National Toxicology Program
U.S. Department of Health and Human Services

PROTOCOL FOR SCOPING REVIEW OF

HEALTH EFFECTS OF NEONICOTINOID PESTICIDES

November 2017

Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

TABLE OF CONTENTS

LIST OF TABLES

BACKGROUND AND SIGNIFICANCE

Background

Neonicotinoid pesticides represent a class of seven chemicals that act as insecticides by neurotoxic effects on the nicotinic acetylcholine receptor (nAChR) which have had increased use in US agriculture over the past decade (Jeschke *et al.* 2011, Douglas and Tooker 2015, Simon-Delso *et al.* 2015). These chemicals persist in the environment, including the human food supply; and they have the potential to impact animals other than their insect targets, including humans (Keil *et al.* 2014, USDA 2014, Yang *et al.* 2014, Gibbons *et al.* 2015). Environmental persistence and irreversible binding to the nAChR raises concern for potential impacts of long term effects on human health even with exposures at low levels (Tennekes and Sanchez-Bayo 2011, Van der Sluijs *et al.* 2015).

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). As patent protection began to expire on these 7 chemicals in 2005, use of generic products have broadened the markets in which they are used, replacing older pesticide classes such as organophosphates and carbamates (Jeschke *et al.* 2011). Neonicotinoids were present in all streams tested near high corn and soybean production areas in the US and levels correlated with rain during crop planting implicating seed treatments as the source (Hladik *et al.* 2014). While levels were well below the US EPA tolerances, neonicotinoid pesticide residues were detectable in many fruits and vegetables tested by the US Department of Agriculture in 2013 (USDA 2014). In addition, neonicotinoids were some of the most commonly detected pesticides (6-31% of samples) in prepared infant and toddler foods in a 2012 study by the FDA, which monitors pesticide residues on prepared foods as part of its Pesticide Monitoring Program (FDA 2015). Neonicotinoids may enter the flesh of the fruit or vegetable, making it difficult to readily wash and remove residues prior to consumption (Chen *et al.* 2014).

Exposure to neonicotinoid pesticides has been associated with adverse health effects in various species, including mammals, honeybees, and other wildlife (Krupke *et al.* 2012, Whitehorn *et al.* 2012, Mason 2013, Gibbons *et al.* 2015, Morrissey *et al.* 2015, Pisa *et al.* 2015, Rundlof *et al.* 2015, Van der Sluijs *et al.* 2015, Cimino *et al.* 2017). Several studies have characterized the potential neurotoxic effects of neonicotinoids (Li *et al.* 2011, Kimura-Kuroda 2012). For example, nicotinic acetylcholine receptors are important for synaptic transmission and learning and memory, and *in vitro* studies using cerebellar neurons from neonatal rats found that neonicotinoid pesticides can affect the neonicotinoid acetylcholine receptors in a similar way as nicotine (Tomizawa *et al.* 2001, Kimura-Kuroda 2012). Neonicotinoids can bind the α4β2 nicotinic acetylcholine receptor subtype (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 nicotinic acetylcholine receptor subtype plays an important role in the developing brain, including the proliferation, migration, and differentiation of neurons and their integration into neural circuits (Role and Berg 1996, Dwyer *et al.* 2009). 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, Gibbons *et al.* 2015).

Significance

The association between neonicotinoid pesticide exposure and potential human health effects was identified as a potential candidate for systematic review. Given the interest and extent of the evidence, the National Toxicology Program (NTP) at NIEHS will conduct a scoping review to identify the extent of evidence available to understand human health effects of neonicotinoid pesticides. The health effects

literature for neonicotinoid pesticides will be 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 evidence mapping will also include studies that may not be directly relevant to human health effects of neonicotinoid pesticides, such as studies in honeybees and pets, but that may be of interest for additional research by other groups. The information contained in this scoping review will be made publicly available in a NTP Research Report, which could be used to support a full systematic review or for consideration of future research on this topic.

