NATURAL RESOURCES DEFENSE COUNCIL

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Comments from the Natural Resources Defense Council (NRDC) on the Draft Pesticide Cumulative Risk Assessment: Framework for Screening Analysis EPA-HQ-OPP-2015-0422

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The following comments are being submitted on behalf of the Natural Resources Defense Council (NRDC). NRDC uses law, science, and the support of more than 1.2 million members and online activists nationwide to protect the planet's wildlife and wild places and to ensure a safe and healthy environment for all living things. NRDC has no direct or indirect financial or fiduciary interest in the manufacture or sale of any chemical that would be the subject of these comments.

These comments address the draft Pesticide Cumulative Risk Assessment: Framework for Screening Analysis. The EPA Office of Pesticide Programs (OPP) describes it as providing, "guidance for screening available information to identify groups of pesticides that may have a common mechanism of toxicity (i.e., candidate CMGs). In addition, this document provides guidance for screening available information on those candidate groups for potential cumulative risks, which may lead to more refined CRAs (cumulative risk assessments)."

Section 408(b)(2)(D)(v) of the Federal Food, Drug, and Cosmetic Act (FFDCA) requires EPA to take into account available evidence concerning the cumulative effects of pesticide residues and other substances that have a common mechanism of toxicity. The OPP has previously developed two guidance documents:

- Guidance For Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity (USEPA, 1999), which describes the process for CMGs;
- Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity (USEPA, 2002), which describes the steps used in conducting CRA. Copies of those two guidance documents can be found at http://www.regulations.gov in docket ID number EPA-HQ-OPP-2015-0422.

Based on OPP's draft pesticide CRA framework, OPP determined that abamectin and emamectin share a similar toxicological profile and a testable hypothesis can be identified. The cumulative assessment was done in conjunction with pending actions for abamectin to expand the use of abamectin on Caneberry subgroup 13-07A, soybeans, sweet corn, ear tags for lactating dairy cattle, and golf course turf. A draft copy of the human health risk assessment considering both aggregate and cumulative risks is included in the docket to provide an example of how the OPP would implement its draft CRA Framework and to allow for public comment.

NRDC comments:

These comments cover the following areas:

A - Specific recommendations for improvement of OPP's draft CRA framework:

1. The pesticide CRA framework should expand the concept of cumulative risk beyond mode of action/adverse outcome pathway (MOA/AOP), to include chemicals that contribute to a common adverse health impact. As currently drafted, it fails to address true cumulative risk.
2. The CRA framework should include critical elements of a systematic review process for evaluating and integrating multiple streams of data.
3. The CRA framework should demonstrate that OPP has conducted a comprehensive literature search.
4. The CRA framework should include a systematic, transparent, and repeatable process for evaluating confidence in a study that addresses study outcome biases (beyond internal validity, reporting quality, or GLP compliance).
5. MOA/AOP frameworks should be used as tools to organize existing *in vivo* and *in vitro* data and to establish common adverse health impacts, not as a way to overly restrict common mechanisms of toxicity, and should be interpreted relative to the plausibility of alternate MOA/AOPs and the established default assumption.

6. Before acceptance of an MOA/AOP, OPP's framework should require rigorous testing and validation, and not use incomplete data sets to downgrade the categorization of chemical hazards.
7. The pesticide CRA framework should require that defaults be maintained for the steps in the risk assessment that require inferences.

B- Specific comments on the case study, abamectin, as a demonstration of the framework

1. OPP did not follow any documented systematic review process.
2. There is no documentation to show whether or not OPP conducted a comprehensive literature search.
3. All endpoints are based on unpublished industry-sponsored studies.
4. OPP failed to consider alternate MOA/AOPs and related agents in the cumulative assessment group.
5. OPP relied on a poorly understood proposed MOA/AOP with very little supporting data.
6. OPP eliminated established default protective factors.

C - A report of the National Research Council (NRC, 2015) identified some serious concerns with the use of predictive toxicology and computational methods at this time.

D- Conclusions

A - RECOMMENDATIONS FOR IMPROVEMENT of OPP's DRAFT CRA FRAMEWORK

1 - The pesticide CRA framework should expand the concept of cumulative risk beyond MOA/AOP, to include chemicals that contribute to a common adverse health impact. As currently drafted, it fails to address true cumulative risk.

The most significant problem with the CRA framework is that it will not address the true cumulative risk posed by pesticide exposures. Even at the time of passage of FQPA in 1996 scientists understood that restricting the CRA to common mechanisms of toxicity is only adequate if all agents in a mixed exposure act solely through the single defined mechanism – a presumption that is rarely if ever true. Agents that act on different pathways leading to toxic or carcinogenic effects may increase risks qualitatively (such as additional target organs) and/or quantitatively (increased potency) to levels greater than that of individual agents. These synergistic effects are not addressed in the single-mechanism risk assessments that the pesticide office continues to do. The biological evidence supporting this issue is highlighted in the international multi-author report of "The Halifax Project" that was published this year in Carcinogenesis (Goodson et al 2015). For example, the authors identify inflammation as having a critical role in tumorigenesis (Goodson et al, 2015; Colotta et al, 2009).

