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NTP Research Report on the Scoping Review of Potential Human Health Effects Associated with Exposures to Neonicotinoid Pesticides

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

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For questions about the reports and studies, please email NTP or call 984-287-3211.

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Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Research Report on the Scoping Review of Potential Human Health Effects Associated with Exposures to Neonicotinoid Pesticides* by letter in February 2019 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

(1) Peer review the Draft *NTP Research Report on the Scoping Review of Potential Human Health Effects Associated with Exposures to Neonicotinoid Pesticides* and comment on the adequacy of the scoping report in identifying and summarizing the relevant literature.

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Conflict of Interest

Individuals identified as authors in the About This Report section have certified that they have no known real or apparent conflict of interest related to neonicotinoid pesticides.

Abstract

Introduction: Neonicotinoid pesticides are commonly used in the United States to control insects on domestic animals and as seed coatings on agricultural crops, such as corn and soybeans. In areas of widespread use, neonicotinoids have been observed in surface waters, produce, and prepared foods. Because these pesticides are neurotoxic to insects through insect nicotinic acetylcholine receptors (nAChRs), concerns have been raised as to whether neonicotinoids may bind to receptors in off-target species, including humans, and result in adverse health effects. The associations between exposures to neonicotinoid pesticides and potential human health effects were nominated by private individuals to the National Toxicology Program (NTP) as a potential candidate for systematic review in 2014. NTP later learned that the U.S. EPA Office of Pesticide Programs (OPP) was also evaluating currently registered neonicotinoid pesticides as part of registration review activities. Thus, NTP consulted with EPA and solicited public comments during problem formulation activities to maximize the utility of this report.

Objective: The objective of the scoping activities was to identify and summarize scientific literature indexed in PubMed reporting exposure to one or more neonicotinoid pesticides registered for use in the United States and any reported outcome relevant to human health effects.

Methods: A scoping review was conducted following the Office of Health Assessment and Translation (OHAT) method for systematic review through an abbreviated data extraction step. A comprehensive search strategy was used to retrieve original research records from PubMed that contained reports of exposure to any of seven neonicotinoid pesticides (acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, thiamethoxam, and dinotefuran) and all reported outcomes relevant to human health effects, including epidemiological, experimental animal, and in vitro model systems. Records were screened at the title-and-abstract level for relevance according to pre-specified inclusion/exclusion criteria. Included records were then screened at the full-text level to verify relevance and manually categorize studies by exposure, outcome, study type, species, and cell type, where appropriate.

Results: A total of 191 studies were included as relevant to human health effects associated with exposure to neonicotinoid pesticides, including six epidemiological studies; 19 human case reports; and 113 experimental animal studies that included rodents, fish, *Caenorhabditis elegans*, and *Drosophila*. An interactive evidence map was prepared to allow exploration of the literature by pesticide, broad health effect categories, and evidence stream (e.g., human, animal, or in vitro). Most of the research focused on imidacloprid (n = 127 records). The most commonly reported outcome category across all evidence streams was neurological effects (n = 86), whereas congenital and developmental effects were investigated in four of the six epidemiological studies. For these health categories with the most records, the study designs including measured endpoints were captured to assess the consistency of measures for potential human health hazard evaluation.

Discussion: In this scoping review, NTP compiled publicly available scientific literature on neonicotinoid exposure and health effects and summarized the state of the science and limitations of the evidence base of these data to support further health hazard and risk assessments. Various health effects have been reported in these data to be associated with exposure to neonicotinoids. However, a limited body of evidence was identified for use in health

hazard assessment, primarily due to the heterogeneity of investigated health outcomes and measured endpoints. This scoping review by design summarizes publicly available scientific literature indexed in PubMed only and does not include any proprietary studies available to EPA, which likely contain relevant toxicological data that would complement these data and allow for more comprehensive health hazard assessment.

Preface

NTP conducts scoping reviews to identify, categorize, and summarize the literature-based evidence evaluating whether exposure to environmental substances (e.g., chemicals, physical agents, and mixtures) may be associated with adverse health effects. These reviews serve as a foundational step in directing potential further inquiry by identifying areas that are data rich or data poor on project-specific key concepts such as: exposures, health effects, mechanisms, experimental model or study design, and evidence stream (human, experimental animal, in vitro models); however, they do not include a synthesis of the data. Depending on the goals and the available evidence, scoping reviews may include: (1) a summary of the research relating to specific questions or relatively broad topic areas, (2) a systematic evidence map—an interactive visual display of research relating to relatively broad topic areas that can be sorted, filtered, and categorized to illustrate the extent and types of evidence, or (3) both.

NTP conducts these health effects evaluations following the first three steps of the general methods outlined in the "Handbook for Conducting a Literature-Based Health Assessment Using the OHAT Approach for Systematic Review and Evidence Integration" [†]: (1) problem formulation, (2) literature search and selection of studies for inclusion, and (3) abbreviated data extraction to categorize published research by key concepts relevant to the goals of the review. The key feature in applying the systematic review approach to scoping reviews is the application of a transparent framework to document the methods.

[†]OHAT is the abbreviation for Office of Health Assessment and Translation, which is within the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences.

Introduction

Neonicotinoid pesticides are a class of insecticides that are neurotoxic to insects through insect nicotinic acetylcholine receptors (nAChRs) (Tan et al. 2007). Because nAChRs are also present in the nervous systems of mammals, there is concern that neonicotinoids may affect animals other than their insect targets, including humans (Keil et al. 2014; USDA 2014; Van der Sluijs et al. 2015; Yang et al. 2014). Globally, seven neonicotinoid pesticides are commercially available: imidacloprid, acetamiprid, clothianidin, thiamethoxam, thiacloprid, nitenpyram, and dinotefuran (Simon-Delso et al. 2015). These pesticides have been used increasingly in U.S. agriculture since 2005 (Douglas and Tooker 2015; Jeschke et al. 2011; Simon-Delso et al. 2015) and persist in the environment, resulting in detection in the human food supply (Bonmatin et al. 2015; Chen et al. 2014; Hladik et al. 2014; USDA 2014; FDA 2016). However, no national-level exposure data (e.g., National Health and Nutrition Examination Survey) are available for these pesticides so exposure levels in the general population are unknown. Environmental persistence and irreversible binding to nAChRs have raised concerns for potential adverse human health impacts from chronic low-level exposures (Tennekes and Sanchez-Bayo 2011; Van der Sluijs et al. 2015). Although it should be noted that binding of some neonicotinoids and their metabolites to some mammalian nAChRs is relatively weak, with lower affinity and efficacy than target nAChRs (EFSA 2013).

Since their introduction in 1990, the neonicotinoid market share increased to 24% for crop protection and 80% for seed treatment by 2008 (Jeschke et al. 2011). The patent protections for these seven neonicotinoids have expired, beginning with imidacloprid in 2005; thus, the introduction of generic versions of these pesticides has broadened the markets where these pesticides are used, including in India and China (Jeschke et al. 2011). Due to widespread use, neonicotinoids were present in all streams tested near high production areas of corn and soybean in the United States; levels correlated with rain during crop planting, implicating seed treatments as the source (Hladik et al. 2014). Neonicotinoids can enter the flesh of the fruit or vegetable, making it impossible to wash and remove residues prior to consumption (Chen et al. 2014). Although below EPA tolerances, neonicotinoid pesticide residues were detectable in many fruits and vegetables tested by the U.S. Department of Agriculture in 2013 (USDA 2014), and were among the most frequently detected pesticides on human foods by the U.S. Food and Drug Administration in 2016, which monitored pesticide residues on prepared foods as part of the Pesticide Monitoring Program (FDA 2016).

Exposure to neonicotinoid pesticides has been associated with adverse health effects in various species, including humans, other mammals, honeybees, and other wildlife (Cimino et al. 2017; Krupke et al. 2012; Mason et al. 2013; Morrissey et al. 2015; Pisa et al. 2015; Rundlof et al. 2015; Van der Sluijs et al. 2015; Whitehorn et al. 2012). Several studies have characterized the potential neurotoxic effects of neonicotinoids (Kimura-Kuroda et al. 2012; Li et al. 2011). For example, nAChRs are important for synaptic transmission and learning and memory (Levin 2002), and in vitro studies using cerebellar neurons from neonatal rats suggested that neonicotinoid pesticides can affect nAChRs in a way similar to nicotine (Kimura-Kuroda et al. 2012; Tomizawa et al. 2001). Neonicotinoids can bind the α4β2 and α7 nAChR subtypes (Li et al. 2011), and perturbation of this receptor subtype is associated with various neurological effects, including depression, schizophrenia, and neurodegenerative diseases like Alzheimer's and Parkinson's disease (Hogg et al. 2003). In addition, the α4β2 nAChR subtype plays an

important role in the developing brain, including the proliferation, migration, and differentiation of neurons and their integration into neural circuits (Dwyer et al. 2009; Role and Berg 1996). Other potential adverse health effects associated with neonicotinoid exposure include developmental and reproductive effects in mammals (Abou-Donia et al. 2008; Gu et al. 2013; Van der Sluijs et al. 2015).

The associations between neonicotinoid pesticide exposures and potential human health effects were identified as a potential candidate for systematic review. Given the interest and extent of the evidence, the National Toxicology Program (NTP) at National Institute of Environmental Health Sciences conducted this scoping review to investigate the extent of the evidence from human and nonhuman studies relevant for evaluating potential human health effects of neonicotinoid pesticides. Publicly available published health effects literature for neonicotinoid pesticides was systematically collected and categorized to develop a systematic evidence map of the key neonicotinoid pesticides (by chemical) and the related health effects, types of evidence, and gaps in research. The information and presentation of the data in this scoping review were developed to support decision-making on this topic by assessing whether the publicly available literature database is sufficient for developing hazard characterization conclusions for one or more health effects in a full systematic review or for consideration of future research on this topic.