OBJECTIVE AND SPECIFIC AIMS

Objective

The objective of this scoping review is to identify and summarize the literature relevant to neonicotinoid pesticide exposure and human health effects including health effect studies in pets.

Specific Aims

- Identify literature reporting exposure(s) to one or more neonicotinoid pesticides registered for use in the U.S. and all outcomes relevant to human health effects, including epidemiological, experimental animal, and *in vitro* model systems.
- Summarize/map relevant health effects and mechanistic data by neonicotinoid pesticide (i.e., the extent and types of health effects evidence available).
- Summarize data available on health effects with a large amount of data (e.g., neurological).

PECO Statement

A PECO statement (**P**opulation, **E**xposure(s), **C**omparator(s), and **O**utcome(s)) (**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. Human PECO (Population, Exposure, Comparator and Outcome) Statement	
Element	**Evidence**
Population	**Human:** All epidemiological studies **Animal:** Non-human animals, including studies in laboratory animals, fish, wildlife (mammalian species), and *C. elegans*. ***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. 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)
Comparators	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.

METHODS

The systematic review techniques in the protocol adhere to the framework developed by Office of Health Assessment and Translation (OHAT) (Rooney *et al.* 2014). The OHAT systematic review framework consists of a 7-step process, and the first 3 are relevant to produce a scoping review; whereas the last 4 are relevant for assessing study quality and synthesizing evidence. Therefore, this protocol is restricted to the first 3 steps: 1) Problem Formulation, 2) Search and Select Studies for Inclusion, and 3) Data Extraction.

Step 1. Problem Formulation

Neonicotinoid pesticides were nominated to NTP for possible evaluation of noncancer health outcomes and exposure summary in two separate nominations in spring of 2015. 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/784117). As a result of the request for information and project scoping, NTP learned that the U.S. EPA Office of Pesticide Programs will be evaluating currently registered neonicotinoid pesticides as part of their Registration Review process[1] performing a risk assessment of neonicotinoid pesticides and that there was an ongoing systematic review activity focused only on the human data. NTP assisted Melissa Perry and Andria Cimino of George Washington University in their review of the epidemiological evidence to address one aspect of the nomination. The collaboration led to subsequent publication of a systematic review of the epidemiological literature on the health effects of neonicotinoids (Cimino et al. 2017). To address other aspects of the nomination, NTP will develop a scoping review product that supports the needs of EPA, promotes data access and data sharing, and avoids duplication of effort. Given the extent of the literature, problem formulation focused on the goal of developing a scoping review to systematically collect and categorize the evidence into a systematic evidence map of the key neonicotinoid pesticide related health effects, types of evidence, and gaps in research. The current protocol was developed using the OHAT Systematic Review framework through step 3, data extraction.

Chemical Selection

Seven chemicals will be considered as members of the class of neonicotinoid pesticides that act on the nicotinic acetylcholine receptor (Jeschke *et al.* 2011). Each of these chemicals are sold under multiple product names and are listed by the percent of US 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%)

[1] Registration Review is a program where all registered pesticides are reviewed by the U.S. EPA at least every 15 years as mandated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Step 2. Search and Select Studies for Inclusion

Literature Search Strategy

A literature search strategy for each chemical was constructed by using (1) common name for the chemical, (2) Chemical Abstract Services Registry Number (CASRN), and (3) and retrieval of synonyms from the ChemIDPlus database which currently contains chemical names and synonyms for over 400,000 chemicals (National Library of Medicine (NLM)2014). Many of the ChemIDPlus database synonyms are ambiguous and could lead to false positives (short alphanumeric sequences that could be confused with arbitrary acronyms or abbreviations, English words that have been used as industrial trade names, or were not found in PubMed). One database (PubMed) will be searched from the beginning of the database entries (full details of the search strategy are presented in **Appendix 1**). No publication year or language limits will be imposed.