We urge the pesticide office to consider the wisdom of the EPA Framework for Cumulative Risk Assessment (2003) that defined a cumulative risk assessment as, "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors" (EPA 2003, p. 6). EPA 2003 highlighted that, "Several key points arise from this definition of cumulative risk. First, cumulative risk involves multiple agents or stressors, which means that assessments involving a single chemical or stressor are not 'cumulative risk assessment' under this definition. Second, there is no limitation that the 'agents or stressors' be only chemicals. 'Agents or stressors' may be chemicals, of course, but they may also be biological or physical agents or even the

absence of a necessity such as habitat. Third, this definition requires that the risks from multiple agents or stressors be combined" (EPA 2003, p. 7) The Food Quality Protection Act (FQPA) of 1996 requires that EPA consider the cumulative effects to human health that can result from exposure to pesticides and other substances that have a common mechanism of toxicity. FQPA did not limit the CRA to only consider mechanism, and certainly not only one mechanism. EPA 2003 recommended a much broader and more inclusive CRA framework.

Building on EPA 2003, the National Academies *Phthalates and Cumulative Risk Assessment (2008)* report recommended that chemicals that contribute to the same adverse health outcome should be considered together in a cumulative risk assessment, not just those chemicals that cause the health outcome by the same specific biological pathway (NRC 2008, p. 11). For example, a number of chemicals can interfere with normal brain development in children, including lead, mercury, brominated flame retardants, and organophosphate pesticides through a variety of different pathways and mechanisms. A cumulative risk assessment focused on the health endpoint of abnormal brain development and function would necessarily consider the impact of a chemical in the context of background exposures to other chemical and non-chemical stressors that also influence brain development, regardless of the specific mechanism involved. Quantitative cumulative risk assessments have been completed using adverse health endpoints to define the cumulative assessment, without knowing the MOA at all. See for example the paper by a Swedish team that used a Hazard Index (HI) approach to assess the cumulative risks from seventeen perfluorinated compounds (Borg et al 2013). Similarly, the Chronic Hazard Advisory Panel (CHAP) provided an expert report to the Consumer Products Safety Commission (CPSC) in July 2014, using a Hazard Index approach to generate quantitative risk estimates from a dozen phthalate chemicals based on common adverse health outcomes, and not on MOA/AOP (CHAP 2014).

In its seminal report, *Science and Decisions (2009)*, the National Academies underscored the key recommendations of the 2008 *Phthalates* report's definition of "agent or stressor" that includes not only chemicals, but biological agents, radiologic agents, physical agents, and psychosocial stressors (NRC 2008, p. 224-229). A broad variety of factors, including nutrition, health status, and psychosocial stress can increase individual vulnerability to toxic chemicals. These factors – and their variability across a population – need to be considered in cumulative risk assessments to protect public health. The National Academies *Science and Decisions* report (2009) noted that, "There is a need for cumulative risk assessments as defined by EPA (EPA 2003)—assessments that include combined risks posed by aggregate exposure to multiple agents or stressors; aggregate exposure includes all routes, pathways, and sources of exposure to a given agent or stressor. Chemical, biologic, radiologic, physical, and psychologic stressors are considered in this definition (Callahan and Sexton 2007)" (NRC 2009, p. 266). As Scammell, et al, note, in the absence of data and methodologies to comprehensively evaluate cumulative risks, tools including indexes, maps, and combined approaches are an important first step in delineating the cumulative context for an assessment (Scammell et al 2014). One approach to incorporating this information into risk assessments is to use such data to quantitatively inform variability and vulnerability factors.

The recommendation to assess chemicals that share a common adverse health endpoint, such as impaired brain development, has not yet been adopted by OPP, and the more far-reaching recommendation from the National Academies *Science and Decisions* (2009) report recommending incorporation of other non-chemical stressors has not been addressed either.

Other offices in the EPA are moving forward with efforts to modernize risk assessment by expanding cumulative risk groups beyond MOA/AOP (Burke and Bahadoori, 2015, personal communication), as well as state Agencies (Dunn and Alexeeff, 2010). The pesticide office is out of step with current scientific discourse and practice, instead proposing a MOA/AOP CRA framework that is so backwards that it even lags behind the EPA 2003 CRA framework of a dozen years ago.

2- The pesticide CRA framework should include critical elements of a systematic review process for evaluating and integrating multiple streams of data.

Government agencies and academic institutions in the US and world-wide are developing coordinated systematic and transparent methods of research synthesis in environmental health, using methods developed in clinical sciences as a model. Systematic reviews integrate information from human epidemiologic data, *in vivo* toxicologic data, *in vitro* cellular and mechanistic data, and *in silico* computational information. Systematic reviews methods for chemical assessments have been developed by the National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT), the EPA Integrated Risk Information System (IRIS) program, the University of California Navigation Guide method (Woodruff and Sutton 2014), and others. Various resources and guides are available, particularly from the NIEHS NTP:

> *Systematic review and evidence integration for literature-based environmental health science assessments.* Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. Environ Health Perspect. 2014 Jul;122(7):711-8. doi: 10.1289/ehp.1307972.
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> *Intersection of Systematic Review Methodology with the NIH Reproducibility Initiative*. Thayer KA, Wolfe MS, Rooney AA, Boyles AL, Bucher JR, Birnbaum LS. Environ Health Perspect. 2014 Jul 1;122(7):A176-7.
>
> *Implementing systematic review at the National Toxicology Program: status and next steps*. Birnbaum LS, Thayer KA, Bucher JR, Wolfe MS. Environ Health Perspect. 2013 Apr;121(4):A108-9.

The "NavGuide" from the University of California is a systemic review method consistent with the OHAT approach:

> Woodruff TJ, Sutton P. *The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes*. Environ Health Perspect. 2014 Oct;122(10):1007-14.

