Section I: Risk assessment

1.1 Assessing risks of pesticides to bees: putting the science into context to inform regulatory decision making[1]

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The U.S. Environmental Protection Agency (EPA) in collaboration with Health Canada's Pest Management Regulatory Agency (PMRA) and the California Department of Pesticide Regulation (CDPR) have developed a guidance for assessing the risks of pesticides to bees. This guidance is based on work conducted in the Europe and through international symposia, and it was externally peer reviewed by the Scientific Advisory Panel process used by EPA to evaluate the emerging science that serves as a foundation for regulatory decision making. EPA has been working with its regulatory counterparts in the Organization for Economic Cooperation and Development (OECD) to ensure the development of tests to support the tiered risk assessment process; these studies include laboratory- and field-based studies examining both exposure and effects to individual bees as well as colonies. For effect studies, multiple measurement endpoints have been identified; however, there is a need to consider the relationship of these endpoints to assessment endpoints and protection goals on which regulatory authorities base decision. Research is needed to develop quantitative linkages between measurement endpoints identified at different levels of biological organization that will enable extrapolation from lower levels of biological organization to apical endpoints at the whole organism, colony, population and community level on which regulatory authorities are likely to base decisions. This presentation provides a general overview of the risk assessment process for bees in the U.S and Canada and emphasizes the need to integrate multiple lines of evidence into the conceptual framework of an Adverse Outcome Pathway (AOP) and to develop a strong foundation for assessing the likelihood and magnitude of an adverse effects, i.e., risk, to bees with which to inform risk management decisions.

Since 2006 when Colony Collapse Disorder was first reported, multiple government reports have been published in the U.S. In 2007, the National Academy of Sciences published a report by the National Research Council on the Status of Pollinators in North America[1] where a number of pollinating species (insects, birds, bats) were reported to be decline. In 2012, the Congressional Research Service reported to Congress on the potential role that pesticides may be having on bee health. In 2013[2], the USDA in collaboration with the EPA published the results of a National Stakeholder meeting on honey bee health[3] where the past 6 years of research was discussed. Although the number of multiple species of pollinators have been reported to be in decline, and in particular the honey bee, the demand for pollination services has continued to increase. For example, California produces 80% of the world's almonds and crop insurances requires almond growers to have 2 colonies per acre. With approximately 800,000 acres devoted to almond production in California, a steady increase since 2004, this means that at this time roughly 1.6 million bee colonies are needed to support almond pollination in California.

Figure 1, from the NASS publication on the Status of Pollinators in North America[4], depicts U.S. Department of Agriculture National Agricultural Statistics Survey (NASS) data[5] on the number of colonies in the US used in honey production. This graph has been used as evidence on managed honey bee declines in the U.S. However, the graph must be interpreted with caution. **Figure 1** indicates that the number of managed colonies used for honey production peaked at roughly 6

[1] The views expressed in this presentation may not reflect those of the U. S. Government, the Canadian Government, the U.S. EPA, or Health Canada's Pest Management Regulatory Agency.

million in 1947, but has declined to roughly 2.5 million by 2006. During the war, sugar was at a premium and many citizens had to rely on honey as a sweetener. After the war, sugar became more plentiful and the demand for honey decreased. As more jobs became available in urban environments and less demand for honey, there were fewer beekeepers.

Figure 2 depicts the NASS data[6] from 1970 to 2012. NASS did not conduct surveys between 1982 and 1987. When NASS resumed the surveys, the methods used to collect information had changed and fewer beekeepers met the criterion for inclusion in the survey; as such, the steep decline depicted in **Figure 1** is to some extent an artifact of how data were being collected. The graph also depicts when Varroa mites (*Varroa destructor*) were introduced into the U.S. around 1989, which was followed by a drop in the total number of colonies. The graph also depicts when CCD was first reported in the U.S. in 2006 and again there was a decrease in the number of colonies associated with honey production. However, the graph indicates that in general, the number of colonies in the U.S. associated with honey production has been relatively constant at around 2.5 million since 1996. What the graph does not depict is the level of effort which beekeepers in the U.S. have had to expend to maintain colonies.

