | **Locomotor activity and emotional reactivity** using the open field test<br>**Anxiety-like behavior** using the elevated-plus-maze test<br>**Depression** using the tail suspension and the splash test<br>**Grooming behavior** using the splash test | In comparison to control group, treated groups showed a:<br>- significant decrease in the time spent in the center and an important increase of the anxiety index in the Holm-Sidak post hoc test<br>- decrease in grooming time in the splash test<br>- significant increase in the immobility time in the 500mg/kg/day treated-group in the open field test |

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| | | | administration for 6 and 12 weeks, respectively | | |
| **Glyphosate** (Glifloglex®) | Gallegos et al. 2016 (Argentina) | Experimental study in male and female adult Wistar rats (90-120 days old)<br>Experimental & control groups:<br>30 mice (10/group) | Pesticide Glifloglex® (Glyphosate concentration: 48 g of Gly isopropy-lamine salt per 100 $cm^3$ product) was given to pregnant rat, from GD0 to weaning on post-gestational day 21, at dietary doses of:<br>-0 mg/kg/day for control group and,<br>-100 mg/kg/day in group 1,<br>-200 mg/kg/day in group 2 | In offsprings:<br>**Sensorimotor development on PND3**:<br>-Righting reflex<br>-Cliff aversion reflex<br>-Negative geotaxis<br>-Eye and auditory canal opening<br>**Motor activity on PND45 and PND90** using the open field test<br>**Anxiety** levels **on PND45** using the open field test and on PND 90 using the Plus maze test | In comparison to control group, offsprings from treated groups showed:<br>- an early onset of cliff aversion reflex and early auditory canal opening. No statistical differences in the development of negative geotaxis and righting reflex.<br>- less squares crossing (only in females and for the highest glyphosate dose at PND45, in both doses and sex at PND90); a decrease in number of rearing; a higher number of grooming episodes in males at PND90.<br>- increased percentage of time spent in open arms in PND90 females, more time in open arms and a higher percentage of entries to open arms in both sex at PND90 |

**Abbreviations:**

**NR**: Not reported in the article

**ADOS**: Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Schedules

**ASD**: Autism spectrum disorder

**BMI**: body mass index, weight (kg)/height $(m)^2$

**CHAMACOS:** Center for the Health Assessment of Mothers and Children of Salinas study

**DDS**: Department of Developmental Services

**CDWR:** California Department of Water Resources

**DSM-IV-R**: Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised

**CA-PUR**: California state Pesticide Use Reporting

**CHARGE** study: Childhood Autism Risks from Genetics and Environment stud

**FFQ**: Food Frequency Questionnaire

**FMB**: Full maternal behavior

**GIS**: Geographic Information System

**NOx**: nitrogen oxides

**WISC-IV**: Wechsler Intelligence Scale for Children, fourth edition

**Table 4. Risk of bias assessment according to the tiered approach risk of bias tool of the NTP/OHAT (NTP/OHAT 2019).**

| | **Epidemiological studies** | | ***In vivo* studies** | | | | | | | | | | | | | | |
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| **Response levels**<br>**Definitely low risk of bias** ++<br>**Probably low risk of bias** +<br>**Probably high risk of bias** - NR<br>**Definitely high risk of bias** -- | | | | | | | | | | | | | | | | | |
| **Bias domains** | **Von Ehreinstein et al. 2019** | **Keil et al. 2014** | **Ait Bali et al. 2018** | **Cattani et al. 2017** | **Ait Bali et al. 2017** | **Bhaskar et al. 2017** | **Khalil et al. 2017** | **Gallegos et al. 2016** | **Godinho et al. 2016** | **Sano et al. 2016** | **Kara et al. 2015** | **Ozdemir et al. 2014** | **Udo et al. 2014** | **Tanaka 2012a** | **Tanaka 2012b** | **Moser et al. 2001** | **Tanaka 2001** |
| **Detection Bias [Key element]:** can we be confident in the exposure characterization? | + | - | ++ | ++ | ++ | ++ | ++ | ++ | ++ | -- | -- | -- | ++ | ++ | ++ | ++ | ++ |
| **Detection Bias [Key element]:** can we be confident in the outcome assessment? | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Confounding Bias [Key element]:** did the study design or analysis account for important confounding and modifying variables? | + | + | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

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| **Selection bias:** did selection of study participants result in appropriate comparison groups? **OR** Was allocation to study groups adequately concealed *(for experimental studies in animals)*? | + | + | + | + | + | + | + | + | + | + | **NR** | **NR** | + | + | + | + | + |
| **Attrition/Exclusion Bias:** were outcome data incomplete due to attrition or exclusion from analysis? | **NA** | **NA** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Reporting Bias:** | | | | | | | | | | | | | | | | | |
| • **Selective reporting bias:** were all measured outcomes reported? | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| • **Conflict of Interest:** was the study free of support from a company, study author, or other entity having a financial interest in any of the treatments studied? | + | + | **NR** | + | + | **NR** | **NR** | + | **NR** | + | + | **NR** | + | **NR** | + | **NR** | **NR** |
| **Performance bias [key element]:** | | | | | | | | | | | | | | | | | |
| • were experimental conditions identical across study groups? | **NA** | **NA** | + | + | + | + | + | + | + | + | - | **NR** | ++ | + | + | ++ | + |
| • were research personnel blinded to the study groups during the study? | **NA** | **NA** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Summary tiered classification** | **T 1** | **T2** | **T1** | **T1** | **T1** | **T1** | **T1** | **T1** | **T1** | **T2** | **T2** | **T2** | **T1** | **T1** | **T1** | **T1** | **T1** |

**NA**: Not applicable

**NR**: Not reported

**Table 5. Summary of results from the evaluation of the level of evidence from epidemiological and *in vivo* studies reporting associations between early exposure to pesticides other than organochlorines, organophosphates, pyrethroids and carbamates and ASD in children or ASD behavioral phenotypes in rodents based on the NTP/OHAT guidelines (NTP/OHAT 2019).**

| | Pesticides | Outcomes | Level of evidence |
|---|---|---|---|
| **Epidemiological studies** | **Azoles** | | |
| | Myclobutanil | ASD risk | Moderate |
| | **Phosphonoglycine** | | |
| | Glyphosate | ASD risk | Moderate |
| | **Neonicotinoids** | | |
| | Imidacloprid | ASD risk | Inadequate |
| ***In vivo* studies** | **Neonicotinoids** | | |
| | Clothianidin | behavioral development impairment | High |
| | Imidacloprid | Learning and memory deficits | High |
| | **Phosphonoglycine** | | |
| | Glyphosate | Depression-like and anxiety presence | High |

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marion Mortamais on behalf of the authors