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The formulation makes the honey bee poison

Christopher A. Mullin *, Jing Chen, Julia D. Fine, Maryann T. Frazier, James L. Frazier

Department of Entomology, Center for Pollinator Research, The Pennsylvania State University, University Park, PA 16802, USA

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ABSTRACT

Dr. Fumio Matsumura's legacy embraced a passion for exploring environmental impacts of agrochemicals on non-target species such as bees. Why most formulations are more toxic to bees than respective active ingredients and how pesticides interact to cause pollinator decline cannot be answered without understanding the prevailing environmental chemical background to which bees are exposed. Modern pesticide formulations and seed treatments, particularly when multiple active ingredients are blended, require proprietary adjuvants and inert ingredients to achieve high efficacy for targeted pests. Although we have found over 130 different pesticides and metabolites in beehive samples, no individual pesticide or amount correlates with recent bee declines. Recently we have shown that honey bees are sensitive to organosilicone surfactants, nonylphenol polyethoxylates and the solvent N-methyl-2-pyrrolidone (NMP), widespread co-formulants used in agrochemicals and frequent pollutants within the beehive. Effects include learning impairment for adult bees and chronic toxicity in larval feeding bioassays. Multi-billion pounds of formulation ingredients like NMP are used and released into US environments. These synthetic organic chemicals are generally recognized as safe, have no mandated tolerances, and residues remain largely unmonitored. In contrast to finding about 70% of the pesticide active ingredients searched for in our pesticide analysis of beehive samples, we have found 100% of the other formulation ingredients targeted for analysis. These 'inerts' overwhelm the chemical burden from active pesticide, drug and personal care ingredients with which they are formulated. Honey bees serve as an optimal terrestrial bioindicator to determine if 'the formulation and not just the dose makes the poison'.

1. Introduction: agrochemicals contribute to bee decline

Colony Collapse Disorder (CCD) and the general decline of pollinators continues to be a major threat worldwide [1,2]. Honey bee (*Apis mellifera* L.) overwintering colony losses in the US have averaged one-third since 2006, and yearly losses are now approaching 50% [3]. It is thought that multiple factors such as pathogens, parasites, malnutrition, and pesticide exposure have a role in CCD. The global diminishing of bees [4,5] and the relative importance of pesticides have both received much attention recently. Foraging bees are exposed to pesticides in agro-ecosystems as they gather nectar and pollen from flowers. Honey bees constitute a terrestrial model *par excellence* for agrochemical sampling in the environment, since their foraging range from a single hive averages about 6 kilometers in radius [6,7]. A comparative study of CCD-affected hives and healthy hives revealed the presence of over 130 different pesticides and metabolites out of 200 analyzed in over 1300 wax, pollen, and bee samples taken from managed hives across the US and Canada, with an average of 6 detections per sample [7–9]. Over 150 different pesticides have been found in samples from apiaries worldwide [10], and hives uncontaminated by pesticides, whether beekeeper-applied or not, are very rare. Managed honey bee colonies are intentionally exposed to miticides in an effort by beekeepers to control *Varroa destructor* Anderson & Trueman [10]. Not surprisingly, coumaphos and fluvalinate (two widely used in-hive miticides) were the two most frequently detected pesticide residues in managed hives [9]. However, no correlation was found between any one pesticide and CCD [5,7–11], suggesting that other more generic formulation ingredients may be involved. The combined effects of insecticides, fungicides and other agrochemical residues on honey bee sociality, foraging dynamics and floral specializations may jeopardize future production of food [1]. The role of agrochemical 'inerts' in the ongoing investigation of CCD/pollinator decline, and their effects on the physiology/behavior of honey bees have only begun to be investigated.

Abbreviations: AT, acquisition trial; CAS, Chemical Abstract Service; CCD, Colony Collapse Disorder; EO_n, polyethoxylate chain of n ethoxy units; FQPA, Food Quality Protection Act; GRAS, generally recognized as safe; HPV, high production volume; LC_{50}, LD_{50}, lethal concentration or dose respectively for 50% of the tested population; LC-ESI-MS, liquid chromatography coupled to electrospray ionization mass spectrometry; MW, molecular weight; NMP, N-methyl-2-pyrrolidone; $NP(EO)_n$, nonylphenol polyethoxylate; $OP(EO)_n$, octylphenol polyethoxylate; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe.

* Corresponding author. Department of Entomology, Penn State University, University Park, PA 16802, USA.
E-mail address: camullin@psu.edu (C.A. Mullin).

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2. Modern formulation technologies and agrochemical safety

Modern pesticide formulations and seed treatments, particularly when multiple active ingredients are blended, require proprietary adjuvants and inert ingredients to achieve high efficacy for targeted pests. An adjuvant, inert (term used in the US) or co-formulant (EU) is an additive (2,000 + in the US) used to enhance the performance or aid in the stability of formulations (20,000+) of active ingredients (1,000+). An adjuvant can be termed a surfactant, penetrant enhancer, activator, spreader, sticker, wetting agent, buffer, antifoaming agent, drift retardant, etc., and usually more than one of these functional descriptors is associated with the same chemical. Adjuvants are much less expensive than the active ingredient, but can reduce the effective pesticide dose needed by as much as 10-fold [12]. A surfactant is a surface active-agent (= detergent, emulsifier, soap) that reduces the surface tension of water. This is achieved by being structurally composed of distinct hydrophilic and hydrophobic moieties, often referred to as head and tail groups, to mediate both the mixing of hydrophobic pesticides with water to form solutions or the dissolution of hydrophobic plant and insect cuticles and membranes to allow active ingredients to penetrate. The head groups, regardless if hydrophilic or hydrophobic, can be neutral, anionic, cationic or amphoteric. An opposite polarity tail moiety, such as a hydrophobic hydrocarbon chain or hydrophilic polyoxyethylene or sugar, completes the surfactant molecule and serves in its classification.