The National Research Council in its 2014 report complimented the EPA IRIS program on its development of systematic review methods for its chemical evaluations. The NRC recommended that a systematic evidence-integration process be developed that considers all lines of evidence (i.e., human, animal, and mechanistic), systematically determine the strength of evidence (not weight of evidence!) considering such aspects as consistency of exposure, evaluates study bias, and treats cancer and non-cancer outcomes more uniformly (that is, do not suppose that non-cancer effects have thresholds below which the risk is negligible) (NRC 2014, Section 6). The IRIS program responded very positively with public workshops that included government and academic experts from the US and Europe, as well as industry representatives (for example, see EPA IRIS workshop October 2014).

Unfortunately, in stark contrast to the chemical assessment programs at EPA IRIS and NIEHS, the pesticide office has not proposed a systematic review process for integrating multiple data streams into

its CRA framework. It is critical that OPP do this in a rigorous and transparent manner. We recommend that the pesticide office fall into step with IRIS and the National Toxicology Program, adopting their systematic review process since it has already undergone significant inter-agency and public review and is now being successfully implemented. These agencies have moved beyond the unrealistically simplistic MOA/AOP CRA of the pesticide office, to include all relevant data streams.

3 - The framework should demonstrate that OPP has conducted a comprehensive literature search

Early in a systematic review process the risk assessor is required to conduct a comprehensive literature search, gathering relevant information from the published, unpublished, and "grey" literature (publicly available government reports, etc.) as part of the literature search (NIEHS NTP 2015). The pesticide office has no systematic or transparent way of doing this, and it often seems as if the only studies that OPP reviews are the ones that the pesticide industry sponsors supply in support of the registration of their pesticide product. This leads to both a deficit in scientific quality and public trust.

The pesticide office has no clear and consistent criteria for excluding studies, and its only inclusion criteria seems to be that studies that are Guideline and GLP-compliant are included, creating a systematic bias favoring industry-sponsored studies and eliminating government-funded or other non-industry studies. Again, this compromises scientific quality and public confidence in the final assessment. NRDC and others have raised these concerns repeatedly over the years, including most recently our comments on OPP's revised human health risk assessment for chlorpyrifos ((Earthjustice et al 2015).

In its recent chlorpyrifos human health risk assessment, the pesticide office disregarded scientific evidence from government-funded academic researchers from Columbia University reporting on neurological impairments in children at exposure levels below those that caused cholinesterase inhibition, the only MOA/AOP that OPP considered (Earthjustice et al 2015). Also in the same assessment, OPP based its risk estimate (dose-response determination) on an industry-sponsored model based on cholinesterase inhibition, rather than a model based on a more sensitive endpoint from the human epidemiologic data that was developed by academic researchers funded by an EPA competitive grant (Hattis et al 2015). A scientific review panel found the industry model to have serious flaws, calling it "problematic," "cursory," "overstated," "inadequate," "inaccurate," "imprecise," and "incomplete." (SAP 2011) Without pre-stated clear exclusion and inclusion criteria such as established by IRIS or NTP, the pesticide office cannot defend itself against accusations that it is biased in favor of industry.

The pesticide office is generating risk assessments that fail to reflect the best available science, instead relying almost exclusively on industry-sponsored data. Without a systematic review framework that includes study inclusion and exclusion criteria, the pesticide office simply cannot defend its data selections.

4- The pesticide CRA framework should include a systematic, transparent, and repeatable process for evaluating confidence in a study that addresses study outcome biases (beyond internal validity, reporting quality, adherence to Guideline study requirements, or GLP compliance).

OPP favors industry-sponsored data because the studies adhere to pre-established "guidelines" and are conducted in laboratories certified to follow Good Laboratory Practices (GLP). In contrast, the NRC 2014 report, in its review and praise of the EPA IRIS systematic review process, noted that GLP criteria fail to

address study bias (NRC 2014, page 63). The NRC report notes that bias is a systematic, not random, flaw in the design and conduct of a study that reduces the validity and reliability of the study results (NRC 2014, pages 62, 63). Industry-sponsored studies have frequently been shown to be "biased by design" to underestimate risks (for documented examples of industry biased studies See Doubt is Their Product: How Industry's Assault on Science Threatens Your Health by OSHA Secretary David Michaels).

GLP is a standard for animal care and data collection required for industry laboratories in response to fraudulent practices documented in the 1970s. Industry labs that produce studies for government review are required by EPA and FDA to follow GLP standards, which include specified approaches to recordkeeping to facilitate audits and reduce fraud (54 Fed. Reg. 34034 August 17, 1989). However, GLP requirements are not necessarily associated with higher quality research, proper study design or correct statistical analysis (Myers et al 2009). In many cases, GLP-compliant studies have not even undergone scientific peer-review and publication.

Guideline studies are often insensitive to health endpoints being measured. Guideline studies are most often designed to identify major toxic effects (apical effects) like cancer. The problem is that major (apical) endpoints will not be predictive or indicate early-warnings of potential toxicity leading to "major" adverse health outcomes. Guideline studies don't necessarily use modern methods for evaluating chemicals and aren't designed to grapple with the problems of low-dose exposures, behavioral or learning effects, or upstream effects like reduced sperm count or reduced anogenital distance which are predictors of infertility.

The NIEHS National Toxicology Program (NTP) systematic review evaluates the risk of outcome-specific bias using five domains: selection bias, performance bias, attrition bias, detection bias, and reporting bias. It considers more than reporting quality (GLP compliance) for a measure of study quality for animal and human studies. Instead, the NTP framework measures internal validity of a study, that is, the "believability" of the study results, rather than simply the articulation or reporting quality, which is a better measure of study quality. EPA OPP has no systematic pre-stated criteria for scoring study bias, making its study selections for calculating risk estimates, or identifying MOA/AOPs, or reducing default protective factors - all based on industry studies - seem scientifically indefensible and arbitrary at best.

