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Spray Toxicity and Risk Potential of 42 Commonly Used Formulations of Row Crop Pesticides to Adult Honey Bees (Hymenoptera: Apidae)

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ABSTRACT To combat an increasing abundance of sucking insect pests, >40 pesticides are currently recommended and frequently used as foliar sprays on row crops, especially cotton. Foraging honey bees may be killed when they are directly exposed to foliar sprays, or they may take contaminated pollen back to hives that maybe toxic to other adult bees and larvae. To assess acute toxicity against the honey bee, we used a modified spray tower to simulate field spray conditions to include direct whole-body exposure, inhalation, and continuing tarsal contact and oral licking after a field spray. A total of 42 formulated pesticides, including one herbicide and one fungicide, were assayed for acute spray toxicity to 4–6-d-old workers. Results showed significantly variable toxicities among pesticides, with LC_{50}s ranging from 25 to thousands of mg/liter. Further risk assessment using the field application concentration to LC_1 or LC_{99} ratios revealed the risk potential of the 42 pesticides. Three pesticides killed less than 1% of the worker bees, including the herbicide, a miticide, and a neonicotinoid. Twenty-six insecticides killed more than 99% of the bees, including commonly used organophosphates and neonicotinoids. The remainder of the 13 chemicals killed from 1–99% of the bees at field application rates. This study reveals a realistic acute toxicity of 42 commonly used foliar pesticides. The information is valuable for guiding insecticide selection to minimize direct killing of foraging honey bees, while maintaining effective control of field crop pests.

KEY WORDS insecticide, spray, toxicity, honey bee, risk assessment

Honey bees (*Apis mellifera* L.) play a vital role in global crop production. In addition to honey production, commercial beekeepers provide millions of honey bee hives for pollinating fruit, nut, seed, oil, and fiber crops (Southwick and Southwick 1992). The annual enhanced crop value from pollination in the United States is estimated at US$16 billion, with 75% of that being attributed to honey bees (Calderone 2012, Johnson and Corn 2014). Honey bee populations are threatened by numerous pests, parasites, and pathogens, including the idiopathic colony collapse disorder (vanEngelsdorp et al. 2006, Potts et al. 2010, Goulson et al. 2015). Additionally, changing agricultural practices have added obstacles to maintaining healthy populations of honey bees (U.S. Department of Agriculture [USDA] 2012). During the past few years, the widespread implementation of transgenic plants has caused a pest status shift from chewing insects to sucking insects on row crops, such as the tarnished plant bug (*Lygus lineolaris* [Palisot de Beauvois]) and stink bugs (*Acrosternum hilare* [Say], *Nezara viridula* [Linnaeus], and *Euschitus servus* [Say]), having a wide range of host plant species (Greene et al. 1999, Lu et al. 2008). This pest status shift, coupled with the development of insecticide resistance in target insects (Zhu et al. 2004, 2012), has resulted in increased foliar sprays of insecticides to control the sucking insects. This also increase the risk of foraging honey bees coming into direct contact with insecticides. Currently, a variety of insecticides from at least four insecticide classes are available for pest control, including pyrethroids, organophosphates, carbamates, neonicotinoids, and other novel insecticides. More than 40 pesticides (including acephate, dicrotophos, thimethoxam, clothianidin, imidacloprid, etc.) are currently recommended by extension specialists for the chemical control of row crop (mainly cotton, soybean, rice, and corn) insects (Brandon and Robinson, 2014, Catchot et al. 2014, Krupke et al. 2014), particularly a variety of insects (e.g., tarnish plant bug and stink bugs) on cotton (Catchot et al. 2014).