Objective and Specific Aims

Objective

The objective of this scoping review was to identify and summarize the literature relevant to neonicotinoid pesticide exposure and human health effects.

Specific Aims

- Identify literature reporting exposure(s) to one or more neonicotinoid pesticides registered for use in the United States and all outcomes relevant to human health effects, including epidemiological, experimental animal, and in vitro model systems.
- Summarize potential health effects and mechanistic data by neonicotinoid pesticide (i.e., the extent and types of health effects studies presented as interactive evidence map and accompanying narrative summary).
- Summarize evidence available on health effects with a large amount of data (e.g., neurological).

Methods

The systematic review techniques applied in this scoping review adhered to the framework developed by Office of Health Assessment and Translation (OHAT) (Rooney et al. 2014). The OHAT Systematic Review framework consists of a seven-step process; the first three are relevant to produce a scoping review, whereas the last four are relevant for assessing study quality and synthesizing evidence. Therefore, this scoping review was restricted to the first three steps: (1) problem formulation, (2) literature search and study selection, and (3) abbreviated data extraction.

Problem Formulation

Neonicotinoid pesticides were nominated in 2014 by members of the public to the National Toxicology Program (NTP) for possible evaluation of noncancer health outcomes and exposure summary. It was unclear from the nominations and initial literature searches whether the extent or nature of the available literature was sufficient to support conclusions as to whether exposure to neonicotinoid pesticides is a hazard to human health. Therefore, as part of the problem formulation activities, NTP requested information about these pesticides on October 7, 2015, in the Federal Register and considered public comments (see https://ntp.niehs.nih.gov/go/796533). NTP learned that an ongoing systematic review activity focused on the human data was underway at George Washington University (Washington, DC). NTP partnered with Drs. Melissa Perry and Andria Cimino in their review of the epidemiological evidence to address one aspect of the nomination, which led to subsequent publication of a systematic review of the epidemiological literature on the health effects of neonicotinoids (Cimino et al. 2017).

Subsequently, NTP learned that the U.S. EPA Office of Pesticide Programs (OPP) would be evaluating currently registered neonicotinoid pesticides as part of registration review activities including risk assessments of currently registered neonicotinoid pesticides. Thus, NTP consulted with EPA to develop a scoping review protocol using the OHAT Systematic Review framework to describe the approach for the citation screen and review, and data extraction to support EPA's registration review process (Appendix B).

Chemical Selection

Seven chemicals were considered as members of the class of neonicotinoid pesticides that act on nicotinic acetylcholine receptors (nAChRs) (Jeschke et al. 2011). Each of these chemicals are sold under multiple product names and are listed by the percent of U.S. market share in 2009 (Jeschke et al. 2011):

- Imidacloprid ($1,091 million, 41%)
- Thiamethoxam ($672 million, 25%)
- Clothianidin ($439 million, 17%)
- Acetamiprid ($276 million, 10%)
- Thiacloprid ($112 million, 4%)
- Dinotefuran ($79 million, 3%)

- Nitenpyram ($8 million, 0.3%)

PECO Statement

A PECO (Population, Exposure[s], Comparator[s], and Outcome[s]) statement (Table 1) was developed to address and understand the potential effects of neonicotinoid pesticides on human health-relevant effects reported in humans, animals, and in vitro model systems (Table 1). The PECO statement is used to help develop the specific research questions, search terms, and inclusion/exclusion criteria for the systematic review (Higgins and Green 2011).

Table 1. PECO (Populations, Exposures, Comparators, Outcomes) Statement

PECO Element	Evidence
Population	**Human**: All epidemiological studies **Animal**: Nonhuman animals, including studies in laboratory animals, fish, wildlife (mammalian species), domestic pets,[a] *Drosophila*, and *C. elegans*. Studies of pesticide effects in insects (e.g., efficacy studies in target pests or off-target lethality in bees) were not considered relevant and excluded. However, studies in *Drosophila* were included since they were used to investigate biological mechanisms relevant to humans. **In vitro**: In vitro models utilizing organs, tissues, cell lines, or cellular components
Exposure	Non-acute exposure to neonicotinoid pesticides based on administered dose or concentration, biomonitoring data (e.g., urine, blood, other specimens), environmental measures (e.g., air, water levels), or job title (e.g., pesticide applicator). Acute poisonings in humans, both accidental and intentional, were not considered representative of exposures in the general population and were excluded. Relevant neonicotinoid pesticides include: Acetamiprid (CASRN 152949-80-9 or 135410-20-7) Clothianidin (CASRN 210880-92-5) Dinotefuran (CASRN 165252-70-0) Imidacloprid (CASRN 138261-41-3 or 105827-78-90) Nitenpyram (CASRN 150824-47-8) Thiacloprid (CASRN 111988-49-9) Thiamethoxam (CASRN 153719-23-4)
Comparator	Both experimental (controlled exposure or treatment) and observational studies (wildlife, ecological) should be included. Experimental studies should include an untreated or vehicle control.
Outcomes	All human health-relevant effects

CASRN = Chemical Abstract Services Registry Number.
[a]Studies of domestic pets were considered if the health of the animal was reported (not product efficacy studies e.g., killing fleas and ticks).

Literature Search

A broad literature search strategy was constructed for each chemical using: (1) common name of the chemical, (2) Chemical Abstract Services Registry Number (CASRN), and (3) retrieval of synonyms from the ChemIDplus database, which currently contains chemical names and synonyms for over 400,000 chemicals (ChemIDplus 2017). No limitations were applied in terms of health outcomes or study designs to retrieve any publications mentioning exposure to any of the included pesticide(s). Because this is a scoping review, a single database (PubMed) was selected that would be most likely to contain the relevant publications on health effects of neonicotinoid pesticides. Rather than a comprehensive search of a systematic review developed to reach hazard conclusions, this scoping review considered a single database sufficient because

the goal was to map the major health effects categories for each evidence stream and identify major gaps in the literature and principal health effects categories that might support further evaluation by a subsequent systematic review. PubMed was most recently searched on April 10, 2018 from the beginning of the database entries (full details of the search strategy are presented in Appendix A). No publication year or language limits were imposed.

Study Selection

Evidence Selection Criteria

Studies were eligible for inclusion if they satisfied the eligibility criteria in the PECO statement. Inclusion and exclusion criteria used to screen articles for relevance and eligibility at both the title-and-abstract and full-text screening stages are summarized in (Table 1). The reason for exclusion at the full-text-review stage was annotated and is reported in a study flow diagram (Figure 1). A study was excluded if it: (1) was a review, commentary, or editorial with no original data; (2) lacked relevant exposure information; (3) lacked health outcome information; (4) focused only on insects (*Droshophila* studies were included if used as a human health model); (5) was a conference abstract, thesis/dissertation; (6) the full text was "not available"; or (7) was not available in English (although the literature search did not limit based on foreign language, for this scoping document these studies were not translated and are therefore considered excluded).

The reference lists of relevant, authoritative reviews or government-authored (state and federal) technical reports identified during the initial search were hand-searched to identify additional studies that were not identified through the electronic searches. These studies were evaluated using the same inclusion and exclusion criteria and processes used for screening the electronic search results. Relevant studies identified through these steps are marked as "references identified from other sources" in the study selection diagram (Figure 1).

Screening Process

DistillerSR®, a web-based, systematic review software program with structured forms and procedures was used to screen articles for relevance and eligibility to ensure standardization of process.[a] The electronic search results were loaded in EndNote and exact article duplicates were removed prior to uploading the references to DistillerSR.

Title/Abstract Review

Two members of the evaluation design team independently conducted a title-and-abstract screen of the search results. Studies were considered possibly relevant if they met either the PECO statement, or if evaluators were unable to determine relevance from the abstract and title. If they were considered possibly relevant, studies were moved to a full-text review. Prior to beginning screening, evaluators were provided project-specific written instructions by the project lead to improve accuracy and consistency among screeners during a pilot training phase. Initially, all articles were screened by each screener and the project lead, with a comparison and discussion of

[a]DistillerSR® (http://systematic-review.net/) is a proprietary project management tool for tracking studies through the screening process and storing data extracted from these studies using user-customized forms.

discrepancies by all screeners. On the basis of these discussions, inclusion and exclusion criteria definitions were refined for clarity. After refinement of the study screening criteria, the title and abstracts of all remaining references were reviewed by any two members of the team, with any conflicts resolved through discussion with the project lead.

Full-text Review

After completion of the title/abstract screen, full-text articles were retrieved for those studies that either clearly met the inclusion criteria or where eligibility to meet the inclusion criteria was unclear. Two members of the evaluation design team independently conducted a full-text screen of the search results to determine whether a reference met the inclusion criteria. The studies possibly relevant to human health effects were then tagged by the evidence stream (e.g., human, animal, or in vitro), exposure type (i.e., neonicotinoid pesticide(s) studied), and health effect category. Substantive disagreements on inclusion of the study, abstracted data or study characteristics were resolved by discussion with the project lead to reach consensus.

Data Extraction

To develop the evidence maps for the scoping review, data extraction was limited to recording and categorizing studies in DistillerSR, Excel®, and Tableau® by key study factors to address the research question, namely evidence stream, study type, exposure, species, and health outcome. Data extraction and categorization was performed by one member of the evaluation team and checked by a second member of the evaluation team for completeness and accuracy. After initial review of the broad health categories, additional study information was extracted for a subset of studies including details on: (1) neurological endpoints measured because neurological effect was the most often-studied health outcome across all evidence streams, and (2) congenital and developmental effects because these outcomes were the most prevalent in human studies.