Searching Other Resources

We will hand-search the reference lists of relevant, authoritative reviews or government-authored (state and federal) technical reports identified during the initial search to identify additional studies that were not identified through the electronic searches. Studies will be evaluated using the same inclusion and exclusion criteria as used for screening records retrieved from the electronic search. Relevant studies identified through these steps will be marked as "provided from other sources" in the study selection flow diagram.

Screening Process

DistillerSR®, a web-based, systematic review software program with structured forms and procedures will be used to screen articles for relevance and eligibility to ensure standardization of process[2]. Initially, results of the literature search will be assembled in EndNote software and exact article duplicates removed prior to uploading the references and within the systematic review software program.

Title/Abstract Review

Two members of the evaluation design team will independently conduct a title and abstract screen of the search results to determine whether a reference meets the inclusion criteria. Studies that are not excluded based on the title and abstract will be screened in a full-text review. Initially, screeners will be trained using project-specific written instructions in a pilot phase undertaken to improve clarity of the inclusion and exclusion instructions and to improve accuracy and consistency among screeners. If changes to the inclusion criteria are made based on the pilot phase, they will be documented in a protocol amendment along with the date modifications were made and the logic for the changes.

[2] 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.

Table 2. Detailed inclusion and exclusion criteria to determine study eligibility		
	Inclusion Criteria	**Exclusion Criteria (or blank if none)**
Participants/Population (Human Studies or Experimental Model Systems)		
human	• No restrictions on sex, age, life stage (including in utero exposure) at time of exposure or outcome assessment • No restrictions on country of residence/origin, lifestyle, race/ethnicity, or occupation	
animal	• No restrictions on sex, age, species (including Drosophila), or life stage at exposure or outcome assessment • Studies in laboratory animals, fish, wildlife (mammalian species), and *C. elegans*	• Amphibians, reptiles, birds, honeybees, other insects, invertebrates (other than *C. elegans*), fungi, plants, bacteria
In vitro	• *In vitro* models utilizing organs, tissues, cell lines, or cellular components	
Exposure		
human, animal, *In vitro*	• 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. • Observational exposure scenarios (e.g., wildlife, ecological) should also be included • Studies that use a neonicotinoid-alone dose group	
Comparators		
human, animal, *In vitro*	• Both experimental (controlled exposure or treatment) and observational studies (wildlife, ecological) should be included	
Outcomes		
human, animal, *In vitro*	• Neurological, developmental, and congenital health effects[a]	• Other health effects, including acute and irritation studies • Environmental impacts
Publications (e.g., language restrictions, use of conference abstracts, etc.)		
human, animal, *In vitro*	• Study must contain original data and must be peer-reviewed • Studies published in a language other than English will be collected and categorized by health effect or mechanism to the extent they can be categorized without full translation as extensive translation and level of effort are beyond the goals of this scoping review.	• Articles with no original data, e.g., editorials, reviews • Non-peer reviewed articles: Conference abstracts or other studies published in abstract form only, grant awards, and theses/dissertations • Retracted articles
*Relevant reviews are used as background and for reference scanning.		

In case of screening conflicts, screeners will independently review their screening results to confirm the inclusion/exclusion decision and, if needed, discuss discrepancies with the other screener(s). Any articles with unresolved screening conflicts at the title and abstract phase will be included in the full text review.

Full-Text Review

After completion of the title/abstract screen, full-text articles will be retrieved[3] for those studies that either clearly met the inclusion criteria or where eligibility to meet the inclusion criteria is unclear. Two members of the evaluation design team will independently conduct a full-text screen of the search results to determine whether a reference meets the inclusion criteria. True disagreements will be resolved by discussion involving another member(s) of the team or, if necessary, through consultation with technical advisors.