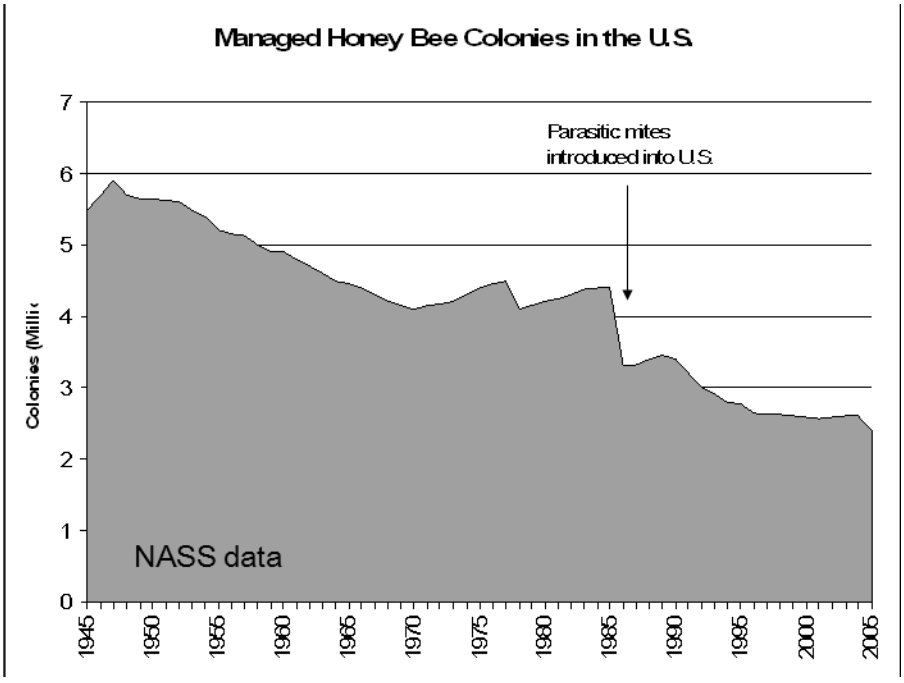

Figure 1 National Agricultural Statistics Survey data on the number of managed honey bee (Apis mellifera) colonies associated with honey production in the United States by survey year. Taken from NAS 2007 report on the Status of Pollinators in North America.

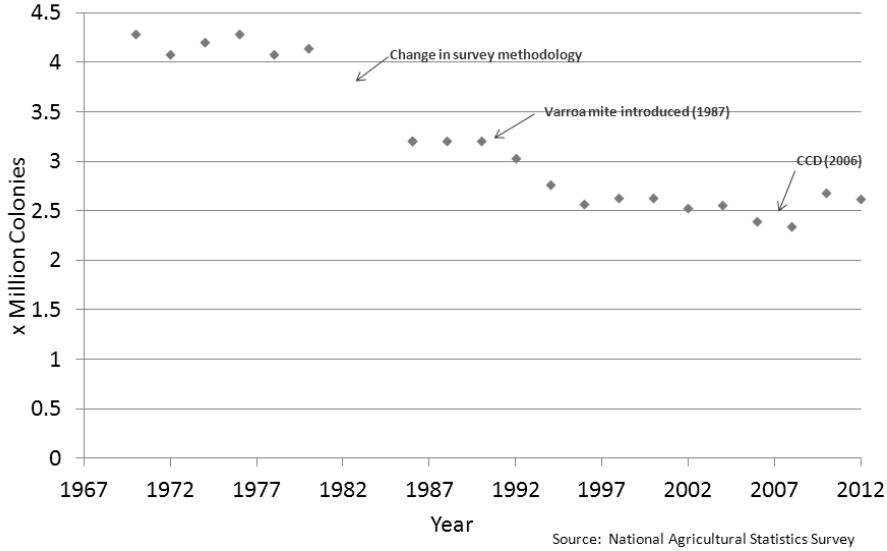

Figure 2 National Agricultural Statistics Survey data on the number of managed honey bee (Apis mellifera) colonies associated with honey production in the United States in survey years 1967 to 2012.

Declines in honey bees has not been limited to the U.S. as reports in the press and in published articles have highlighted losses in pollinators in Europe as well[7, 8, 9]. In Potts *et al*. 2010[10], researchers reported in the Journal of Apicultural Research decreased numbers of colonies in many countries within Europe from 1985 – 2005 except for those along the Mediterranean coast. Also during this period the number of beekeepers declined in most of these countries.