Residues of >150 pesticides were detected at various levels in wax, pollen, bee, or honey (Johnson et al. 2010, Mullin et al. 2010). The possible relationships between honey bee colony losses and sublethal effects of pesticide residues have received considerable attention, and published data indicated that pesticide residues

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may pose either serious adverse impact (Di Prisco et al. 2013, Larson et al. 2013, Sanchez-Bayo and Goka 2014) or very low to no risk (Cutler and Scott-Dupree 2007, Pilling et al. 2013, Cutler et al. 2014) to honey bees. While the collective data from these studies has generally provided inconclusive results, however, many pesticides used in mid-south agriculture, such as dicrotophos and acephate, have high acute toxicity to honey bees. Yet, they have received relatively less experimental examination. In addition, when farmers come to select insecticides for insect pest control, it is difficult to determine which are relatively more or less toxic for honey bees, because very limited bee-toxicity warning is provided in pesticide use recommendation, or a similar or even identical warning is marked in pesticide labeling for most chemicals. Therefore, honey bees and other pollinators may be indiscriminately exposed to a variety of pesticides in row crops.

Previous assessments of pesticide acute toxicity to honey bees have mostly been made from topical application or using an artificial feeder with pesticides incorporated into sugar solution (Faucon et al. 2005, Lu et al. 2012, Hardstone and Scott 2010). Insecticides can exhibit contact toxicity, systemic toxicity, and/or both toxicities (Thomson 1989, Mahajna et al. 1997, Fishel 2013). One-time topical treatment with technical grade pesticide excludes continuous exposure via tarsal contact and oral licking after spray and ignores potential synergistic toxicity from formulating materials (Zhu et al. 2014, Mullin et al. 2015). Such tests may not provide adequate information about formulated pesticides for growers to choose that may be both effective to target pests and less harmful to honey bees if such products are available. Recent study evaluated the risk of a neonicotinoid insecticide to bumble bees and found clothianidin was hazardous to bumble bees after spraying (Larson et al. 2013) because foraging bees took the contaminated pollen. Thus, research is promptly needed to simulate foliar sprays to better understand the potential risk of pesticide exposure in the field because foraging honey bees are not only directly exposed to spraying pesticides, but also through multiple routes simultaneously including direct contact, inhalation, and ingestion (Johnson 2015). To provide spray toxicity data and potential risk of field exposures, we simulated field spray and assessed toxicities of 42 commonly used formulated pesticides (Catchot et al. 2014, Krupe et al. 2014) to honey bees, using a modified Potter Spray Tower.

Materials and Methods

Pesticides. A total of 42 formulated pesticides were examined for spray toxicity to honey bee workers, including 40 insecticides and miticides, one herbicide, and one fungicide. These studied were chosen from recommended crop protection chemicals listed in extension bulletins (Brandon and Robinson 2014, Catchot et al. 2014, Krupke et al. 2014), recommendations from extension entomologists and pathologists, and direct information from cotton farmers. Pesticides used for these bioassays were provided by the manufacturers or purchased from local agro-chemical suppliers. When multiple formulations made by different companies were marketed, the selection of a formulation for testing was based exclusively on availability without preference. All chemicals were stored at approximately 10°C in a refrigerator.

Honey Bees and Cage Design. Colonies and brood were supplied by local beekeepers in Arkansas. Hives were inspected and certified to be apparently disease free by the Arkansas State Plant Board. Combs with >50% coverage of healthy brood were transferred to an incubator (33 ± 0.5°C; 65% $\pm$ 3 RH) with no light. Twenty-five newly emerged bees were transferred to a cage and maintained at 33°C in an incubator for at least 4 d before being used for bioassays. The cage was made of a 500-ml round wide-mouth polypropylene jar (D by H: 9.3 by 10 cm). The lid of the jar was cut to make an 8.9-cm-diameter (d) hole and covered with 8-mesh metal screen to facilitate spray applications. Four holes were made at the bottom of the jar: two 1.27 cm (d) holes for bee entry and ventilation and two 2.54 cm (d) holes for holding sugar solution and water vials. A piece of plastic comb foundation (3.81 by 8.9 cm) was glued to the bottom and side of the jar for bees to congregate and reach the feeding vials. Each cage, containing 25 workers (4–6 d old), was supplied with a piece (1 by 1 by 2 cm^3) of Global Patties (purchased from Betterbee Inc., Greenwich, NY), and 20 ml each of sugar syrup (50%, V/V) and d-H_2O in scintillation vials.