Multiple publications of same data

Multiple publications with overlapping data for the same study (e.g., publications reporting subgroups, additional outcomes or exposures outside the scope of an evaluation, or longer follow-up) will be identified by examining author affiliations, study designs, cohort name, enrollment criteria, and enrollment dates. If necessary, study authors will be contacted to clarify any uncertainty about the independence of two or more articles. OHAT will include all publications on the study, select one study to use as the primary, and consider the others as secondary publications with annotation as being related to the primary record during data extraction. The primary study will generally be the publication with the longest follow-up, or for studies with equivalent follow-up periods, the study with the largest number of cases or the most recent publication date. OHAT will include relevant data from all publications of the study, although if the same outcome is reported in more than one report, OHAT will include a single instance of the data (and avoid more than one, i.e. duplicate instances of the data). Although only one study is identified as the primary study, relevant information will be considered from other publications. For example, when a study refers to a previous publication for additional details of the methods, those citations will be identified and considered with the primary citation for data extraction and risk of bias evaluation.

Tracking study eligibility and reporting the flow of information

The reason for exclusion at the full-text-review stage will be annotated and reported in a study flow diagram in the final report. Studies will be excluded if: (1) is a review, commentary, or editorial with no original data; (2) lacks relevant exposure information; (3) lacks relevant health outcome information; (4) is a conference abstract, thesis/dissertation, or (5) full text is "not available".

Step 3. Data Extraction and Content Management

Data extraction will be managed with structured forms and stored in a database format using Health Assessment Workspace Collaborative (HAWC), an open source, web-based interface[4]. Data extraction elements are listed in appendices for human (**Appendix 2**), experimental animal (**Appendix 3**), and *in vitro* studies (**Appendix 4**). Data will be extracted only for health effects that are relevant to the PECO

[3] OHAT will initially attempt to retrieve a full-text copy of the study using an automated program, such as QUOSA, when possible, and NIH library services (NIH subscriptions and interlibrary loans). For publications not available through NIH, OHAT will search the Internet and/or may attempt to contact the corresponding author. Studies not retrieved through these mechanisms are excluded and notated as "not available."

[4] HAWC (Health Assessment Workspace Collaborative): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals (http://hawcproject.org).

statement, however, other health effects will be identified for potential future use and/or use by other groups. Study information collected during data extraction will be visualized, when appropriate (e.g., when there are data on the same or health effects evaluated across multiple studies), and made publicly available upon publication of the finalized report.

The extracted data will be used to summarize study designs and findings. The content of the data extraction may be revised following the identification of the studies included in the review. Data extraction will be performed by one member of the evaluation team and checked by a second member of the evaluation team for completeness and accuracy. Data extractors from the evaluation team will be trained using project-specific written instructions in an initial pilot phase using a subset of studies. Any discrepancies in data extraction will be resolved by discussion or consultation with a third member of the evaluation team. Information that is inferred, converted, or estimated during data extraction will be annotated (e.g., using brackets [n=10]).

Step 4. Study Results and Summaries

The results of all included human, animal, and *in vitro* studies will be summarized by outcome and chemical in text and tables to develop a systematic evidence map of the evidence by health effect and types of evidence, and identify data gaps in available research. In addition, visualizations using the extracted data in HAWC will also be generated to summarize the data.

SCOPING REVIEW: OUTLINE

The NTP Scoping Review on the health effects of neonicotinoid pesticides will include the following information:

Introduction

This section will provide a brief background on the topic.

Methodology

This section will provide a brief overview of the methodologies used in the review process, including:

- the research question
- the search strategy used to identify and retrieve studies
- the process for selecting the included studies
- the methods of data extraction

Results

This section will include the results from the scoping efforts. Results will be presented in tables or figures as appropriate using HAWC. The results from the included studies will be discussed by outcome. This will include a description of:

- the number of studies identified that reported the outcome
- the full list of excluded studies, with reasons for exclusion documented for studies excluded at the full text review stage
- the results and summaries for each included study (including files in downloadable format)
- the visualization of result summaries for included studies (generated using HAWC)

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.

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.

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.

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.

Dwyer JB, McQuown SC, Leslie FM. 2009. The dynamic effects of nicotine on the developing brain. *Pharmacol Ther* 122(2): 125-139.