Since 2006, the USDA, and more recently the Bee Informed Partnership, has conducted a survey[11] of beekeepers to determine the percent winter loss. Over the past seven years winter loss have ranged between 22% to 36% compared to what survey respondents indicated was an acceptable winter loss rate of roughly 15%[12]. The winter loss numbers do not reflect losses that occur at other times during the year. Based on estimates from the Bee Informed Partnership, total annual losses from April 2012 – April 2013 averaged around 49%[13].

As alluded to earlier, a number of factors have been associated with honey bee losses and according to researchers at the USDA, which has been tasked by Congress as the lead federal agency for determining the causes of CCD and declines in honey bee health, the factors include diseases/pests, agricultural practices where lands are converted to extensive monocultures that may not support honey bees or urbanization where forage habitats are also lost, both of which can lead to nutritional deficits for bees. Pesticides have also been identified as a factor as well as bee management practices (e.g., moving colonies thousands of miles). Although multiple factors have been associated with declines, no single factor has been identified as a "cause". USDA has coined the term 'the three Ps" to characterize the "primary factors" including: pests/disease, pesticides, and poor nutrition.

Regulatory agencies such as EPA and PMRA are responsible for evaluating the potential risks from of a wide range of chemicals that can vary greatly in their physical, chemical, and biological properties. In the case of ecological risk assessment for each chemical, there are thousands of species to account for potential adverse effects which can differ vastly in their biology (and susceptibility) as well as their potential for exposure to a given chemical. In human health risk assessment, there are many different types of organ, tissue, and other biological systems to account for as well as variation in susceptibility based on biology or demographics. Moreover, for

each combination of a chemical and species or aspect of human biology, there is a wide range of possible adverse effects (or outcomes) to account for when evaluating risk.

In the U.S. there are 16,683 registered conventional pesticide representing roughly 672 active ingredients[14]. In 2014 alone, the Registration Division processed 1,391 actions related to the registration of pesticides. The Pesticide Reregistration Division processed 4,414 actions related to pesticide registrations. For a single chemical that was recently evaluated, there were 58 environmental fate studies and 107 ecological effect studies submitted; of the ecological effect studies, 33 were on aquatic organisms and 74 on terrestrial organisms, of which 39 were on honey bees. The honey bee studies ranged from laboratory-based studies on individual organism to semi-field controlled exposure studies on the whole colony. In the face of the scientific challenges associated with assessing risk there is finite time and resources allocated to completing reviews. Risk assessors must be able to develop methods/technologies to produce chemical risk assessments that are timely (continue to meet work milestones), efficient (use best available and most relevant scientific information in a targeted manner to reduce the use of resources and animals and take maximum advantage of existing data), transparent (make scientific assumptions and linkages clear), and high quality (results are reliable and of the highest scientific standard). At the same time, it is critical that improvements in risk assessment process must be able to support sound regulatory decisions that are protective of both human health and the environment.

Figure 3 depicts the general framework followed by regulatory agencies such as EPA and PMRA in conducting ecological risk assessments across taxa and this process is codified at EPA through formal Agency guidance[15]. The process consists of three phases, *i.e.*, problem formulation, analysis and risk characterization. Problem formulation is the initial phase where protection goals and their associated assessment endpoints are identified, a risk hypothesis articulated and a conceptual model of potential routes of exposure and effects are depicted and an overall plan for conducting the risk assessment is outlined. The box to the far left of **Figure 3** (entitled Planning Dialog) is considered a critical component of the risk assessment process since it is where risk management goals (aka protection goals) are defined and the risk manager is informed regarding potential risks associated with the chemical under evaluation. Following problem formulation, the analysis phase begins where, based on submitted studies, the environmental fact (exposure profile) and ecological effects (stressor-response profile) are characterized. Once estimates of exposure and effects are developed, the risk assessment proceeds into the risk characterization phase where point estimates of exposure and effects are then used to form a quantitative risk estimate which is then further characterized with other lines of evidence to provide risk managers with an understanding of the potential magnitude and likelihood of adverse effects to particular taxa. Although the process depicted in Figure 3 appears to be relatively uni-directional, it is intended to be iterative.