Data Availability

Interactive versions of each figure can be accessed directly using the link included beneath each figure. In addition, all interactive figures and additional study details can be viewed online and can be downloaded from Tableau in Microsoft Excel format here: https://doi.org/10.22427/NTP-DATA-002-00069-0001-0000-8 (NTP, 2019).

Results

Literature Search Results

The screening results and reasons for exclusion are outlined in the study selection diagram (Figure 1). The electronic database searches retrieved 3,068 individual references, and eight additional references were identified by reviewing the reference lists of published reviews. After duplicate removal, a total of 3,056 individual references were screened for study inclusion in the title-and-abstract screen. Of these, 2,706 studies were excluded as not relevant to study eligibility criteria, including more than half that mentioned relevant neonicotinoid exposures but did not contain information relevant to human health effects as defined by the eligibility criteria. These studies included pesticide efficacy studies in pets that do not report on the health of the animals, as well as studies of effects on honeybees or other nontarget organisms that did not contain information directly relevant to human health or were not designed to model human health effects (e.g., ecological hazard studies). More information on effects of the neonicotinoid pesticides clothianidin, imidacloprid, and thiamethoxam on bees is available in technical reports and risk assessments performed by the European Food Safety Authority (https://www.efsa.europa.eu/en/press/news/180228).

Of the 247 studies identified as potentially relevant to human health effects and reviewed in the full-text screen, 53 were excluded due to lack of human health effects information and 191 studies were considered relevant to study eligibility criteria and binned according to evidence stream including 25 studies in humans and 86 in mammals (Figure 1).

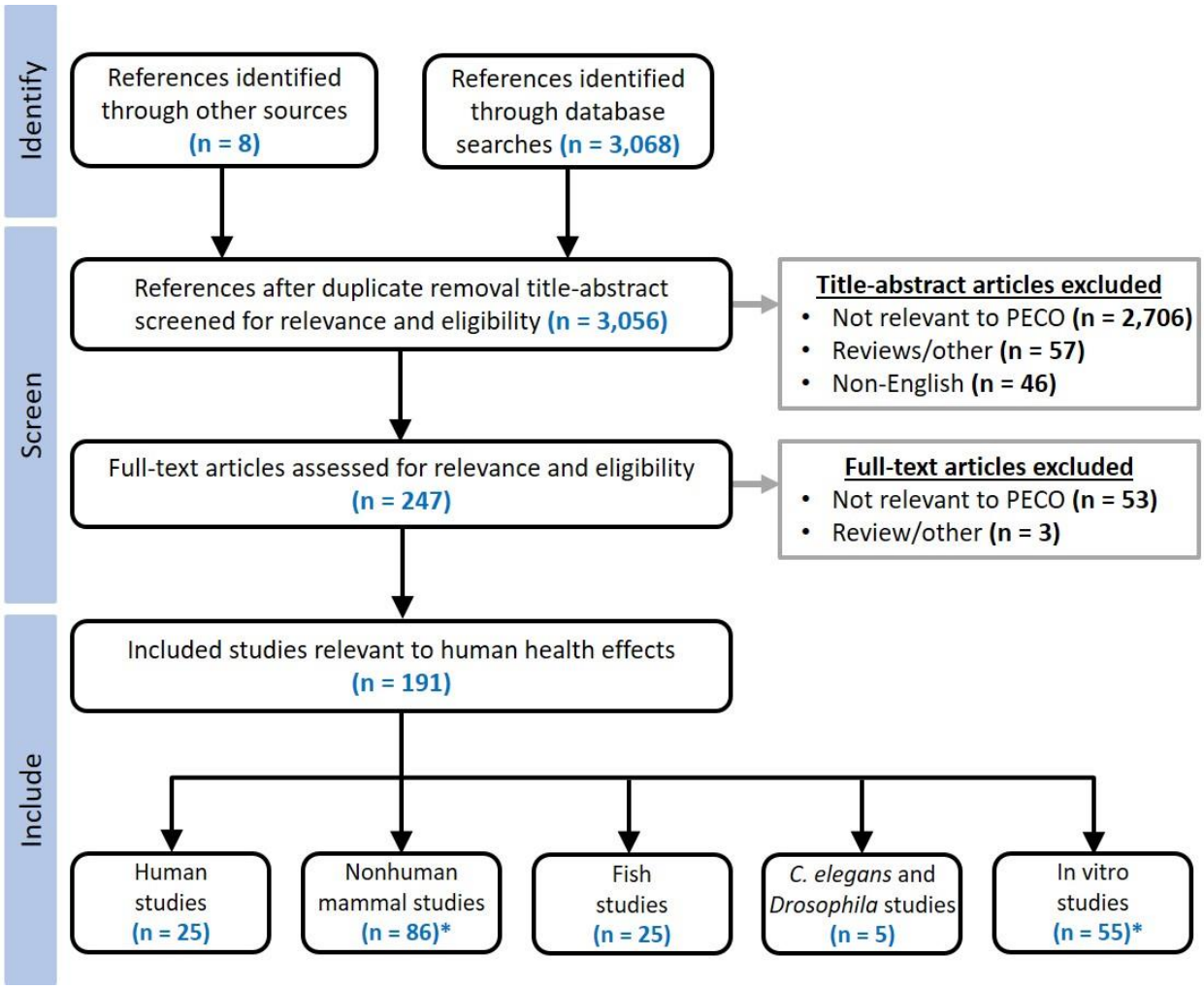

Figure 1. Study Selection Diagram

*Five publications contained data relevant to both experimental mammal studies and in vitro studies.

Human Health-relevant Studies

The 191 included studies were classified by broad health effects categories, evidence stream, and the neonicotinoid(s) studied (Figure 2); the studies were further classified by animal species and/or study type (e.g., case reports or epidemiological studies in humans) in the interactive version of the figure here: https://doi.org/10.22427/NTP-DATA-002-00069-0001-0000-8 (NTP, 2019). Note that some studies evaluated multiple health effects or evidence streams and may be listed multiple times.

		Neonicotinoid Studied							
Health Effect	Evidence Stream	Acetamiprid	Clothianidin	Dinotefuran	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Grand Total
Cancer	Animal				1			1	2
	In vitro				1		1	1	1
Cardiovascular	Animal				4		1		5
	Human case report	3	1		8		1		11
Dermal	Animal			1	3				4
	Human case report				1				1
Developmental/congenital	Animal	1	3		3	1	4	1	11
	Human epi				4				4
	In vitro	2	1		4		2	3	5
Digestive	Animal				3				3
	Human case report	3	1		9		1		12
Endocrine	Animal	1			11		1		13
	Human case report						1		1
	In vitro				3		1	1	3
Hematological	Animal	1			10		1		12
	Human case report	1			5				6
	Human epi				1				1
Immune	Animal	2		1	2	1		1	5
	In vitro		1						1
Immunological	Animal	1			10		1	2	14
	Human case report				3				3
	In vitro				1				1
Liver	Animal	1			22	1	1	7	32
	Human case report	2	1		3				4
	Human epi				1				1
	In vitro	2	2		3	1	1	2	3
Musculoskeletal	Animal				2				2
	Human case report				1				1
	In vitro				2				2
Neurological	Animal	4	8	2	24	1	3	4	42
	Human case report	3	1		14		2		18
	Human epi	1	1		3	1	1	1	3
	In vitro	8	5		20	2	4	4	24
Other	Animal	4		1	11	1	1	1	17
	Human case report				2				2
	In vitro	5	3		14		3	1	19
Renal/urinary	Animal				13				13
	Human case report				2		1		3
	Human epi				1				1
Reproduction	Animal	4	5		13		1		23
	In vitro	2			4		2	2	4
Respiratory	Animal				5				5
	Human case report	2	1		9				10
	In vitro				1				1
Grand Total		34	23	4	127	7	20	23	191

Figure 2. Number of Studies Evaluating Neonicotinoid Exposures and Outcomes in Humans and Animals

Note: Some studies may have characterized health effects of neonicotinoids in multiple evidence streams and therefore may be listed multiple times. Row and column totals and grand total shown in the figure represent counts of distinct references. Interactive figure and additional study details in Tableau (NTP, 2019).

Neurological effects were the most commonly studied health effect (86 studies with one study containing both animal and in vitro data) across all evidence streams with 42 animal studies, three human epidemiological studies, and 18 human case reports (Table 2). The majority of in vitro studies investigated endpoints associated with neurological effects (24 studies), followed by five studies of developmental effects, and 19 in vitro studies classified as "Other" effects that consisted mainly of cytotoxicity and genotoxicity studies. Aside from case reports, the largest pocket of human epidemiological evidence consisted of four studies reporting developmental or congenital outcomes. Several alternative model organism studies were identified including 25 fish studies reporting a range of effects, and three studies of *Caenorhabditis elegans* reporting developmental, reproductive, neurological, and other effects. Overall, imidacloprid was the most commonly studied neonicotinoid (127 studies), followed by acetamiprid (34 studies), clothianidin (23 studies), thiamethoxam (23 studies), thiacloprid (20 studies), nitenpyram (7 studies), and dinotefuran (4 studies).

Neurological and Congenital/Developmental Effects

On the basis of the initial categorization of health effects identified in Table 2, additional study characteristics were captured for the health outcomes with the highest number of studies to evaluate the homogeneity of the endpoints reported and determine whether enough similar evidence would be available for future hazard evaluation of any exposure-outcome pair. Therefore, the remainder of this scoping review focuses on neurological effects (the health effect with the highest number of studies across all evidence streams) and congenital/developmental effects (the highest number of human non-case report studies). Case reports were not included in the focused evaluation because they are generally a medical report on a single subject after an acute high exposure to neonicotinoids rather than a chronic, low-level exposure that may be more representative of exposures to the general population; however, the case reports may provide supporting evidence for potential health effects following high exposures and thus were included in the evidence map.