Modified Spray Tower. The Potter-Precision Laboratory Spray Tower was purchased from Burkard Scientific (Uxbridge, Middx, United Kingdom). For laboratory safety and working efficiency purposes, the spray tower was reconstructed with Plexiglas to fit into a fume hood. The modified spray tower, containing the original spray nozzle and nearly the same pressure air delivering and regulating systems as those in Potter Spray Tower, significantly reduces the time for sample handling and cleaning between chemicals. With the spray settings at volume = 0.5 ml, air pressure = 69 kpa (10 psi), and spray distance = 22 cm, the sprayer delivers a stream of mist into the cage which forms a thin layer that uniformly covers all inner sides of the cage without forming visible droplets.

Dose Response Bioassay. To obtain the median lethal concentration (LC_{50}) data, each chemical was diluted in d-H_2O to 6–7 concentrations, plus a water (d-H_2O) only as control. Bees at 4–6 d old were used for dose-response bioassays. Each cage (containing 25 bees) was treated as a replication, and three replications were used for each concentration. Dead bees were recorded before treatment, and cages with more than three dead bees were not used for bioassays. The modified spray tower at the setting described above was used to spray bees with 500 µl of pesticide solution. After spraying, bees were maintained at 33°C and 48-h mortality was recorded. For some slow action and/or low-toxicity pesticides, the incubation was extended to 7 d to ensure the 48-h mortality is truly representative.

Data Processing and Statistical Analysis. SAS (version 9.2) probit analysis (SAS Institute Inc. 2008) was conducted to calculate LC_{50} (lethal concentration that kill 50% honey bee workers) values and 95%

fiducial limits. Chi-square tests were applied to ensure the goodness-of-fit of the models. If a given bioassay failed the goodness-of-fit chi-square test, the experiment was repeated. Some pesticides have low toxicity to honey bees, and their 95% fiducial limits could not be calculated. The assays for these chemicals were repeated until similar mortality patterns were reached over a similar dose range.

Risk Assessment. LC_{50} values are often used as a toxic parameter for revealing the comparative toxicity of chemicals tested. In field, pesticides are used at different rates or concentrations. Therefore, two chemicals having the same LC_{50}s may pose different risks to foraging bees if they are used at different field concentrations. To correctly assess the risk of all 42 pesticides, we obtained the field use rates from extension recommendations (Brandon and Robinson, 2014, Catchot et al. 2014, Krupke et al. 2014). A field use (or application) concentration (mg/liter) was calculated by dividing the field use rate by average use volume (e.g., 10 gallon per acre; Catchot et al. 2014) for each pesticide. The toxic risk of each pesticide to bees was assessed by using the ratio calculated by dividing the field use concentration by toxic parameter (LC_1, LC_{50}, or LC_{99} to bees). If their ratios of field use concentration to LC_1 are less than 1, these pesticides are relatively safe to bees. If their ratios of field use concentration to LC_{99} are greater than 1, those pesticides are highly toxic. The remaining pesticides may have intermediate toxicity to bees if their ratios don't fall into the low range or high range. The greater the ratio is the greater the risk to honey bees. In addition, each LC_{50} value of all 42 formulations was converted to amount of active ingredient. Relative toxicity of the active ingredient to honey bees was ranked and compared to the toxicity rank of respective formulation.

Conversion of LC_{50} to LD_{50}. Honey bee workers (16 d old) were immobilized by placing bees in freezer (−20°C) until all bees fell to the bottom of cage (some bees with barely moving legs). Five bees (as a group) were weighed immediately before and after being sprayed with of d-H_2O using spray tower. The spray volume, distance, and pressure were set the same as describe above. The sprays were repeated eight times with different bees. Average spray weight per bee was calculated by assuming 1 ml of d-H_2O is equal 1 gram. In order to compare relative toxicity in term of active ingredient among 42 pesticides, the percentage of active ingredient from pesticide label was used to calculate LC_{50} in active ingredient. LD_{50} for either formulation or active ingredient was estimated by using average weight of spray solution deposited on each honey bee.