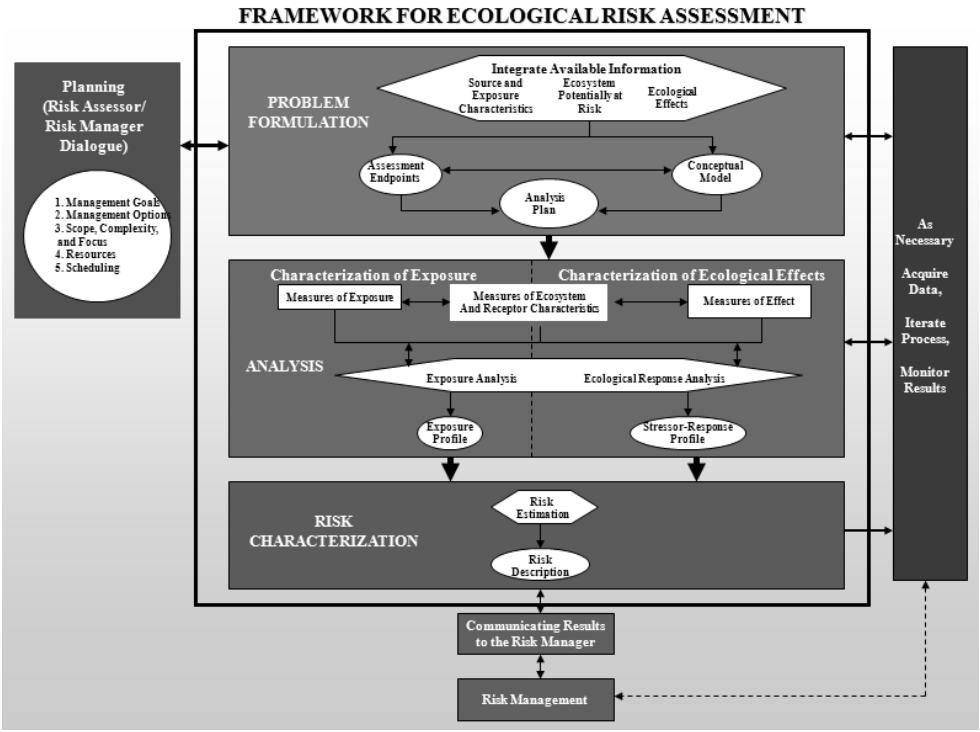

Figure 3 Generic ecological risk assessment framework.

With respect to assessing the potential risks of pesticides to insect pollinators and more specifically to bees (honey bees), in September 2012, the EPA OPP in collaboration with Health Canada's Pest Management Regulatory Agency (PMRA) and the California Department of Pesticide Regulation (CDPR) presented a White Paper[16] on a proposed risk assessment framework for bees to a FIFRA Scientific Advisory Panel (SAP). In June of 2014, a final harmonized guidance document[17] was published. Up until this point, EPA relied on a qualitative process for evaluating the potential hazard that pesticides represent to beneficial insects using the honey bee as a surrogate. The harmonized guidance describes a process whereby the potential risks of pesticide uses can be quantified using the deterministic risk quotient approach similar to that used by EPA for quantifying risks to other taxa.

The risk assessment framework described in the harmonized guidance is predicated on efforts that were underway in Europe as described by the European and Mediterranean Plant Protection Organization (EPPO) scheme[18] and the 2014 European Food Safety Authority (EFSA) guidance[19] as well as the Society of Environmental Toxicology and Chemistry (SETAC) global Pellston Workshop[20] held in 2011.