Human Studies

The study designs and outcomes for the six epidemiological studies, including five case-control studies and one cross-sectional study, are summarized in Table 2**.** All studies evaluated exposure to imidacloprid, and a general neonicotinoid exposure category. Three case-control studies used statewide pesticide use records in California and proximity to residence of participant's mother during pregnancy to estimate exposures to imidacloprid and neonicotinoids as a group in participants of the California Birth Defects Monitoring Program and reported on different types of birth defect outcomes (Carmichael et al. 2014; Shaw et al. 2014; Yang et al. 2014). A fourth case-control study conducted in California investigated the occurrence of autism spectrum disorder in children participants of the Childhood Autism Risks from Genetics and Environment (CHARGE) study whose mothers reported using flea or tick control on pets within the household during pregnancy or the participant's childhood (Carmichael et al. 2014; Keil et al. 2014; Khan et al. 2010; Shaw et al. 2014; Yang et al. 2014). The remaining case-control study compared levels of neonicotinoids in the urine (including imidacloprid, acetamiprid, thiacloprid, nitenpyram, clothianidin, and thiamethoxam) of subjects in Japan with nicotinic symptoms including muscle pain, weakness, spasm, finger tremor, or memory loss to those subjects without symptoms (Marfo et al. 2015). The only cross-sectional study measured imidacloprid levels and

evaluated biochemical parameters and clinical symptoms including dizziness and shortness of breath in pesticide workers in Pakistan compared with controls (Khan et al. 2010).

Table 2. Epidemiological Studies Evaluating Neonicotinoid Exposures and Effects

Study Design (Location/Subjects) [n]	Exposure Assessment (Life Stage at Exposure)	Outcomes	Results	Study
Case-control (California/Infants or fetuses with congenital heart defects from the California Birth Defects Monitoring Program) [101 cases, 785 controls]	Imidacloprid; neonicotinoid pesticide group Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero)	Group of 4 congenital heart defects (Tetralogy of Fallot)	↑* adjORs for imidacloprid and neonicotinoid pesticide group exposures and congenital heart defects	Carmichael et al. (2014)
Case-control (California/Child participants of the Childhood Autism Risks from Genetics and Environment study) [407 cases, 262 controls]	Imidacloprid Exposure estimated categorically (never/ever; and prenatal never/ever/consistent/occasional) based on maternal-reported household usage of flea or tick control on pets (in utero through childhood)	Autism spectrum disorder	↑* adjOR in consistent users; ↑ exposure window analysis found nonsignificant increase in adjORs during pregnancy (results were considered imprecise)	Keil et al. (2014)
Cross-sectional (Pakistan/adult pesticide industrial workers; number of industries included not reported) [238 males; 184 exposed, 54 unexposed]	Multiple pesticides including imidacloprid Exposure groups estimated based on industrial size and production of pesticides as opposed to specific chemical exposure, but imidacloprid was measured in plasma of 184 industrial workers (adult)	Hematology and clinical chemistry (several endpoints); clinical symptoms; plasma cholinesterase; oxidative stress/ inflammatory markers (4 endpoints)	↑* liver enzymes, oxidative stress markers, inflammatory markers; ↓ plasma cholinesterase levels, total antioxidants; ~~A~~ plasma bilirubin, albumin, total proteins, urea, creatinine; clinical symptoms including headache followed by dizziness, vomiting, shortness of breath, skin rash were commonly reported (in about 2–20% depending on clinical symptom and size of industry)	Khan et al. (2010)

Study Design (Location/Subjects) [n]	Exposure Assessment (Life Stage at Exposure)	Outcomes	Results	Study
Case-control (Japan/child and adult with neonicotinoid symptoms) [35 cases, 50 controls]	Multiple insecticides including acetamiprid, thiacloprid, imidacloprid, nitenpyram, clothianidin, thiamethoxam measured in spot urine samples (exposure timing unknown)	Nicotinic symptoms (headache, general fatigue, palpitations or chest pain, abdominal pain, muscle pain/weakness/spasm, cough, postural finger tremor, recent memory loss, and fever)	↑ Nicotinic symptoms in subjects with increased levels of thiamethoxam and DMAP, a metabolite of acetamiprid	Marfo et al. (2015)
Case-control (California/Infants or fetuses with gastroschisis from the California Birth Defects Monitoring Program) [156 cases, 785 controls]	Imidacloprid; neonicotinoid pesticide group Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero)	Birth defect (gastroschisis)	↑ adjORs for imidacloprid and neonicotinoid pesticide group exposures and gastroschisis	Shaw et al. (2014)
Case-control (California/Child participants of the California Birth Defects Monitoring Program) [590 cases, 785 controls]	Neonicotinoid pesticide group including imidacloprid Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero)	Birth defects (anencephaly, spina bifida, cleft lip with or without cleft palate, or cleft palate only)	↑ adjORs for anencephaly, cleft lip with or without cleft palate, cleft palate only no odds ratio calculated for spina bifida	Yang et al. (2014)

*: statistically significant change in effect reported by authors.
adjOR = adjusted odds ratio.
↑: increased effect reported by authors.
↓: decreased effect reported by authors.
~~A~~: no change in effect reported by authors.

Animal Studies

The available neurological and developmental or congenital health outcomes in animal models are summarized in Figure 3 and Figure 4, respectively. The majority of the studies were conducted in rats or mice, of various ages and strains, exposed via the oral route of exposure or injection. A few studies were reported in other animal models (fish, bats, *C. elegans*, and *Drosophila*). One animal study that mentioned neurological effects was not included as it was a case series on three dogs and, consistent with human studies, case reports were not included. Data extraction was limited to brief descriptions of measured endpoints due to the heterogeneity of endpoints and exposure concentrations investigated across the studies.

Neurological Effects

Although 41 experimental animal studies evaluated neurological effects, the data were heterogeneous with few studies using the same chemical while evaluating the same or similar endpoints (Figure 3). Acetylcholinesterase (AChE) levels after imidacloprid exposure were evaluated in the largest number of studies (nine studies in rats and two studies in fish). Learning and memory in rats and mice were also evaluated in five studies that used disparate methods to characterize different facets of learning and memory including the Morris water maze (Kara et al. 2015; Özdemir et al. 2014), T-maze (Bhaskar et al. 2017), Y-maze (Yoneda et al. 2018), and a behavioral flexibility paradigm (acquisition task followed by reversal tasks) (Sano et al. 2016). Eighteen experimental animal studies assessed the effect of neonicotinoids on motor and sensory function, including general locomotor activity, motor strength, coordination, reflexes, and pain response in rodents or zebrafish, flight path in bats, and different motor endpoints in *C. elegans* and *Drosophila*. Other neurological endpoints included anxiety, social behavior, and depression. Twenty-two animal studies investigated neurochemical or other histopathological, structural, or gene expression changes in the brain following exposure to different neonicotinoid pesticides, but as noted, there is little overlap in the specific endpoints evaluated (Figure 3).

			Neonicotinoid Studied							
Outcome Category	Specific Outcome	Species	Acetamiprid	Clothianidin	Dinotefuran	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam	Grand Total
Anxiety	Elevated plus maze	Mouse		1						1
		Rat							1	1
	Movement in light compartment	Mouse	1							1
	Novel tank exploration	Fish				1				1
	Predator avoidance	Fish				1				1
	Time spent in center zone (open field)	Mouse			1					1
		Rat				1				1
Depression	Forced swim test	Mouse			1					1
		Rat				1				1
	Tail suspension test	Mouse			1					1
Learning and Memory	Alternation behavior (Y-maze)	Mouse			1					1
	Behavioral flexibility (discrimination learning)	Mouse	1							1
	Escape time/latency and probe test (Morris water maze)	Rat		1		1				2
	Time to reach goal and path efficiency (T-maze)	Mouse				1				1
Motor and Sensory Function	Chemosensory ability	Nematode						1		1
	Flight path	Bat				1				1
	Irregular swimming, immobility, and insensitivity to stimulation	Rat				1				1
		Fish				1				1
	Motor activity (e.g., open field, activity cage, larval motility)	Mouse		3	1					4
		Rat				3			1	4
		Fish				1			1	2
		Fly				1				1
	Pain response	Mouse				1		1		1
		Rat				1				1
	Reflexes, motor strength, coordination	Mouse		2						2
		Rat	1			1				2
		Fish				1				1
	Thrashing, body bend rates	Nematode		1			1	2		2
Neurochemical	Acetylcholinesterase (e.g., activity, binding, expression)	Rat	1			7			1	9
		Fish				2				2
	Brain ATP and adenosine	Fish							1	1
	Dopamine levels or release	Rat		2					1	2
	Inflammatory cytokines	Rat				1				1
		Fish				1				1
	Oxidative stress (e.g., ROS levels, antioxidant enzyme activity)	Rat	2			4				6
		Fish				4				4
	Serotonin	Mouse			1					1
Social Behavior	Aggression (male aggressive behavior)	Mouse	1							1
	Sexual behavior	Mouse	1							1
	Shoaling	Fish				1				1
	Social interaction	Fly				1				1
Other	Brain histopathology	Mouse	2							2
		Rat	1			4				5
		Fish				1				1
	Brain weight	Mouse	1		1	1				3
		Rat	1			2				3
	Motorneuron expression	Fish							1	1
	Neuronal structure/function (e.g., hippocampal neuron density/ morphology, EEG)	Mouse				1				1
		Rat				1			1	2
	Other (e.g., gene expression, apoptosis)	Mouse	1	1	1					3
		Rat	2	1		3				6
		Fish				2				2
		Bat				1				1
		Nematode						1		1
	Repetative behavior	Fly				1				1
Grand Total			4	8	2	23	1	3	4	41

Figure 3. Number of Studies Evaluating Neonicotinoid Exposures and Neurological Outcomes in Animals

Note: Some studies may have characterized multiple neurological health effects or multiple neonicotinoids and therefore may be listed multiple times. Row and column totals and grand total shown in the figure represent counts of distinct references. Interactive figure and additional study details in Tableau (NTP, 2019).