The pesticide office should implement the NTP or IRIS systematic review process that establishes criteria for identifying and excluding very low-quality studies, as the NTP systematic review does. To do otherwise would be to skew or bias the outcome of the assessment. The pesticide office should exclude or downgrade null association studies that are underpowered if they are inconsistent with the whole body of literature. The NTP systematic review framework identifies some study aspects that would lead to downgrading the confidence rating in that study include: risk of bias, unexplained inconsistency, indirectness in the relationship between a measured outcome and a health effect, imprecision, and publication bias serious enough to significantly decrease confidence in the body of evidence. Ultimately, conclusions should be based on the whole body of literature, excluding the lowest-confidence studies. The pesticide office does the opposite, largely ignoring the whole body of literature, and drawing its industry-favorable conclusions from industry-sponsored studies that are often insensitive and underpowered, potentially leading to biased assessments that are inadequately protective and put public health at risk.

5- The use of MOA/AOP data should be interpreted relative to the plausibility of alternate MOA/AOPs and the established default assumption.

OPP's draft CRA framework relies heavily on the determination of a MOA/AOP (mode of action/ adverse outcome pathway). The term adverse outcome pathway (AOP) was introduced into the risk assessment rhetoric rather recently in a paper proposing a conceptual framework for using new biological and computational sciences to generate mechanistic information that could inform ecological risk assessments (Ankley et al 2010). In their paper, the authors define an AOP as, "a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment" (Ankley et al 2010). The authors propose that the use of AOPs, "can focus toxicity testing in terms of species and endpoint selection, enhance across-chemical extrapolation, and support prediction of mixture effects … [and] facilitate use of molecular or biochemical endpoints (sometimes referred to as biomarkers) for forecasting chemical impacts on individuals and populations" (Ankely et al 2010). The shift to integrate *in vitro* (cellular, mechanistic) and *in silico* (computational) information into CRAs is consistent with the AOP framework. In its draft pesticide CRA framework, OPP describes its use of AOP as "conceptually similar to establishing key events in a MOA [mode of action] or for establishing a CMG [common mechanism group] under the FFDCA [Federal Food Drug and Cosmetic Act]" (EPA draft CRA Framework, p. 4).

AOPs are a double-edged sword. Used appropriately they can increase the robustness and utility of a risk assessment by allowing more of the available information to be used during evidence integration. For example, the prototype AOP is skin sensitization, developed by the OECD, which integrates non-testing information including read-across and Quantitative Structure Activity Relationship (QSAR), which can be very informative and reliable (Patlewicz et al, 2014; NRC 2014). However, with more complex endpoints such as cardiovascular disease, neurodevelopmental disorders, or cancer, the pathways are likely to be less linear, and more likely to be numerous. For example, chemicals may exacerbate processes that are already underway, or the development and progression of the disease may be delayed for many years after the initiating events take place or even occur in the next generation (for example, a latency period of thirty or more years between asbestos exposure and disease, or transgenerational effects of the pesticide vinclozolin) (Guerrero-Bosagna et al, 2013). For these reasons, AOPs should not be relied upon as the sole determinant of a common grouping of agents within a CRA framework.

The use of mechanistic data to inform an MOA/AOP must be interpreted relative to the plausibility of established default assumptions, and not as if the alternative to the proposed mechanism/MOA were no proposed mechanism/MOA. For example, carcinogens are presumed to be no-threshold (a linear low-dose response), so moving away from that health-protective default assumption should not be done unless it proves to be a better fit to the available data than the default linear dose-response and all other plausible dose-response curves. When abandoning a protective default assumption like no-threshold, the assessment should evaluate and quantify how the protections for human health and the environment have been altered.

When information is missing or unreliable, the framework should be clear and consistent that its approach is to use scientifically-based default assumptions that will protect health to improve the timeliness of the chemical assessment and decision-making process. It should set clear scientifically-based criteria for when to depart from these assumptions (NRDC 2012). In the landmark "Science and Decisions" report, the NAS committee concluded that, "established defaults need to be maintained for the steps in the risk assessment that require inferences" (NRC 2009 p.7). The framework should also evaluate and quantify how using a default versus the chosen alternative assumption affects the decisions that protect the environment and public health.

Although AOPs offer the possibility for improving risk assessments, such as by incorporating human variability, multiple exposures, and cumulative risk, there are many significant limitations to relying on AOPs (see Section C below). Without an established systematic review process of the IRIS or NIEHS-NTP chemical assessment programs for evidence evaluation and integration, the pesticide office simply should not be relying on MOA/AOPs.

6- Before acceptance of a MOA/AOP, OPP's framework should require rigorous testing and validation, and not use incomplete data sets to downgrade the categorization of chemical hazards.

Before acceptance of an MOA/AOP, OPP's framework should require rigorous testing and validation, and not use incomplete data sets to downgrade the categorization of chemical hazards. OPP's framework must also consider whether differences in mechanistic events among species are truly qualitative rather than quantitative in nature. For example, in the case study (below), the pesticide office eliminated the FQPA factor (reduced to 1X) for abamectin with the explanation that the activity of P-glycoprotein is different in the mouse and human, and therefore the neurotoxicity observed in mice would not manifest in humans. However, the activity of p-glycoprotein may be quantitatively different in mice and humans (despite the fact that OPP has almost no human data) but there is no evidence that this leads to a qualitatively different neurotoxicity profile. For quantitative differences, OPP's framework should also require information on the range of parameter variability in exposed humans so that sensitive subpopulations are not ignored in these categorizations.