As indicated, problem formulation serves as the basis of the risk assessment process and working in concert with risk managers, who have defined specific protection goals, Agency assessment endpoints are then identified that are complimentary to the protection goals. Measurement endpoints that reflect assessment endpoints are also defined. In the White Paper presented to the SAP[21], several protection goals were identified for honey bees and these goals are consistent with those identified in the EFSA guidance[22] and by the SETAC Pellston Workshop[23]. These goals consist of insuring pollination services, continued production of hive products (*e.g.*, honey, wax, propolis) and contributing to pollinator biodiversity. Assessment endpoints related to those protection goals include population survival, growth and reproduction and are typically referred to as apical

endpoints. For bees, measurement endpoints to inform our understanding of assessment endpoints that are of regulatory interest include measures taken on both individual bees and on the honey bee colony. While bees have measurement endpoints that are common to other taxa such as lethality (*i.e.*, impaired forage bee or colony survival), decreased growth (*e.g.*, reduced weight of individual bees/colony weights), decreased reproduction (*e.g.*, reduced numbers of developing brood), there are an increasing number of measurements endpoints (*e.g.*, behavioral, histological) where the relationship to assessment endpoints may not be clear.

As with the protection goals, the risk assessment framework itself described in the harmonized guidance is predicated on the efforts of EPPO, EFSA, and the SETAC Pellston. Some of the attributes of EPA/PMRA/CDPR risk assessment process is that it is tiered. At the most basic level used for screening large numbers of compounds, relatively conservative estimates of exposure and effects are used. These are typically based on laboratory-based measures on individual bees. In moving up to higher levels of refinement, there is an increasing need for data that are intended to reflect greater realism and transition from individual-based effects to colony-level effects. While the process makes use of existing guideline toxicity studies, it also draws on studies that are under development such as the chronic adult and larval bee toxicity tests. Also, while there are many potential routes of exposure for bees, the risk assessment focuses on what are considered to be major routes of exposure (*i.e.*, contact and ingestion of residues in pollen/nectar). Also the process distinguishes risks from foliarly applied compounds versus soil/seed treatment.

The screening-level (Tier 1) is using conservative estimates of exposure (contact and oral) and effects to individual bees are evaluated to derive risk estimates. If risk exceeds threshold values referred to as Levels of Concern (LOCs), the risk manager can request that the assessment proceed to Tier 2 where more refined measures of exposure are considered and effects are determined at the colony rather than individual bee level. At Tier 2 effects are still assessed under relatively controlled conditions. At the highest level of refinement (Tier 3) data are intended to reflect potential effects at the colony level under actual use conditions.

At Tier 1, the risk assessment process for bees relies heavily on lethality as a measurement endpoint for assessing acute toxicity. Guideline studies though require the reporting of sublethal measurement endpoints and sufficient information may be available in the study to support the calculation of a median effect dose (*i.e.*, ED_{50}) or depending on the study, a median effect concentration (*i.e.*, EC_{50}). A broader range of endpoints are typically considered for assessing chronic risks where study designs are hypothesis-based and generate a no-observed adverse effect concentration (NOAEC) and a lowest-observed adverse effect concentration (LOAEC). Typically, these endpoints are based on impaired survival, growth or reproduction which are all known to have effects at the population level. As the final phase of the risk assessment process, point estimates of exposure based on maximum application rates and point estimates of the most sensitive toxicity endpoints are expressed as a ratio referred to as the risk quotient.

Toxicity tests to support risk assessment are continuing to evolve. Well in advance of the risk assessment framework, EPA issued an interim guidance[24] in 2011 for risk assessors on data to consider when evaluating the potential for adverse effects to bees. However, with the release of the EPA/PMRA/CDPR harmonized guidance, the battery of tests that serve as a foundation for the screening-level assessment, *i.e.*, laboratory-based studies of individual bees and more refined colony level studies under field conditions, are being required depending in the amount of information the risk manager may need. These data requirements have focused on the understanding that the honey bee colony represents a complex superorganism consisting of bees in different stages of development, different genders, and amazingly different roles. Data requirements have attempted to address these different aspects by first determining the extent to which chemicals may be toxic to individual adult and larval bees on an acute and chronic exposure basis. At higher levels of refinement, toxicity testing examines potential effects to whole colonies under relatively controlled conditions (semi-field studies) and then under actual use conditions when bees are free-foraging.

Regulatory authorities have been working with the Organization for Economic Cooperation and Development (OECD) as well as the International Committee on Plant-Pollinator Relationships (ICP-PR) who are in the process of developing toxicity testing protocols that can be used in a regulatory context, *i.e.*, study designs that are sufficiently detailed and tested to insure that the methods can be readily reproduced and that data are generated in a way that is consistent. So, efforts are underway to advance testing protocols for individual bees. Relative to larval toxicity testing, the challenge has been to develop suitable methods to allow the study to be extended beyond the larval development stage to include pupation and emergence of the young adult bees. These tests examine a much broader span of honey bee brood development. High mortality rates have in the past limited these longer test designs in the past but progress is being made.