Developmental Effects

A total of 11 animal studies were identified that reported developmental or congenital effects after exposure to a neonicotinoid pesticide (Figure 4**)**. No studies examined the effects of dinotefuran. Six rodent studies characterized the developmental effects from neonicotinoid exposure during different critical developmental windows, including gestation-only (Babelova et al. 2017; Gawade et al. 2013), gestation and lactation (Gawade et al. 2013; Sano et al. 2016; Tanaka 2012b), lactation-only (Bhaskar and Mohanty 2014), or gestation through adolescence (Gawade et al. 2013; Tanaka 2012a); one study tested imidacloprid effects at three time periods from gestation through adolescence (Gawade et al. 2013). The primary endpoints measured in these rodent studies were body weight (five studies), survival (four studies), skeletal malformations (one study), and sex ratio (one study). Neurodevelopmental effects from these studies are included with neurological effects (Figure 3)**.**

Five studies using alternative toxicological model organisms were also identified. Three zebrafish (*Danio rerio*) studies and one common carp study assessed the effects of embryonic thiamethoxam, thiacloprid, or imidacloprid exposure on hatching success, embryo development, and morphological abnormalities (Liu et al. 2018; Osterauer and Kohler 2008; Scheil and Kohler 2009; Velisek and Stara 2018). A single *C. elegans* study exposed hermaphrodite adults and their eggs to clothianidin, thiacloprid, or nitenpyram and characterized the larval development of the offspring (Kudelska et al. 2017).

		Neonicotinoid Studied						
Specific Outcome	**Species**	**Acetamiprid**	**Clothianidin**	**Imidacloprid**	**Nitenpyram**	**Thiacloprid**	**Thiamethoxam**	**Grand Total**
Body Weight	Mouse	1	2	1				4
	Rat			1				1
Embryo or Larval Development	Mouse					1		1
	Fish			1		2	1	4
	Nematode		1		1	1		1
Larvae Abnormalities	Fish					1		1
Sex Ratio	Mouse		1					1
Skeletal Malformations, Runts	Rat			1				1
Survival (fetal, postnatal, or larval)	Mouse		2					2
	Rat			1				1
	Fish					1		1
Grand Total		1	3	3	1	4	1	11

Figure 4. Number of Studies Evaluating Neonicotinoid Exposures and Developmental Outcomes in Animals

Notes: No studies examined developmental or congenital effects of dinotefuran, therefore it is not shown here. Some studies may have characterized multiple developmental health effects or multiple neonicotinoids and therefore may be listed multiple times. Row and column totals and grand total shown in the figure represent counts of distinct references. Interactive figure and additional study details in Tableau (NTP, 2019).

In Vitro Studies

In total, 29 in vitro studies were identified as relevant to human neurological or developmental outcomes, because the studies investigated relevant mechanisms or processes (e.g., effects on

nicotinic acetylcholine receptors [nAChRs] or embryonic development) or were conducted in relevant tissue models (e.g., neuron cultures). Eleven of these were related to evaluating the effects on nAChRs as this is the known mechanism in target insects (see Table 3). Eighteen in vitro studies were identified that evaluated other types of endpoints relevant to human neurological or developmental outcomes, and the details of these studies are summarized in Table 4. The majority of these 29 studies tested imidacloprid, and several studies also evaluated the effects of acetamiprid, clothianidin, thiacloprid, or thiamethoxam. Most of the studies evaluating endpoints other than nAChR (n = 10) used primary neurons or neuronal cell lines including human neuroblastoma SH-SY5Y and the rat neuroblastic cell line PC12. These studies also commonly characterized the effects of different neonicotinoids on electrophysiological properties (Alloisio et al. 2015; Camlica et al. 2018; Kimura-Kuroda et al. 2012; Meijer et al. 2015; Meijer et al. 2014), cell viability or morphology (Bal et al. 2010; Camlica et al. 2018; Christen et al. 2017; Kimura-Kuroda et al. 2016; Meijer et al. 2015; Senyildiz et al. 2018; Skandrani et al. 2006), or changes in gene or protein expression (Kawahata and Yamakuni 2018; Kimura-Kuroda et al. 2016; Skandrani et al. 2006; Sugiyama et al. 2015). Five studies evaluated the effect of neonicotinoids on developmental endpoints using a human adrenocortical cell line in conjunction with either primary human umbilical vein endothelial cells (Caron-Beaudoin et al. 2016) or a human placental choriocarcinoma cell line (Caron-Beaudoin et al. 2017), mouse or rabbit embryos (Babelova et al. 2017), mouse oocytes (Gu et al. 2013), or primary mouse Sertoli cells (Babelova et al. 2017; Caron-Beaudoin et al. 2016; Caron-Beaudoin et al. 2017; Gu et al. 2013; Kugathas et al. 2016).

Table 3. In Vitro Studies Evaluating Neonicotinoid Effects on Nicotinic Acetylcholine Receptors

Exposure	Concentrations Tested	Cell Types	Endpoints Evaluated	Study
Imidacloprid	0.01 to 1 mM	Frog oocytes expressing hybrid nAChR with subunits from various species (rat, chicken, or insect)	Evoked currents; effects of mutations on potency	Bao et al. (2016)
Acetamiprid, Clothianidin, Thiamethoxam	0.00001 to 0.01 M	Frog oocytes expressing rat recombinant nAChR	Effects on ACh-induced currents	Cartereau et al. (2017)
Imidacloprid	5 to 95 nM	*Drosophila* S2 cells expressing hybrid nAChR with subunits from various species (rat and *Drosophila*)	Affinity of agonist and antagonist binding	Lansdell and Millar (2000)
Imidacloprid	Specific concentrations not reported; only IC50 concentration of 155 nM provided	Sf9 insect cells expressing rat recombinant nAChR	Receptor binding IC50	Latli et al. (1999)
Imidacloprid; Clothianidin	Up to 300 μM	HEK 293 cell line stably expressing human nAChR	Receptor activation; evoked currents; dose-response relationship of receptor antagonism	Li et al. (2011)

Exposure	Concentrations Tested	Cell Types	Endpoints Evaluated	Study
Imidacloprid	About 10 to 500 μM	Frog oocytes expressing nAChRs from embryonic rat muscle	Membrane and single-channel currents	Methfessel (1992)
Imidacloprid	10 μM	Rat adrenal PC12 cell line	nAChR-channel modifications	Nagata et al. (1996)
Imidacloprid	10 μM; 0.01, 0.1, 10, 30 μM	Rat brain PC12 cell line	nAChR-channel modifications; whole-cell current electrical activity	Nagata et al. (1998)
Imidacloprid	Specific concentrations not reported; only IC50 of 14 μM provided	Human neuroblastoma SH-SY5Y cell line; rodent brain membranes	Structure activity relationships for receptor binding	Tomizawa and Casida (1999)
Imidacloprid; Thiacloprid	Up to 0.1 mM	Mouse fibroblast M10 cell line stably expressing chicken nAChR	Receptor binding affinity	Tomizawa and Casida (2000)
Imidacloprid; Thiacloprid	Up to about 1 mM	Mouse fibroblast M10 cell line	Receptor binding affinity; agonist potency; antinociceptive effects	Tomizawa et al. (2001)

nAChR = nicotinic acetylcholine receptors; HEK = human embryonic kidney; PC12 = pheochromocytoma.

Table 4. In Vitro Studies Evaluating Neonicotinoid Exposures and Neurological and Developmental Effects

Exposure	Concentrations Tested	Cell Types	Endpoints Evaluated	Study
Neurological Endpoints				
Imidacloprid	1, 10, 100 pM; 1,10, 100 μM	Primary rat cortical neurons	Spontaneous electrical activity	Alloisio et al. (2015)
Imidacloprid	1, 10, 50, 100, 1000 μM	Primary mouse brain stellate cells	Cell membrane activity	Bal et al. (2010)
Acetamiprid	1, 10, 100, 1000 μM	Frog sciatic nerves	Nerve action potential amplitude and area; nerve histopathology	Camlica et al. (2018)
Acetamiprid, Clothianidin, Imidacloprid, Thiamethoxam	1, 10, 100 μM	Rat PC12 cell line	Neurite outgrowth; gene expression	Christen et al. (2017)
Acetamiprid, Clothianidin, Thiacloprid, Thiamethoxam	1 μM to 1 mM (specific concentrations not reported for each compound)	Purified electric eel AChE	Inhibition of AChE activity; AChE binding kinetics	Gyori et al. (2017)
Imidacloprid	3, 30, 100 μM	Rat adrenal medulla PC12D cell line; primary rat adrenal chromaffin cells	TH and PNMT gene and protein expression; catecholamine secretion; dopamine content; adrenaline biosynthesis	Kawahata and Yamakuni (2018)
Acetamiprid, Imidacloprid	1, 10, 100 μM	Rat neonatal cerebellar neurons	Ca^{2+} influxes; neural excitation	Kimura-Kuroda et al. (2012)
Acetamiprid, Imidacloprid	1 μM	Rat neonatal cerebellar neurons	Gene expression; neuron branching	Kimura-Kuroda et al. (2016)
Imidacloprid	10 μM	Rat adrenal PC12 cell line	Basal Ca^{2+} levels; inhibition of depolarization-evoked Ca^{2+}	Meijer et al. (2014)
Imidacloprid	0.1, 1, 10, 100 μM (cell viability) 10 μM (depolarization)	Rat adrenal PC12 cell line	Cell viability; inhibition of depolarization-evoked Ca^{2+}	Meijer et al. (2015)
Acetamiprid, Clothianidin, Imidacloprid, Thiacloprid, Thiamethoxam	0.125, 0.25, 0.5, 1, 2, 4 mM (cytotoxicity, cell viability) 50, 100, 200, 500 μM (DNA damage)	Human neuroblastoma cell line (SH-SY5Y)	Cytotoxicity; DNA damage	Senyildiz et al. (2018)