The criteria for reducing health protections by calling a chemical non-hazardous are intentionally stringent for agencies tasked with the responsibility of protecting health and the environment (see for example IARC Monograph classifications and the NTP framework for systematic review). Dr. Melnick, retired career NIEHS scientist, warned that serious public health consequences may follow if chemicals are misclassified as less toxic or non-toxic based on untested mechanistic hypotheses, poorly validated tests, or incomplete data sets "Declaring a chemical as not hazardous, or reducing a level of health protection, should require validation, not speculation" (Melnick et al 2003).

7 - The pesticide CRA framework should require that defaults need to be maintained for the steps in the risk assessment that require inferences.

The pesticide CRA framework should invoke established defaults that will protect health, and set stringent criteria for when to depart from health-protective defaults. In *Science and Decisions*, the NAS committee concluded that, "established defaults need to be maintained for the steps in the risk assessment that require inferences" (NRC 2009, p. 7). The NAS committee recommended that EPA and other agencies update default factors and assumptions based on the best current science, identify where unstated or implicit assumptions are used, and replace these with explicit assumptions. These recommendations push EPA to, "continue and expand use of the best, most current science to support or revise its default assumptions," making the assumptions stronger, rather than reducing reliance on them (NRC 2009, p. 207). In fact, the committee specifically recommended that EPA develop "clear standards for departures from defaults" (NRC 2009, 199). The committee also noted that establishing, "clear criteria for departure from defaults can provide incentives for third parties to produce research" that can reduce uncertainty and, over time, result in more accurate assessments. Importantly, by using the established defaults more often, EPA avoids "the delay entailed by having to re-examine generic information with every new risk assessment" (NRC 2009, p. 191).

B- SPECIFIC COMMENTS ON ABAMECTIN

1- OPP did not follow any documented systematic review process

The application of OPP's proposed draft guidance for pesticide CRA is in the Abamectin (2015) human health risk assessment Appendix H: Abamectin and Emamectin Cumulative Screening Assessment (DP No. 426599). OPP states that the framework supplements two existing guidances, the Guidance for identifying pesticides that have a common mechanism of toxicity (EPA 1999), and the Guidance on cumulative risk assessment for pesticides with common mechanisms of toxicity (EPA 2002) (Framework p.3, 58). However, none of these guidances contain the critical elements of a robust and comprehensive systematic review framework that represents the state of the science. Without this, OPP's risk assessment appears to result from a closed-door process – more like a negotiated effort with the chemical industry – whose outcome is neither replicable nor defensible. Details are below.

2- There is no documentation to show whether or not OPP conducted a comprehensive literature search.

OPP states that since the last assessment in 2012 it has "re-evaluated the entire abamectin and emamectin toxicological database along with additional studies published in the open literature." (EPA 2015, p. 5, 17) Our search of the National Library of Medicine online "pubmed" database for studies with both "abamectin" and "toxicity" in the title or abstract generated 136 hits. Did OPP review any of these? Importantly, did OPP review any of the papers that suggested alternate MOA/AOPs for the pesticides, other than what was provided to OPP by the pesticide registrant?

The NTP systematic review framework requires an initial literature search to be as comprehensive as reasonably possible, by gathering the published, unpublished, and "grey" literature (publicly available government reports, etc.). And, the search is documented and publicly available.

3- All toxicity endpoints are based on unpublished industry-sponsored studies

In the Abamectin human health risk assessment (EPA 2015) Appendix A lists the toxicology profile of abamectin and emamectin with all the studies OPP used for toxicity determinations and endpoints. All of them are industry-sponsored Guideline studies. OPP provides in tabular form the extracted NOAEL and LOAEL values and a few words or a sentence to indicate the adverse effects that formed the basis of the endpoint (EPA 2015 Appendix A.3).

This is in stark contrast with Appendix B, which appears to be the only location where non-guideline studies are listed, and then they are listed by citation only. No information is provided to indicate whether or how the study informed OPP s assessment, whether data were extracted from the studies, and if so what data was extracted, how it was used, and what it was based upon. There is no public documentation at all on the use – if any – of non-Guideline studies.

For extracting data from studies, the NIEHS-NTP systematic review framework recommends template forms customized for the type of study (animal, human, in vitro) and the specific needs of the evaluation. Quality control is built into the process during this step, and all data extraction files are made publicly available. This level of transparency would increase agency credibility as well as meaningful public participation and communication.

4- OPP failed to consider alternate MOA/AOPs and related agents in the cumulative assessment group.

The family of macrolide lactones includes abamectin, milbemectin, milbemycins, avermectins, and ivermectins. In addition to anti-parasitic effects, they also have anti-inflammatory effects that appear to be the result of down regulating Nuclear Transcription Factor kappa-B (NF-kB) and the Mitogen-Activated Protein Kinase (MAPK) activation pathway, targeting leukocytes and neutrophils (Zhang et al 2011; Culić et al 2001). Abamectin has been reported to inhibit the production of pro-inflammatory cytokines TNF-α (which is regulated by NF-kb) and interleukin-6 (IL-6) in lung tissue in BALB/c mice, both of which can recruit neutrophils and macrophages (Zhang et al 2011). Because abamectin alters the activity of inflammatory cells and pro-inflammatory cytokines, which have many and varied functions in the cell, the pesticide office's presumption of a single MOA/AOP is unrealistic. A 1989 book reviewing abamectin and ivermectin literature at the time concluded that "it is not possible to assign a single mechanism of action for [abamectin] in the various systems that have been studied….. in addition to their interaction with chloride channels, several other actions of [abamectin] have been proposed; the importance of these remains to be resolved" (Campbell et al, 1989).