Efforts are also underway to develop a chronic toxicity test with adult bees. The 10-day adult bee toxicity testing protocol is one such test. Semi-field testing protocols currently exist in Europe (*e.g.*, OECD 75[25]); however, this particular test protocol focuses on brood development and there is a broader interest in the overall functioning of the colony. EPA has already started to require semi-field testing to support chemical registration. Semi-field tests provide an opportunity to not just examine effects at the colony level, but effects on individual bees as well can be assessed, *e.g.*, behavior/foraging activity, for various castes within the colony. The semi-field studies also provide an opportunity to measure potential exposure by looking at residues in foliage, pollen and nectar of treated plants and comparing those residues to what are contained in the bee colony as bee bread and royal/brood jelly. While sublethal effects may be reported in laboratory-based studies, *e.g.*, proboscis extension reflex or biochemical measures of immune response, a large array of endpoints are increasingly reported in semi- and full-field testing conducted to support higher tier refinements, and whether effects observed at the individual laboratory-based level are significant at the whole colony level. The White Paper and guidance document discuss these measurement endpoints for the honey bee as with other taxa, the utility of these endpoints has typically been in the qualitatively characterizing risk estimates that are primarily based on more apical endpoints such as impaired survival, growth and reproduction.

The FIFRA SAP that reviewed the framework on assessing risks to bees encouraged the consideration many of these sublethal effects in the future when suitable linkages have been identified between these measurement endpoint and impaired survival, growth and reproduction. One of the concepts that was discussed in the White Paper as a means of developing suitable linkages between multiple levels of biological organization has been the conceptual framework of an Adverse Outcome Pathway[26] (AOP). The conceptual framework has been invoked in a number of EPA activities, the most recent being the Endocrine Disrupting Screening Program. The AOP provides a systematic framework to support the integration of diverse types of data in hazard/risk assessment. Once such a framework has been established, information obtained from lower levels of biological organization, for example, structure-activity relationships and in vitro studies can then be used to predict and potentially screen for outcomes at higher levels of biological organization including the population level. The key to making AOPs work, is the ability to establish clear linkages (or causal quantitative relationships) between lower and higher levels of biological organization. There are numerous advantages for using AOPs in chemical risk assessment. In general, AOPs allow us to use the information we do have more effectively and to build better predictive tools in cases where potential effects are not empirically measured, *i.e.*, for which study data are not available.

In keeping with the conceptual framework of AOPs and recommendations from the SAP, efforts are underway on the development of simulation models for honey bee colonies. These models may provide a means to establish linkages between sublethal effects and more apical endpoints that are used as assessment endpoints. These models may also serve as a means of fine tuning toxicity testing methods to focus on measurement endpoints that have the highest likelihood of impacting the colony and/or provide the best means of addressing particularly uncertainties that

have been identified. Also, simulation models may provide a means of more consistently integrating colony-level measurement endpoints from Tier 2 and Tier 3 testing to support qualitative characterizations of Tier 1 RQ values. EPA has been working collaboratively with the USDA Agricultural Research Service on expanding the BeePop (VarroaPop[27]) model to include a pesticide module for determining the effects that pesticides may play on colony survival when other factors (*e.g.*, Varroa mites; *Varroa destructor*) are affecting colonies as well. We are also aware of efforts in the Europe to examine the utility of the BEEHAVE honey bee model[28] as well as other simulation models.

One of the important components of the proposed risk assessment process is the consideration of other lines of evidence. These multiple lines of evidence are considered in terms of their consistency/coherence and biological plausibility. A challenge faced by risk assessors is the role of sublethal effects that have been reported with increasing frequency in the open literature and their relationship to assessment endpoints of impaired survival, growth and reproduction at the colony. Multiple lines of evidence are considered in the risk assessment in an effort to place quantitative estimates of risk (RQ values) based on laboratory studies of individual bees into the context of potential effects on the whole colony under what may be more realistic exposure conditions.

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