Exposure	Concentrations Tested	Cell Types	Endpoints Evaluated	Study
Imidacloprid	50 to 800 μg/mL	Human neuronal cell line (SH-SY5Y)	Cell growth; GRP expression, HSP expression; SH-SY5Y toxicity	Skandrani et al. (2006)
Nitenpyram	0.1, 1, 10, 100 μM	Cortical neurons	GluR2 protein expression; glutamate toxicity	Sugiyama et al. (2015)
Developmental Endpoints				
Acetamiprid, Clothianidin, Thiacloprid, Thiamethoxam	0.1, 1, 10, and 100 μM	Fertilized mouse and rabbit embryos	Embryo development; blastomere number; cell death; blastocyst quality	Babelova et al. (2017)
Imidacloprid, Thiacloprid, Thiamethoxam	0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 μM (varied by chemical and endpoint)	Human adrenocortical carcinoma cell line (H295R) and primary HUVEC cells	Aromatase activity; CYP19 expression; cell viability	Caron-Beaudoin et al. (2016)
Imidacloprid, Thiacloprid, Thiamethoxam	0.1, 0.3, 3, 10 μM (varied by chemical and endpoint)	Human adrenocortical carcinoma cell line (H295R) and human placental choriocarcinoma cell line (BeWo)	CYP19 catalytic activity (aromatase activity); hormone production; CYP3A7 and SULT2A1 expression	Caron-Beaudoin et al. (2017)
Acetamiprid, Imidacloprid	0.5, 5 mM	Mouse oocytes, sperm, fertilized zygotes, fertilized embryos	Sperm DNA integrity; sperm fertilization capability; embryo development; zygote development	Gu et al. (2013)
Imidacloprid	1 nM to 0.1 mM (only IC50 reported, not specific concentrations)	Primary juvenile mouse Sertoli SC5 cells	Anti-androgenic activity; COX enzyme expression	Kugathas et al. (2016)

PC12 = pheochromocytoma; PC12D = chromaffin-cell tumor; AChE = acetylcholinesterase; GRP = glucose regulated protein; HSP = heat shock protein; HUVEC = human umbilical vein endothelial; COX = cyclooxygenase; TH = tyrosine hydroxylase; PNMT = phenylethanolamine N-methyltransferase.

Discussion

Using systematic review methods, this scoping review of publicly available literature indexed in PubMed identified a heterogenous body of evidence on potential health effects data associated with neonicotinoid exposures. The interactive evidence map allows researchers to sort and explore the literature by pesticide, broad health effect categories, and evidence stream (Figure 2). Although 191 studies were identified as potentially relevant to the human health effects of neonicotinoids, the studies varied considerably by endpoints examined and evidence stream as well as by study design, including human observational studies, case series and case reports in humans and animals, and dozens of experimental studies in animals, including rodents, primates, fish, *C. elegans*, and *Drosophila*. The clear majority of the research has been performed on the most widely used neonicotinoid—imidacloprid (127 publications)—with as few as four publications identified for each of the other six chemicals (n = 4–34 publications). Neonicotinoids are insecticides that are neurotoxic to insects through insect nicotinic acetylcholine receptors (nAChRs) and, as might be expected from this mechanism, neurological effects were the most-studied health effects across all evidence streams including an array of endpoints measured in experimental animal studies (Figure 3).

Twenty-five studies of humans mentioned neonicotinoid exposure in relation to specific health effects. The majority of the human evidence is limited to case reports (n = 19), which covered a range of observed human health effects from neurological effects to hematological and cardiovascular effects after acute, high-exposure scenarios. In addition to the case reports, there were five case-control studies and one cross-sectional study (Table 2). While four of the case-control studies evaluated congenital/developmental effects, they all evaluated different developmental effects, including various birth defects, such as congenital heart defects (Carmichael et al. 2014; Keil et al. 2014; Shaw et al. 2014; Yang et al. 2014), gastroschisis (Shaw et al. 2014), and anencephaly, cleft lip, and spina bifida (Yang et al. 2014). One measured autism spectrum disorder, which was also considered a neurological effect (Keil et al. 2014). The remaining case-control and cross-sectional studies reported neurological symptoms and biochemical measures (Khan et al. 2010; Marfo et al. 2015). The limited number of epidemiological studies is a critical data gap for assessing the potential health effects of neonicotinoids, as described in more detail in a previously published systematic review (Cimino et al. 2017). Additional high-quality studies on the same or related endpoints would help develop bodies of evidence for reaching conclusions on the potential association between exposure to neonicotinoids and any specific health effect.

All animal models were considered relevant to this review if used to investigate mechanisms potentially relevant to human health (e.g., *Drosophila* studies). However, studies focused solely on the efficacy of pesticides to insect pests or those that described effects on nontarget insects were not included in this review. Using these criteria, a relatively large body of animal evidence was discovered with 86 studies identified that used nonhuman mammalian models, 25 that used fish, and five using *C. elegans* or *Drosophila* (Figure 2). Despite these relatively high numbers, considerable heterogeneity was observed in study designs and investigated health effects. The largest pocket of studies within a related health category was for neurological outcomes (Figure 3). Although 42 of the studies evaluated neurological effects (one of which was a case series in three dogs), the endpoints varied with few covering the same category of neurological

effect (e.g., anxiety, motor function, or learning and memory) or specific endpoint measured (e.g., acetylcholinesterase, escape time in the Morris maze, flight path in bats).

Although 29 in vitro studies were also relevant to neurological or developmental effects, 11 of these studied the effects on mammalian nAChR only (Table 3**)**, and too few studies investigating other similar endpoints were available to evaluate any additional potential specific mechanism or mode of action for biological plausibility using in vitro data (Table 4). The nAChR-dependent mechanism by which neonicotinoids exert effects on target organisms (insects) is well established; however, interactions of neonicotinoids with human receptors are less clear. Case reports of acute poisonings have described nicotinic-like symptoms (see human case reports in Figure 2), and decreased plasma cholinesterase levels and nicotinic symptoms were reported in subjects with higher measured neonicotinoid levels in plasma or urine, respectively (Table 2) (Khan et al. 2010; Marfo et al. 2015). However, these data are limited to a small number of exposed cases outside of the United States and included acute exposures and co-exposures to multiple pesticides not limited to neonicotinoids.

In addition to the studies considered relevant for human health effects, a large body of evidence was also available from studies on neonicotinoids that did not meet the study eligibility criteria due to the lack of endpoints directly relevant to human health. The majority of excluded neonicotinoid studies that did not report human health effects-related endpoints reported target insect responses and included efficacy studies in pets (e.g., killing fleas on pets). A large proportion of studies evaluated ecological effects including effects on off-target insects or other invertebrates, such as honeybees, which are beyond the scope of this review of human health effects as their nAChRs are distinct from mammals. However, a few studies focused on exposure and/or pharmacokinetics (i.e., absorption, distribution, metabolism, or excretion in human, animal, or in vitro studies) for neonicotinoids that could be useful when evaluating human health effects.

Limitations of the Scoping Review

There were several limitations to the approaches used to generate this scoping review and systematic evidence map. Because this is a scoping review, only one database, PubMed, was queried to address extent of evidence, major health effects studied, and likelihood that publicly available data would support a systematic review to reach hazard conclusions. If performing a full systematic review, searching of multiple scientific literature databases would be required to ensure inclusion of all published literature, and could also include searches of grey literature to complement the publicly available literature base.

The study selection strategy was limited to publications in English and did not include review of 46 studies published in languages other than English that may have provided additional useful data. Any further attempts to perform systematic review should include screening of these publications.

The coding of specific study details and categorization of factors in addition to evidence stream and broad health categories were focused only on the health outcomes with the most studies, neurological and congenital/developmental, to evaluate consistency across specific reported health endpoints. Because the total numbers of studies in other health outcome categories were so few, it is unlikely that health effects data from the open literature associated with

neonicotinoid exposure in outcome categories other than neurological and congenital/developmental could be synthesized for human health hazard evaluation. Because this was a scoping review and not a full systematic review, individual study quality or risk of bias was not considered.

Summary

Almost 200 studies were identified in the publicly available scientific literature as providing relevant information to inform the understanding of the potential human health effects associated with exposures to neonicotinoid pesticides. To facilitate future health hazard assessments of these pesticides, more studies using consistent measurement of endpoints across health outcome categories on neonicotinoid pesticides other than imidacloprid are needed.