FDA (Food and Drug Administration). 2015. *Pesticide Monitoring Program: Fiscal Year 2012 Pesticide Report*. Available: http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/Pesticides/UCM432758.pdf [accessed 28 April 2017].

Gibbons D, Morrissey C, Mineau P. 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res Int* 22(1): 103-118.

Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, Yuan Y, Xu Y, Liu M, Shi HJ, Xu WP. 2013. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro. *PLoS One* 8(7): e70112.

Higgins J, Green S. 2011. Cochrane handbook for systematic reviews of interventions. Version 5.1.0 (updated March 2011). http://handbook.cochrane.org/ [accessed 3 February 2013].

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.

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.

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.

Kimura-Kuroda JK, 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.

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.

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.

Mason RT, 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.

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.

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, Morrissey CA, Noome DA, Settele J, Simon-Delso N, Stark JD, Van der Sluijs JP, Van Dyck H, Wiemers M. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res Int* 22(1): 68-102.

Role LW, Berg DK. 1996. Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* 16(6): 1077-1085.

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.

Rundlof M, Andersson GK, Bommarco R, Fries I, Hederstrom V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* 521(7550): 77-80.

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, Mineau P, Mitchell EA, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR, Wiemers M. 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ Sci Pollut Res Int* 22(1): 5-34.

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, Cowan A, Casida JE. 2001. Analgesic and toxic effects of neonicotinoid insecticides in mice. *Toxicol Appl Pharmacol* 177(1): 77-83.

USDA (U.S. Department of Agriculture). 2014. *Pesticide Data Program—Annual Summary, Calendar Year 2013*. (AMS) AMS. Available: http://www.ams.usda.gov/pdp [accessed 10 September 2015].

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, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Simon-Delso N, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Whitehorn PR, Wiemers M. 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.

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.

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.

ABOUT THE PROTOCOL

Contributors

Evaluation teams are composed of federal staff and contractor staff. Contractor staff members are screened for potential conflicts of interest. Federal staff members should do a self-evaluation. Technical advisors were screened for conflict of interest prior to their service and did not report any conflicts of interest. Epidemiologists and toxicologists on OHAT evaluation teams should have at least three years' experience and/or training in reviewing studies, including summarizing studies and critical review (e.g., assessing study quality and interpreting findings). Team members should have at least a master's degree or equivalent experience in epidemiology, toxicology, environmental health sciences, or a related field.

Federal Staff

Name	Affiliation
Windy Boyd	NIEHS/NTP, Project Lead
Andrew A. Rooney	NIEHS/NTP
Vickie Walker	NIEHS/NTP
Abee L. Boyles	NIEHS/DERT, former NTP Project Lead

Contract Support Staff: Will assist in screening and data extraction

Name	Affiliation
Robyn Blain	ICF
Anna Engstrom	ICF
Canden Byrd	ICF
Pamela Hartman	ICF

Technical Advisors

Technical advisors are outside experts retained on an as-needed basis to provide individual advice to the NTP for a specific topic. The technical advisors were selected for their ongoing work with, or experience in neonicotinoid pesticides and environmental health.

Name	Affiliation
Monique Perron	U.S. EPA

Sources of Support

National Institute of Environmental Health Sciences/Division of the National Toxicology Program

Protocol History and Revisions

Date	Activity or revision
January 10, 2018	Protocol posted publicly at (https://ntp.niehs.nih.gov/go/nachrs)

APPENDICES

Appendix 1. Literature Search Strategy

The strategy for this search is broad for the consideration of all endpoints and comprehensive for neonicotinoid pesticides as an exposure or treatment in order to ensure inclusion of relevant papers.