Abalis et al (1986) is mentioned by OPP as support for its proposed AOP (EPA 2015 Appendix H 2.3), but no full reference is provided (EPA 2015 Appendix B). However, we believe that OPP is referring to the publication by Abalis, Eldefrawi and Eldefrawi 1986. That paper reported that in 'microsac' preparations from rat brain, avermectin B1a activated (opened) two distinctly separate chloride channels, $GABA_A$-receptor chloride channels, and also voltage-gated chloride channels that were totally insensitive to GABA (Abalis et al 1986). This biochemical study demonstrates that – under the experimental conditions reported – avermectin has complex actions on rat brain cells, and at least two distinct AOPs.

Based on the available data, and on the gaps in understanding of the mechanisms leading to toxicity in humans, the data do not support a conclusion that only one MOA/AOP is operable. Importantly, the data also do not support OPP's conclusion that the one AOP that the agency identified is any more or less true than other possible AOPs. . An over-reliance on AOPs – as the proposed CRA does - can be really detrimental to the CRA process, and can result in chemical evaluations that too narrowly define the relationships that could exist between substances.

Other macrocyclic lactones include commonly used antibiotics such as erythromycin, azithromycin, and others. Has OPP considered the interactions and cumulative exposures of abamectin with these agents? Why has OPP not included all of the macrocyclic lactones in the Cumulative Assessment Group (CAG) with abamectin? Has OPP considered the interactions of cumulative exposure to ivermectin and azithromycin, as demonstrated in the study on healthy human volunteers by Amsden et al (2007)? OPP has failed to address the true cumulative risks from exposure to related chemicals.

5- OPP relied on a poorly understood proposed MOA/AOP with very little supporting data.

OPP's framework for screening analysis establishes a candidate CMG by considering chemical structural similarities, hazard profiles, and mechanism of toxicity. As reported by OPP in the 2015 Abamectin human health assessment, the insecticidal MOA of the macrocyclic lactones of the avermectin class (abamectin and emamectin) is mediated by interaction with the glutamate-gated chloride channels and gamma-aminobutyric acid A ($GABA_A$) gated chloride channels in insects (EPA 2015 Appendix H 2.3). OPP states that this is "presumed to be the insecticidal mechanism of action of emamectin and abamectin as

well" (EPA 2015 Appendix H 2.3). OPP provides only two citations for its proposed AOP (Abalis et al 1986; Huang and Casida 1997). Abalis et al (1986) reported that in a biochemical preparation from rat brain, avermectin B1a opened two distinct chloride channels through two distinct receptor-binding activities, one GABA-ergic and one completely insensitive to GABA. Huang and Casida is a receptor binding study conducted in an in vitro tissue culture system of rat cerebellar granule neuron cells. Huang and Casida report that in their tissue culture system Avamectin binds to two different sites on the chloride channel with different effects, activating the channel at lower concentrations when binding to the high-affinity site, and blocking the channel at higher concentrations when it binds to the lower-affinity site (Huang and Casida 1997).

OPP also notes that abamectin interferes with GABA-mediated neurotransmission (EPA 2015 p. 10). While binding to GABA receptors may lead to neurotoxicity, other consequences of altered GABAergic signaling (e.g., physiological changes due to interplay with other receptor systems) in conjunction with other exposures should be considered in a cumulative risk assessment. GABA-mediated toxicity is a very complicated concentration-dependent effect, poorly understood, and poorly supported proposed toxicity mechanism, operating through at least two distinct AOPs.

6- OPP eliminated established default protective factors.

For abamectin the protective FQPA factor had been 10X in the pre-2011 abamectin assessments due to the lack of a developmental neurotoxicity study (EPA 2015, p. 23). In 2011 it was reduced to 3X for all repeat exposure (chronic) risk assessments because two DNT studies were submitted. Interestingly, the OPP reviewers at the time noted that in both reproduction and developmental neurotoxicity studies of rat pups there was a very narrow dose range between the dose that caused reduced body weight (0.2 mg/kg/day) and death (0.4 mg/kg/day). OPP also noted increased susceptibility of the young animals in rats, CD-1 mice, and rabbits. The FQPA factor was removed for the acute dietary scenarios because the endpoint was from a dog study where the dose-response range was not so steep. OPP noted at the time that the CD-1 mice are the most sensitive due to the lack of P-glycoprotein (EPA 2011, p. 24)

In 2012 OPP reviewed abamectin again, but still retained the 3X FQPA for the same reasons as previously described. The acute dietary endpoint was based on the dog study and an acute neurotoxicity study in rats, and all other endpoints (chronic dietary, short term oral, dermal, inhalation) were based on combined data from three reproduction studies and two developmental neurotoxicity studies (NOAEL 0.12 mg/kg/day, FQPA 3X, UF 100X) (EPA 2012, p.29). Those studies are in multiple species: rats; rabbits; and CD-1 mice that are most sensitive.

In 2015 OPP eliminated the FQPA factor (reduced to 1X). OPP's rationale is that in mammals the observed neurotoxicity is mediated through the P-glycoprotein (P-gp), which is in the cell membrane and acts to move xenobiotics (like pesticides) from within the cell to outside the cell, where the body can excrete them in bile, urine, or fecal waste (EPA 2015 p. 20). This supposedly prevents accumulation of these pesticides in the brain and gonads and fetus (EPA 2015 p. 20). "Therefore," writes OPP, "test animals with genetic polymorphisms that compromise P-gp expression are particularly susceptible to abamectin induced neurotoxicity," citing Lankas et al 1997, as evidence that the CF-1 mouse is uniquely sensitive to abamectin and emamectin neurotoxicity because some CF-1 mice have no or diminished P-gp activity (EPA 2015 p. 20). Lankas et al (1997) reported that in subpopulations of CF-1 mice, levels of P-gp levels correlated well with whether the mice were highly sensitive (low or no P-gp) or insensitive (high P-gp levels) to the toxic effects of abamectin and ivermectin (Lankas et al 1997, 1998). The authors are employed by Merck Research Laboratories, which makes abamectin. OPP provides no systematic

assessment of the quality of these studies, limitations, analysis of bias, etc. OPP also doesn't seem to have made any attempt to adjust the doses across studies and to humans, despite evidence in whole animal studies that avermectins have different effects at different doses (Abalis et al 1986; Huang and Casida 1997), and how P-gp addresses toxicity across the dose range is unknown.