References

Abou-Donia MB, Goldstein LB, Bullman S, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA. 2008. Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exposure. J Toxicol Environ Health A. 71(2):119-130. http://dx.doi.org/10.1080/15287390701613140

Alloisio S, Nobile M, Novellino A. 2015. Multiparametric characterisation of neuronal network activity for in vitro agrochemical neurotoxicity assessment. Neurotoxicology. 48:152-165. http://dx.doi.org/10.1016/j.neuro.2015.03.013

Babelova J, Sefcikova Z, Cikos S, Spirkova A, Kovarikova V, Koppel J, Makarevich AV, Chrenek P, Fabian D. 2017. Exposure to neonicotinoid insecticides induces embryotoxicity in mice and rabbits. Toxicology. 392:71-80. http://dx.doi.org/10.1016/j.tox.2017.10.011

Bal R, Erdogan S, Theophilidis G, Baydas G, Naziroglu M. 2010. Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording. Neurotoxicology. 31(1):113-120. http://dx.doi.org/10.1016/j.neuro.2009.10.004

Bao H, Shao X, Zhang Y, Cheng J, Wang Y, Xu X, Fang J, Liu Z, Li Z. 2016. IPPA08 allosterically enhances the action of imidacloprid on nicotinic acetylcholine receptors. Insect Biochem Mol Biol. 79:36-41. http://dx.doi.org/10.1016/j.ibmb.2016.10.010

Bhaskar R, Mishra AK, Mohanty B. 2017. Neonatal exposure to endocrine disrupting chemicals impairs learning behaviour by disrupting hippocampal organization in male Swiss albino mice. Basic Clin Pharmacol Toxicol. http://dx.doi.org/10.1111/bcpt.12767

Bhaskar R, Mohanty B. 2014. Pesticides in mixture disrupt metabolic regulation: In silico and in vivo analysis of cumulative toxicity of mancozeb and imidacloprid on body weight of mice. Gen Comp Endocrinol. 205:226-234. http://dx.doi.org/10.1016/j.ygcen.2014.02.007

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EA et al. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ Sci Pollut Res Int. 22(1):35-67. http://dx.doi.org/10.1007/s11356-014-3332-7

Camlica Y, Bediz SC, Comelekoglu U, Yilmaz SN. 2018. Toxic effect of acetamiprid on Rana ridibunda sciatic nerve (electrophysiological and histopathological potential). Drug Chem Toxicol. 42(3):264-269.

Carmichael SL, Yang W, Roberts E, Kegley SE, Padula AM, English PB, Lammer EJ, Shaw GM. 2014. Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. Environ Res. 135:133-138. http://dx.doi.org/10.1016/j.envres.2014.08.030

Caron-Beaudoin E, Denison MS, Sanderson JT. 2016. Effects of neonicotinoids on promoter-specific expression and activity of aromatase (CYP19) in human adrenocortical carcinoma (H295R) and primary umbilical vein endothelial (HUVEC) cells. Toxicol Sci. 149(1):134-144.

Caron-Beaudoin E, Viau R, Hudon-Thibeault AA, Vaillancourt C, Sanderson JT. 2017. The use of a unique co-culture model of fetoplacental steroidogenesis as a screening tool for endocrine disruptors: The effects of neonicotinoids on aromatase activity and hormone production. Toxicol Appl Pharmacol. 332:15-24. http://dx.doi.org/10.1016/j.taap.2017.07.018

Cartereau A, Martin C, Thany SH. 2017. Neonicotinoid insecticides differently modulate acetycholine-induced currents on mammalian alpha7 nicotinic acetylcholine receptors. Br J Pharmacol. 175(11):1987-1998. http://dx.doi.org/10.1111/bph.14018

ChemIDplus. 2017. ChemIDplus – a TOXNET database. Bethesda, MD: National Institutes of Health, National Library of Medicine.https://chem.nlm.nih.gov/chemidplus/.

Chen M, Tao L, McLean J, Lu C. 2014. Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures. J Agric Food Chem. 62(26):6082-6090. http://dx.doi.org/10.1021/jf501397m

Christen V, Rusconi M, Crettaz P, Fent K. 2017. Developmental neurotoxicity of different pesticides in PC-12 cells in vitro. Toxicol Appl Pharmacol. 325:25-36. http://dx.doi.org/10.1016/j.taap.2017.03.027

Cimino AM, Boyles AL, Thayer KA, Perry MJ. 2017. Effects of neonicotinoid pesticide exposure on human health: A systematic review. Environ Health Perspect. 125(2):155-162. http://dx.doi.org/10.1289/EHP515

Douglas MR, Tooker JF. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. Environ Sci Technol. 49(8):5088-5097. http://dx.doi.org/10.1021/es506141g

Dwyer JB, McQuown SC, Leslie FM. 2009. The dynamic effects of nicotine on the developing brain. Pharmacol Ther. 122(2):125-139. http://dx.doi.org/10.1016/j.pharmthera.2009.02.003

European Food Safety Authority (EFSA). 2013. Scientific opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid. EFSA Journal. 11(12):3471. https://dx.doi.org/10.2903/j.efsa.2013.3471

Gawade L, Dadarkar SS, Husain R, Gatne M. 2013. A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. Food Chem Toxicol. 51:61-70. http://dx.doi.org/10.1016/j.fct.2012.09.009

Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, Yuan Y, Xu Y, Liu M, Shi HJ et al. 2013. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro. PLoS One. 8(7):e70112. http://dx.doi.org/10.1371/journal.pone.0070112

Gyori J, Farkas A, Stolyar O, Szekacs A, Mortl A, Wehovsky A. 2017. Inhibitory effects of four neonicotinoid active ingredients on acetylcholine esterase activity. Acta Biol Hung. 68(4):345-357. http://dx.doi.org/10.1556/018.68.2017.4.1

Higgins J, Green S. 2011. Cochrane handbook for systematic reviews of interventions. The Cochrane Collaboration.

Hladik ML, Kolpin DW, Kuivila KM. 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. Environ Pollut. 193:189-196.

Hogg RC, Raggenbass M, Bertrand D. 2003. Nicotinic acetylcholine receptors: From structure to brain function. Rev Physiol Biochem Pharmacol. 147:1-46. http://dx.doi.org/10.1007/s10254-003-0005-1

Jeschke P, Nauen R, Schindler M, Elbert A. 2011. Overview of the status and global strategy for neonicotinoids. J Agric Food Chem. 59(7):2897-2908. http://dx.doi.org/10.1021/jf101303g

Kara M, Yumrutas O, Demir CF, Ozdemir HH, Bozgeyik I, Coskun S, Eraslan E, Bal R. 2015. Insecticide imidacloprid influences cognitive functions and alters learning performance and related gene expression in a rat model. Int J Exp Pathol. 96(5):332-337. http://dx.doi.org/10.1111/iep.12139

Kawahata I, Yamakuni T. 2018. Imidacloprid, a neonicotinoid insecticide, facilitates tyrosine hydroxylase transcription and phenylethanolamine N-methyltransferase mRNA expression to enhance catecholamine synthesis and its nicotine-evoked elevation in PC12D cells. Toxicology. 394:84-92. http://dx.doi.org/10.1016/j.tox.2017.12.004

Keil AP, Daniels JL, Hertz-Picciotto I. 2014. Autism spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: The CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Environ Health. 13(1):3.

Khan DA, Hashmi I, Mahjabeen W, Naqvi TA. 2010. Monitoring health implications of pesticide exposure in factory workers in Pakistan. Environ Monit Assess. 168(1-4):231-240. http://dx.doi.org/10.1007/s10661-009-1107-2

Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M, Kawano H. 2012. Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats. PLoS One. 7(2):e32432. http://dx.doi.org/10.1371/journal.pone.0032432

Kimura-Kuroda J, Nishito Y, Yanagisawa H, Kuroda Y, Komuta Y, Kawano H, Hayashi M. 2016. Neonicotinoid insecticides alter the gene expression profile of neuron-enriched cultures from neonatal rat cerebellum. Int J Environ Res Public Health. 13(10):987. http://dx.doi.org/10.3390/ijerph13100987

Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One. 7(1):e29268. http://dx.doi.org/10.1371/journal.pone.0029268

Kudelska MM, Holden-Dye L, O'Connor V, Doyle DA. 2017. Concentration-dependent effects of acute and chronic neonicotinoid exposure on the behaviour and development of the nematode Caenorhabditis elegans. Pest Manag Sci. 73(7):1345-1351. http://dx.doi.org/10.1002/ps.4564

Kugathas S, Audouze K, Ermler S, Orton F, Rosivatz E, Scholze M, Kortenkamp A. 2016. Effects of common pesticides on prostaglandin D2 (PGD2) inhibition in SC5 mouse Sertoli cells, evidence of binding at the COX-2 active site, and implications for endocrine disruption. Environ Health Perspect. 124(4):452-459. http://dx.doi.org/10.1289/ehp.1409544

Lansdell SJ, Millar NS. 2000. The influence of nicotinic receptor subunit composition upon agonist, alpha-bungarotoxin and insecticide (imidacloprid) binding affinity. Neuropharmacology. 39(4):671-679. http://dx.doi.org/10.1016/S0028-3908(99)00170-7

Latli B, D'Amour K, Casida JE. 1999. Novel and potent 6-chloro-3-pyridinyl ligands for the alpha4beta2 neuronal nicotinic acetylcholine receptor. J Med Chem. 42(12):2227-2234. http://dx.doi.org/10.1021/jm980721x

Levin ED. 2002. Nicotinic receptor subtypes and cognitive function. J Neurobiol. 53(4):633-640.

Li P, Ann J, Akk G. 2011. Activation and modulation of human alpha4beta2 nicotinic acetylcholine receptors by the neonicotinoids clothianidin and imidacloprid. J Neurosci Res. 89(8):1295-1301. http://dx.doi.org/10.1002/jnr.22644

Liu X, Zhang Q, Li S, Mi P, Chen D, Zhao X, Feng X. 2018. Developmental toxicity and neurotoxicity of synthetic organic insecticides in zebrafish (Danio rerio): A comparative study of deltamethrin, acephate, and thiamethoxam. Chemosphere. 199:16-25. http://dx.doi.org/10.1016/j.chemosphere.2018.01.176

Marfo JT, Fujioka K, Ikenaka Y, Nakayama SM, Mizukawa H, Aoyama Y, Ishizuka M, Taira K. 2015. Relationship between urinary N-desmethyl-acetamiprid and typical symptoms including neurological findings: A prevalence case-control study. PLoS One. 10(11):e0142172. http://dx.doi.org/10.1371/journal.pone.0142172

Mason R, Tennekes H, Sánchez-Bayo F, Jepsen PU. 2013. Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. J Environ Immun Tox. 1(1):3-12. http://dx.doi.org/10.7178/jeit.1

Meijer M, Brandsema JA, Nieuwenhuis D, Wijnolts FM, Dingemans MM, Westerink RH. 2015. Inhibition of voltage-gated calcium channels after subchronic and repeated exposure of PC12 cells to different classes of insecticides. Toxicol Sci. 147(2):607-617. http://dx.doi.org/10.1093/toxsci/kfv154

Meijer M, Dingemans MM, van den Berg M, Westerink RH. 2014. Inhibition of voltage-gated calcium channels as common mode of action for (mixtures of) distinct classes of insecticides. Toxicol Sci. 141(1):103-111. http://dx.doi.org/10.1093/toxsci/kfu110

Methfessel C. 1992. Effect of imidacloprid on the acetylcholine receptor of rat muscle. Pflanzenschutz-Nachr Bayer. 45:369-380.