Database	Search Terms
PubMed	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 2. Data Extraction Elements for Human Studies

HUMAN	
Funding	Funding source(s)
	Reporting of conflict of interest (COI) by authors (*reporting bias)
Subjects	Study population name/description
	Dates of study and sampling timeframe
	Geography (country, region, state, etc.)
	Demographics (sex, race/ethnicity, age or lifestage and exposure and outcome assessment)
	Number of subjects (target, enrolled, n per group in analysis, and participation/follow-up rates) (*missing data bias)
	Inclusion/exclusion criteria/recruitment strategy (*selection bias)
	Description of reference group (*selection bias)
Methods	Study design (e.g., prospective or retrospective cohort, nested case-control study, cross-sectional, population-based case-control study, intervention, case report, etc.)
	Length of follow-up (*information bias)
	Health outcome category, e.g., neurodevelopment
	Health outcome, e.g., memory (*reporting bias)
	Diagnostic or methods used to measure health outcome (*information bias)
	Confounders or modifying factors and how considered in analysis (e.g., included in final model, considered for inclusion but determined not needed (*confounding bias)
	Substance name and CAS number
	Exposure assessment (e.g., blood, urine, hair, air, drinking water, job classification, residence, administered treatment in controlled study, etc.) (*information bias)
	Methodological details for exposure assessment (e.g., HPLC-MS/MS, limit of detection) (*information bias)
	Statistical methods (*information bias)

HUMAN (continued)	
Results	Exposure levels (e.g., mean, median, measures of variance as presented in paper, such as SD, SEM, 75th/90th/95th percentile, minimum/maximum); range of exposure levels, number of exposed cases
	Statistical findings (e.g., adjusted β, standardized mean difference, adjusted odds ratio, standardized mortality ratio, relative risk, etc.) or description of qualitative results. When possible, OHAT will convert measures of effect to a common metric with associated 95% confidence intervals (CI). Most often, measures of effect for continuous data are expressed as mean difference, standardized mean difference, and percent control response. Categorical data are typically expressed as odds ratio, relative risk (RR, also called risk ratio), or β values, depending on what metric is most commonly reported in the included studies and on OHAT's ability to obtain information for effect conversions from the study or through author query.
	If not presented in the study, statistical power can be assessed during data extraction using an approach that can detect a 10% to 20% change from response by control or referent group for continuous data, or a relative risk or odds ratio of 1.5 to 2 for categorical data, using the prevalence of exposure or prevalence of outcome in the control or referent group to determine sample size. For categorical data where the sample sizes of exposed and control or referent groups differ, the sample size of the exposed group will be used to determine the relative power category. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power as "appears to be adequately powered" (sample size for 80% power met), somewhat underpowered (sample size is 75% to < 100% of number required for 80% power), "underpowered" (sample size is 50% to < 75% of number required for 80% power), or "severely underpowered" (sample size is < 50% of number required for 80% power).
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, non-monotonic)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, and statistical result conversions, etc.

Appendix 3. Data Extraction Elements for Animal Studies

ANIMAL	
Funding	Funding source(s)
	Reporting of COI by authors and/or translators (*reporting bias)
Animal Model	Sex
	Species
	Strain
Treatment	Chemical name and CAS number
	Source of chemical
	Purity of chemical (*information bias)
	Dose levels or concentration (as presented and converted to mg/kg bw/d when possible)
	Other dose-related details, such as whether administered dose level was verified by measurement, information on internal dosimetry (*information bias)
	Vehicle used for exposed animals
	Route of administration (e.g., oral, inhalation, dermal, injection)
	Age or lifestage at start of dosing and at health outcome assessment
	Duration and frequency of dosing (e.g., hours, days, weeks when administration was ended, days per week)
Methods	Study design (e.g., single treatment, acute, subchronic (e.g., 90 days in a rodent), chronic, multigenerational, developmental, other)
	Guideline compliance (i.e., use of EPA, OECD, NTP or another guideline for study design, conducted under GLP guideline conditions, non-GLP but consistent with guideline study, non-guideline peer-reviewed publication)
	Number of animals per group (and dams per group in developmental studies) (*missing data bias)
	Randomization procedure, allocation concealment, blinding during outcome assessment (*selection bias)
	Method to control for litter effects in developmental studies (*information bias)
	Use of negative controls and whether controls were untreated, vehicle-treated, or both
	Endpoint health category (e.g., reproductive)
	Endpoint (e.g., infertility)
	Diagnostic or method to measure endpoint (*information bias)
	Statistical methods (*information bias)