While there is no reason to believe the study results of Lankas et al are not reliably reported, what OPP does next with this small bit of data from a single laboratory as the only basis for an otherwise unsupported hypothesis is unjustified. OPP then presumes – with no supporting references – that not only would P-gp have the same activity in humans of all ages, but that it is the only detoxifying/elimination mechanism and that it is a completely effective elimination mechanism. OPP notes that P-gp is undetectable in the neonatal rat brain until a week after birth (PND 7), and that this must be the explanation for why abamectin induced toxic effects including death in the reproductive and DNT tests. OPP does not address the fact that toxic effects were seen in mice, rats, and rabbits, and does not report P-gp levels in fetal mice or rabbits. OPP then notes – without providing any supporting references - that in the developing human fetus P-gp was found as early as 22 weeks of gestation (5.5 months). OPP makes the following unsupported presumptions: that the human fetus has active P-gp; that it would effectively and efficiently eliminate avamectins; that it would do this at all relevant doses; and, that it would do this even for the first 5.5 months where brain and gonadal development occurs but there are no data to inform whether or not P-gp is present or active. OPP dismisses all evidence from whole animal studies of developmental and reproductive toxicity, concluding that, "the Agency, at this time, does not believe that the early post-natal findings in the rat are relevant to human newborns or young children" (EPA 2015 p. 20). OPP uses data from the beagle dog to support the risk estimates. OPP does not provide any scientific support for this conclusion – they are purely speculative. For example, OPP should provide: a quantitative analysis of active P-gp levels across relevant species including dogs; a developmental and DNT study in what OPP believes to be a relevant species such as dogs; some evidence that P-gp is an effective elimination mechanism before dismissing evidence of harm from whole animal studies; an explanation *with evidence* for why evidence of susceptibility in mice and rabbits that supported the 3X FQPA for abamectin (EPA 2009) is disregarded (MRIDs 44179901 and 00130819).

OPP's elimination of the FQPA factor is certainly not valid if exposures to P-gp inhibitors are also occurring, such as ivermectin (Didier and Loor, 1996; Lacher et al, 2015) and many drugs. OPP did not consider this in its cumulative risk assessment, although it should have been included.

Contrary to OPP's presumption that polymorphism of the MDR-1 gene in human populations does not result in a loss of P-gp function similar to that found in CF-1 mice (Macdonald & Gledhill, 2007; EPA 2015, p. 21), it appears that MDR1 polymorphism (3435CT) is associated with reduced intestinal P-gp expression (Hoffmeyer et al, 2000). This means that some people may be more like the subpopulation of sensitive CF-1 mice. Further, this reduced expression is the result of a single-nucleotide polymorphism (SNP) in exon 26 of the MDR1 gene, and at present over twenty SNPs have been identified in the MDR1 gene. Some of these SNPs result in altered P-gp function (Kim et al 2001; Yang et al 2008). These may have clinical implications, given that a polymorphism of C3435T is also reported to be a risk factor for inflammatory bowel diseases, Parkinson's disease and renal epithelial tumor (Sakaeda et al 2003). Brisson et al (2015) identified MDR1 polymorphisms as linked with elevated risk of childhood leukemia, particularly when associated with environmental exposures to pesticides and insecticides. OPP's presumption provides only a single reference to support it, and that reference clearly notes in the abstract that, "there is no clear consensus on whether or not SNPs, or combinations of SNPs,

reduce human p-glycoprotein functionality" (Macdonald and Gledhill 2007), demonstrating that the pesticide office may have mis-read or over-interpreted the article.

In contrast to the little evidence that OPP required to adopt a speculative AOP for abamectin, with its recent chlorpyrifos human health risk assessment, OPP dismissed multiple, robust, peer-reviewed studies providing epidemiologic evidence showing that the AOP the pesticide office was using to support its risk estimates (cholinesterase inhibition) was not sensitive enough to protect kids that were exposed prenatally (Earthjustice et al 2015). How can the pesticide office justify this difference? In both cases, the outcome was favorable to industry by reducing protections for people, and permitting more pesticide.

For the purposes of these comments, the main point is that OPP provides very little scientific data supporting its removal of the default FQPA factor, while dismissing evidence of harm in whole animal studies across multiple species in the face of a proposed hypothesis with no evidence – quantitative or qualitative – of its relevance in humans. OPP relied on studies without any systematic review for study quality and bias, and OPP applied those studies without a pre-explained method for extracting data and with no dose-response analysis for relevancy. Further, most of the steps in the proposed AOP were speculative only, including dubious relevance to early-life stage humans.

Established protective default factors like the FQPA are backed by medical and scientific information and understanding gathered from cellular, whole animal, and human epidemiologic studies, clinical reports, poisoning incident data, developmental biology, etc. (EPA 2006 Supplemental; NRC 1993). Protective default factors like FQPA should not be dismissed on the basis of poorly-supported, partially understood proposed AOPs. "Declaring a chemical as not hazardous, or reducing a level of health protection, should require validation, not speculation" (Melnick et al 2003).