Morrissey CA, Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, Liber K. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environ Int. 74:291-303. http://dx.doi.org/10.1016/j.envint.2014.10.024

Nagata K, Aistrup GL, Song JH, Narahashi T. 1996. Subconductance-state currents generated by imidacloprid at the nicotinic acetylcholine receptor in PC 12 cells. Neuroreport. 7(5):1025-1028. http://dx.doi.org/10.1097/00001756-199604100-00014

Nagata K, Song JH, Shono T, Narahashi T. 1998. Modulation of the neuronal nicotinic acetylcholine receptor-channel by the nitromethylene heterocycle imidacloprid. J Pharmacol Exp Ther. 285(2):731-738.

National Toxicology Program (NTP). 2019. Tableau data on the scoping review of health effects of neonicotinoid pesticides. https://doi.org/10.22427/NTP-DATA-002-00069-0001-0000-8.

Osterauer R, Kohler HR. 2008. Temperature-dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (Danio rerio). Aquat Toxicol. 86(4):485-494. http://dx.doi.org/10.1016/j.aquatox.2007.12.013

Özdemir HH, Kara M, Yumrutas O, Uckardes F, Eraslan E, Demir CF, Bal R. 2014. Determination of the effects on learning and memory performance and related gene expressions of clothianidin in rat models. Cogn Neurodyn. 8(5):411-416. http://dx.doi.org/10.1007/s11571-014-9293-1

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M et al. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res Int. 22(1):68-102. http://dx.doi.org/10.1007/s11356-014-3471-x

Role LW, Berg DK. 1996. Nicotinic receptors in the development and modulation of CNS synapses. Neuron. 16(6):1077-1085. http://dx.doi.org/10.1016/S0896-6273(00)80134-8

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. 122(7):711-718. http://dx.doi.org/10.1289/ehp.1307972

Rundlof M, Andersson GK, Bommarco R, Fries I, Hederstrom V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J et al. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature. 521(7550):77-80. http://dx.doi.org/10.1038/nature14420

Sano K, Isobe T, Yang J, Win-Shwe TT, Yoshikane M, Nakayama SF, Kawashima T, Suzuki G, Hashimoto S, Nohara K et al. 2016. In utero and lactational exposure to acetamiprid induces abnormalities in socio-sexual and anxiety-related behaviors of male mice. Front Neurosci. 10:228. http://dx.doi.org/10.3389/fnins.2016.00228

Scheil V, Kohler HR. 2009. Influence of nickel chloride, chlorpyrifos, and imidacloprid in combination with different temperatures on the embryogenesis of the zebrafish Danio rerio. Arch Environ Contam Toxicol. 56(2):238-243. http://dx.doi.org/10.1007/s00244-008-9192-8

Senyildiz M, Kilinc A, Ozden S. 2018. Investigation of the genotoxic and cytotoxic effects of widely used neonicotinoid insecticides in HepG2 and SH-SY5Y cells. Toxicol Ind Health. 34(6):375-383. http://dx.doi.org/10.1177/0748233718762609

Shaw GM, Yang W, Roberts E, Kegley SE, Padula A, English PB, Carmichael SL. 2014. Early pregnancy agricultural pesticide exposures and risk of gastroschisis among offspring in the San Joaquin Valley of California. Birth Defects Res A Clin Mol Teratol. 100(9):686-694. http://dx.doi.org/10.1002/bdra.23263

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V et al. 2015. Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. Environ Sci Pollut Res Int. 22(1):5-34. http://dx.doi.org/10.1007/s11356-014-3470-y

Skandrani D, Gaubin Y, Beau B, Murat JC, Vincent C, Croute F. 2006. Effect of selected insecticides on growth rate and stress protein expression in cultured human A549 and SH-SY5Y cells. Toxicol In Vitro. 20(8):1378-1386.

Sugiyama C, Kotake Y, Yamaguchi M, Umeda K, Tsuyama Y, Sanoh S, Okuda K, Ohta S. 2015. Development of a simple measurement method for GluR2 protein expression as an index of neuronal vulnerability. Toxicol Rep. 2:450-460. http://dx.doi.org/10.1016/j.toxrep.2014.12.014

Tan J, Galligan JJ, Hollingworth RM. 2007. Agonist actions of neonicotinoids on nicotinic acetylcholine receptors expressed by cockroach neurons. Neurotoxicology. 28(4):829-842. http://dx.doi.org/10.1016/j.neuro.2007.04.002

Tanaka T. 2012a. Effects of maternal clothianidin exposure on behavioral development in F(1) generation mice. Toxicol Ind Health. 28(8):697-707. http://dx.doi.org/10.1177/0748233711422726

Tanaka T. 2012b. Reproductive and neurobehavioral effects of clothianidin administered to mice in the diet. Birth Defects Res B Dev Reprod Toxicol. 95(2):151-159. http://dx.doi.org/10.1002/bdrb.20349

Tennekes HA, Sanchez-Bayo F. 2011. Time-dependent toxicity of neonicotinoids and other toxicants: Implications for a new approach to risk assessment. J Environment Analytic Toxicol. S4:001.

Tomizawa M, Casida JE. 1999. Minor structural changes in nicotinoid insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. Br J Pharmacol. 127(1):115-122. http://dx.doi.org/10.1038/sj.bjp.0702526

Tomizawa M, Casida JE. 2000. Imidacloprid, thiacloprid, and their imine derivatives up-regulate the alpha 4 beta 2 nicotinic acetylcholine receptor in M10 cells. Toxicol Appl Pharmacol. 169(1):114-120. http://dx.doi.org/10.1006/taap.2000.9057

Tomizawa M, Cowan A, Casida JE. 2001. Analgesic and toxic effects of neonicotinoid insecticides in mice. Toxicol Appl Pharmacol. 177(1):77-83. http://dx.doi.org/10.1006/taap.2001.9292

U.S. Department of Agriculture (USDA). 2014. Pesticide Data Program—Annual Summary, Calendar Year 2013.

U.S. Food and Drug Administration (FDA). 2016. Pesticide Monitoring Program: Fiscal Year 2012 Pesticide Report. Food and Drug Administration.http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/Pesticides/UCM432758.pdf. [Accessed: 28 April 2017]

Van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bijleveld van Lexmond MFIJ, Bonmatin JM, Chagnon M, Downs CA, Furlan L, Gibbons DW, Giorio C et al. 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ Sci Pollut Res. 22:148-154. http://dx.doi.org/10.1007/s11356-014-3229-5

Velisek J, Stara A. 2018. Effect of thiacloprid on early life stages of common carp (Cyprinus carpio). Chemosphere. 194:481-487. http://dx.doi.org/10.1016/j.chemosphere.2017.11.176

Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 336(6079):351-352. http://dx.doi.org/10.1126/science.1215025

Yang W, Carmichael SL, Roberts EM, Kegley SE, Padula AM, English PB, Shaw GM. 2014. Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California. Am J Epidemiol. 179(6):740-748. http://dx.doi.org/10.1093/aje/kwt324

Yoneda N, Takada T, Hirano T, Yanai S, Yamamoto A, Mantani Y, Yokoyama T, Kitagawa H, Tabuchi Y, Hoshi N. 2018. Peripubertal exposure to the neonicotinoid pesticide dinotefuran affects dopaminergic neurons and causes hyperactivity in male mice. J Vet Med Sci. http://dx.doi.org/10.1292/jvms.18-0014

Appendix A. Literature Search Strategy

Table A-1. Literature Search Strategy

Database	Search Terms
PubMed (Date of search: July 20, 2015; 2,051 results. Literature updates: November 17, 2015 (136 results); March 24, 2017 (672 results); April 10, 2018 (454 results))	Acetamiprid[nm] OR acetamiprid[tiab] OR mospilan[tiab] OR clothianidin[tiab] OR "((e)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine)"[nm] OR Dantop[tiab] OR Dinotefuran[nm] OR dinotefuran[tiab] OR 165252-70-0[rn] OR "1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine"[tiab] OR Imidacloprid[nm] OR imidacloprid[tiab] OR 105827-78-9[rn] OR premise-75[tiab] OR "1-((6-Chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine"[tiab] OR comodor[tiab] OR confidor[tiab] OR coretect[tiab] OR couraze[tiab] OR imicide[tiab] OR proagro[tiab] OR provado[tiab] OR Nitenpyram[nm] OR nitenpyram[tiab] OR Capstar[tiab] OR Thiacloprid[nm] OR thiacloprid[tiab] OR Biscaya[tiab] OR Thiamethoxam[nm] OR thiamethoxam[tiab] OR 153719-23-4[rn] OR Actara[tiab]

Appendix B. Supplemental Files

The following supplemental files are available at https://doi.org/10.22427/NTP-DATA-002-00069-0001-0000-8.

B.1. Protocol Information

Protocol
nachrs_protocol_508.pdf

B.2. Tableau Dataset

Excel Data File
neonics_tableau_data.xlsx

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