Modern nonionic surfactants include alkyl and aryl ethoxylates, organosiloxanes, sorbitans and fatty acid esters [13–15]. Common formulation anionics include sodium dodecylsulfate, sulfonates, lauric and other fatty acids, glycolic acid ethers and phosphates. Cationics are exemplified by tallow amines and trialkylammonium salts. Other inerts function as co-solvents, higher-boiling liquids (antifreezes) used to keep formulation components in solution such as butanol, diethoxol, methylcyclohexanone, N-methyl-2-pyrrolidone (NMP), propylene glycol, and xylene. Emulsifiable and soluble concentrates are the most commonly used agrochemical formulations and are often a dynamic blend of nonionic with either anionic or cationic surfactants to produce micro-emulsions or solutions of mutiple components in a tank mix. Typical formulations contain less than 50% active ingredients with the remainder surfactants and solvents. Adjuvant use has evolved (Fig. 1) from focus on alkylphenol, alcohol, fatty acid and sorbitan ethoxylates in combination with sulfonates to new technologies comprising fatty (tallow) amine and organosilicone ethoxylates and co-solvents like NMP [16–18].

3. Formulations inerts and impacts on non-target species

Adjuvants are largely assumed to be biologically inert and are usually not included in risk assessments required to register a pesticide in the U.S. [19–21]. Of the 20 toxicological tests required to register a new pesticide in the US, 13 are conducted with only the active ingredient(s); only 7 short-term acute mammalian and avian toxicity tests use the entire formulation [22,23]. Medium- and long-term toxicity tests only examine the active ingredient(s). Little data exist concerning the toxicity of 'inert' ingredients on honey bees, likely because bee toxicity information for pesticide formulations is not currently required by the US EPA as part of the pesticide registration process in contrast to the EU where toxicity for representative formulations is mandatory [24]. Moreover, the specific ingredients that make up spray adjuvants are considered trade secrets of the chemical companies that manufacture them and are therefore usually not disclosed [12,23]. In response to public concerns, the US EPA Inert Ingredient Assessment Branch recently conducted an open commentary period that ended on April 23, 2010 (Docket ID: EPA-HQ-OPP-2009-0635; cf [25]) to consider the disclosure of inert ingredients on pesticide product labels. EPA action on hundreds of responses is still pending. More label disclosure would allow increased user and consumer awareness of all potentially toxic chemicals in pesticide formulations as well as more thorough testing of the potential biological impacts.

Co-formulants and supplemental adjuvants that can be used in tank mixes often enhance the pesticidal efficacy as well as inadvertently the non-target effects of the active ingredient after application [26,27]. Numerous studies have found that pesticide active ingredients elicit very different physiological effects on non-target organisms when combined with their formulation ingredients [28]. Indeed, systemic movement of the top pesticide used globally, glyphosate, is determined by its formulation inerts. Glyphosate has negligible ecotoxicity without tallow amines and other adjuvants [18,29], including its toxicity to humans [30–32]. Formulation inerts often increase pesticide toxicity to aquatic insects, fish and amphibians [33,34]. The nonionic surfactant R-11 synergized the acute toxicity of the insecticides spinosad [35] and imidacloprid [36] on aquatic crustaceans, and in the absence of an insecticide reduced the growth rate of *Daphnia pulex* at concentrations found after application near aquatic systems at recommended field rates [21]. Clearly the formulation components themselves have a lot to do with the potency of the poison.

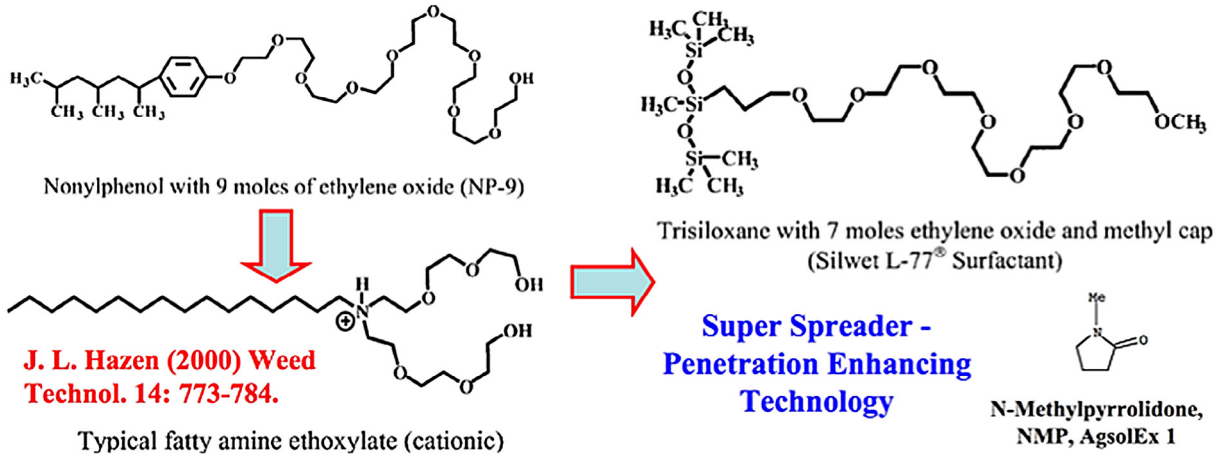

Fig. 1. Brief chemical history of modern adjuvant development.