7- The cumulative assessment group is too restrictive.

The macrocyclic lactones (avermectins and milbemycins) are products or chemical derivatives of soil microorganisms belonging to the genus Streptomyces. OPP included only two chemicals in the macrocyclic lactones of the avermectin class, i.e. abamectin and emamectin. There are five avermectins in commercial use: ivermectin, abamectin, doramectin, eprinomectin, and selamectin. Commercially available milbemycins are milbemycin oxime and moxidectin. (Merck Veterinary Manual, 2014) Ivermectin, eprinomectin, abamectin, doramectin, and moxidectin are variously available for use in cattle including dairy cattle, sheep, pigs, goats, and horses as an antiparasitic drug treatment.

Abamectin and emamectin share a similar chemical structure and act as agonists and antagonists (depending on the concentration) of the gamma-aminobutyric acid (GABA) neurotransmitter (EPA 2015 Appendix A and I; Huang and Casida 1997). Acute toxicity studies for eprinomectin found shared similar neurotoxicity symptoms observed in abamectin studies, such as ataxia, tremors, loss of righting reflex, ptosis, and bradypnoea among CD-1 mice (Bagdon and McAfee, 1990). Since these additional compounds meet OPP's own framework, they should not be excluded from the assessment.

Although abamectin and emamectin are currently the only members of this group with active registrations of pesticides, individuals can still be exposed to the other chemicals via diet. The FDA has set maximum residue limits (MRLs) for ivermectin, doramectin, and eprinomectin in cattle meat (specifically liver and muscle) and milk. For example, the maximum amount of eprinomectin allowed in cattle milk is 12 ppb. Thus, ivermectin, doramectin, and eprinomectin residues should be taken into

consideration in OPP's calculations of cumulative exposure. The assessment should also account for exposure to other pesticides such as lindane, cyclodienes, and fipronil which act on GABA-gated channels (Ratra et al 2001; Bloomquist 2003; Sunol et al 1998).

In addition, effects of co-exposure to pyrethroids, which affect voltage-gated sodium channels, should be considered. Abamectin is documented to be used in combination with bifenthrin and may also be used in combination with other pyrethroids (Cloyd 2009). Workers and communities in agricultural areas are likely to be exposed to combinations of many pesticides.

C - NRC IDENTIFIED SERIOUS CONCERNS WITH THE USE OF CURRENT PREDICTIVE AND COMPUTATIONAL TOX METHODS

Recent advances in computational and biological tools have allowed for the rapid evaluation of chemical toxicity at the molecular and cellular levels. These tools have been implemented in large-scale agency projects, including the Toxicity Forecaster (ToxCast™) and Tox21 projects at EPA and NIEHS. While the hope for these tools to aide in decision-making is high, the current applications for emerging data streams are less clear. Based largely upon non-native cell types (e.g., immortalized cell lines) and isolated cellular machinery (e.g., nuclear receptors), these tools can miss processes that require higher levels of cellular functioning. The ability of new tools to predict chemically-induced perturbations at the tissue, whole organism, and population levels have yet to be determined.

A report of the National Academies released this summer details some of the concerns associated with the use of predictive toxicology methods as they currently exist (NRC 2015). The committee warned that, "In vitro assays, alternative animal models, and other emerging technologies described here and in more detail later in the committee's report hold promise, but some important limitations or considerations should be noted:

a. *In vitro* assays for predicting acute toxicity have focused primarily on non-mechanistic indicators of toxicity, such as cytotoxicity; they were not developed with a quantitative linkage to any phenotype (acute or chronic).
b. Existing assays do not cover the full range of exposure or toxicity routes, and do not include first pass metabolism.
c. Most current *in vitro* assays do not account for important pharmacokinetic characteristics, such as metabolism, that can influence *in vivo* toxicity.
d. Cellular systems commonly use immortalized cancer cell lines, which might fail to detect chemical activity or effects that might occur in normal (non-tumor) differentiated cells.
e. Cells can have different levels of activity or responsiveness, depending on whether they are primary cells, differentiated cells, or immortalized cells and on how many times they have been cultured, so assay reproducibility can be a problem.

The limitations highlighted by the NRC report demonstrate that the mechanistic information obtained from emerging tools should be used be used cautiously and judiciously. The lack of biological and chemical coverage, the inability to mimic or replicate metabolism, the absence of clear links to long- or short-term human health outcomes, and the uncertainties associated with high-throughput screening data could lead to an underestimate, or complete missing, of the toxicity of chemicals in our environment. OPP should use mechanistic information to strengthen the public health protectiveness of its overall evaluation, but should not be considered as more or less valuable than other evidentiary data.

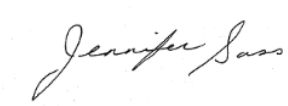
Mechanistic data should be treated as a parallel stream of data, and can be very helpful in interpreting human and animal data, but should not be seen as either necessary or sufficient for interpreting or evaluating other data. Importantly, it should not be used to dismiss evidence of harm. Strong evidence from mechanistic/MOA studies could support a conclusion and raise it to a level of increased concern.

CONCLUSION

The chemical and agrochemical industry and OPP have dismissed evidence of harm from whole animal studies, instead positing inadequately tested MOA or AOPs. The result is denied or delayed regulatory limits that fail to account for cumulative risk, and fail to protect vulnerable populations. OPP should adopt the systematic review framework of the NTP or IRIS programs to provide consistency and transparency, reduce speculation, and hopefully eliminate the industry-favorable bias in the way the pesticide office gathers, reviews, and utilizes scientific evidence.

Thank you for the opportunity to submit comments.

Respectfully,



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