Results

Acute Spray Toxicity (LC_{50}). A total of 142 dose response assays were conducted. The assay was repeated at least twice for each pesticide to obtain overlapped 95% fiducial limits. LC_{50} values and statistical analyses for all 42 pesticides are summarized in Table 1. These chemicals showed a wide range of LC_{50} values (Table 1). Five insecticides had an LC_{50} value below 100 mg/liter, suggesting higher toxicity to bees than those with higher LC_{50} values. Thirteen insecticides had an LC_{50} range from 100 to 300 mg/liter. Thirteen insecticides had an LC_{50} range from 300 to 7,000 mg/liter and the remaining 11 chemicals had LC_{50} values greater than 7,000 mg/liter. All 42 pesticides were sorted into ascending order according to their LC_{50} value, while their toxicities to bees were in descending order in Table 1 because more toxic pesticides need less amount of the chemical to kill the same percentage (50%) of bees. The slope of the dose response curve indicates the sensitivity of honey bees to chemicals. The higher slopes indicate the greater sensitivity. Twenty-five pesticides had slopes between 1 and 2. Nine chemicals had slopes below 1, while eight pesticides, including all four organophosphorus insecticides, had slopes greater than 2.

Comparison of Different Toxicity Parameters. The average fresh body weight for 16-d-old worker bees was 0.125 g. The average volume of pesticide solution deposited on each bee was 1.575 µl or mg per bee. By using these two numbers, lethal concentration (LC_{50}: mg/liter) and lethal dose (LD_{50}: µg/bee) of formulation and active ingredient were obtained (Table 2). The toxicity ranks of 42 pesticides were sorted by LC_{50}s of formulations from 1, the most toxic, to 42, the least toxic pesticides. The toxicity ranks were also sorted by LC_{50}s of active ingredient (column 5, Table 2). Re-calculating LC_{50}s to active ingredient changed toxicity ranks substantially for some pesticides, and the toxicity of the chemical, different percentages of active ingredient in formulations, and other potential factors may account for the discrepancy (please see discussions). LC_{50}s were converted to LD_{50}s based on average weight of pesticide solution deposited on each bee and average fresh weight of bee body. Comparison of 12 LD_{50}s from this study with corresponding 12 LD_{50}s from Hardstone and Scott (2010) indicated that four insecticides had similar range of LD_{50}s and other eight insecticides had different LD_{50}s between our data and the data from Hardstone and Scott (2010). Among the eight insecticides, five insecticides from this study had lower LD_{50}s or higher toxicity and three insecticides had lower toxicity than the same insecticides from Hardstone and Scott (2010).

Risk Assessment. The field-use rates recommended by the Delta Agricultural Digest (Brandon and Robinson, 2014) are different for different target pests, and have a range for one specific target. We used a median rate as the field use rate (oz./acre in Table 3) for each pesticide. Ratios of field use concentration to LC_1 or LC_{99} were used as an indication of the toxicity risk of the various chemicals to honey bees. Results in Table 3 indicated that three chemicals (Acetamiprid, Etoxazole, and Glyphosate) had ratios of field use concentration to LC_1 less than 1, suggesting these chemicals are relatively safe to foraging bees because they may kill less 1% bees at the field use rate. When the field use concentrations were compared with corresponding LC_{99} values, 26 chemicals (with gray background in Table 3) have a

Table 1. Spray toxicity of 42 commonly used pesticides to honey bees, measured with formulated pesticides and spray tower