ANIMAL (continued)	
Results	Measures of effect at each dose or concentration level (e.g., mean, median, frequency, measures of precision or variance) or description of qualitative results. When possible, OHAT will convert measures of effect to a common metric with associated 95% confidence intervals (CI). Most often, measures of effect for continuous data will be expressed as percent control response, mean difference, or standardized mean difference. Categorical data will be expressed as relative risk (RR, also called risk ratio).
	No observed effect level (NOEL), lowest observed effect level (LOEL), benchmark dose (BMD) analysis, statistical significance of other dose levels, or other estimates of effect presented in paper. **Note:** The NOEL and LOEL are highly influenced by study design, give no quantitative information about the relationship between dose and response, and can be subject to author's interpretation (e.g., a statistically significant effect might not be considered biologically important). Also, a NOEL does not necessarily mean zero response. Ideally, the response rate or effect size at specific dose levels is used as the primary measure to characterize the response.
	If not presented in the study, statistical power can be assessed during data extraction using an approach that assesses the ability to detect a 10% to 20% change from control group's response for continuous data, or a relative risk or odds ratio of 1.5–2 for categorical data, using the outcome frequency in the control group to determine sample size. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power. Studies will be considered adequately powered when sample size for 80% power is met.
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, nonmonotonic)
	Data on internal concentration, toxicokinetics, or toxicodynamics (when reported)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, statistical result conversions, etc.

Appendix 4. Data Extraction Elements for *In Vitro* Studies

In vitro	
Funding	Funding source(s)
	Reporting of COI by authors and/or translators (*reporting bias)
Cell/Tissue Model	Cell line, cell type, or tissue
	Source of cells/tissues (and validation of identity)
	Sex of human/animal origin
	Species
	Strain
Treatment	Chemical name and CAS number
	Concentration levels (as presented and converted to µM when possible)
	Source of chemical
	Purity of chemical (*information bias)
	Vehicle used for experimental/control conditions
	Duration and frequency of dosing (e.g., hours, days, weeks when administration was ended, days per week)
Methods	Guideline compliance (i.e., use of EPA, OECD, NTP or another guideline for study design, conducted under GLP guideline conditions, non-GLP but consistent with guideline study, non-guideline peer-reviewed publication)
	Randomization procedure, allocation concealment, blinding during outcome assessment (*selection bias)
	Number of replicates per group (*information bias)
	Percent serum/plasma in medium
	Use of negative controls and whether controls were untreated, vehicle-treated, or both
	Report on data from positive controls – was expected response observed? (*information bias)
	Endpoint health category (e.g. neurological and thyroid)
	Endpoint or assay target (e.g., T3, T4, TSH levels).
	Name and source of assay kit
	Diagnostic or method to measure endpoint (e.g., reporter gene)(*information bias)
	Statistical methods (*information bias)
Results	Measures of effect at each dose or concentration level (e.g., mean, median, frequency, and measures of precision or variance) or description of qualitative results. When possible, OHAT will convert measures of effect to a common metric with associated 95% confidence intervals (CI). Most often, measures of effect for continuous data will be expressed as mean difference, standardized mean difference, and percent control response. Categorical data will be expressed as relative risk (RR, also called risk ratio).
	No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), statistical significance of other concentration levels, AC50, or other estimates of effect presented in paper. Note: The NOEC and LOEC are highly influenced by study design, do not give any quantitative information about the relationship between dose and response, and can be subject to author's interpretation (e.g., a statistically significant effect may not be considered biologically important). Also, a NOEC does not necessarily mean zero response.
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, non-monotonic)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, statistical result conversions, etc.