No[a]	Chemical name	Toxicity to bees LC_{50} mg/liter	95% Fiducial limits[b]	$Pr > \chi^2$	Slope ± SE
1	Dicrotophos	24.92	23.00–27.01	0.1521	2.3563 ± 0.2064
2	Thiamethoxam	62.56	55.94–70.78	0.1524	1.2913 ± 0.1106
3	Emamectin Benzoate	65.51	59.54–71.80	0.2251	1.6940 ± 0.1380
4	Clothianidin	67.27	61.16–74.07	0.9550	1.5763 ± 0.1213
5	Abamectin	68.32	62.54–74.69	0.1586	1.7667 ± 0.1354
6	Thiamethoxam + l-cyhalothrin	107.32	94.50–121.29	0.8311	1.0861 ± 0.0951
7	Acephate	126.43	117.48–136.11	0.2531	2.5512 ± 0.2139
8	Zeta-cypermethrin	138.31	125.07–152.65	0.7471	1.5101 ± 0.1197
9	Chlorpyrifos	141.10	131.92–150.99	0.9934	3.0920 ± 0.2940
10	Dimethoate	142.78	132.83–153.61	0.7934	2.6167 ± 0.2283
11	Methomyl	179.38	164.43–196.07	0.1218	1.8928 ± 0.1520
12	Cyfluthrin	182.54	163.77–203.81	0.6137	1.3089 ± 0.1061
13	Bifenthrin + avermectin	197.21	180.00–215.67	0.2139	1.8317 ± 0.1458
14	Permethrin	198.25	179.74–217.68	0.6024	1.6974 ± 0.1441
15	Imidacloprid + b-cyfluthrin	213.12	185.78–247.50	0.0747	1.4408 ± 0.1437
16	Oxamyl	214.03	200.06–229.22	0.9953	2.9926 ± 0.2780
17	Sulfoxaflor	229.50	212.77–247.57	0.1200	2.4437 ± 0.2093
18	Bifenthrin	258.30	228.35–290.80	0.8196	1.2348 ± 0.1091
19	Spinosad	302.26	262.47–349.07	0.0541	1.5402 ± 0.1564
20	Beta-cyfluthrin	312.15	281.60–349.40	0.3671	1.5585 ± 0.1726
21	Cypermethrin	332.55	301.04–369.49	0.4391	1.4907 ± 0.1239
22	Bifenthrin + Zeta-cypermethrin	498.31	457.22–541.56	0.3883	2.0673 ± 0.1831
23	Imidacloprid	552.20	454.54–663.32	0.0618	1.2036 ± 0.1229
24	Gamma-Cyhalothrin	575.31	525.19–628.69	0.9853	1.9169 ± 0.1645
25	Lambda-Cyhalothrin	575.41	447.72–881.42	0.0633	1.1281 ± 0.1772
26	Methoxyfenozide + spinetoram	712.21	634.69–787.34	0.8863	1.7831 ± 0.1869
27	Carbaryl	895.21	817.28–982.13	0.3290	1.7916 ± 0.1432
28	Indoxacarb	1,140	1008–1281	0.1008	1.3800 ± 0.1267
29	Esfenvalerate	1,500	1369–1643	0.9432	1.7216 ± 0.1327
30	Thiodicarb	1,774	1600–1976	0.1365	1.5687 ± 0.1318
31	l-cyhalothrin + chlorantraniliprole	2,059	1912–2218	0.4423	2.5668 ± 0.2266
32	Tetraconazole	8,105		0.7419	2.0618 ± 8.3697
33	Fenpyroximate	1.46E + 04	8359–568554	0.7894	1.3667 ± 0.5200
34	Acetamiprid	2.51E + 05	25160–1.356E38	0.9168	0.4153 ± 0.1996
35	Flonicamid	9.76E + 05		0.0501	0.3255 ± 0.1963
36	Etoxazole	1.30E + 06		0.4737	0.3191 ± 0.2277
37	Novaluron	4.03E + 07		0.5257	0.2634 ± 0.3340
38	Propargite	8.06E + 07		0.2440	0.2128 ± 0.2408
39	Flubendiamide	5.96E + 08		0.7018	0.1733 ± 0.2193
40	Chlorantraniliprole	2.93E + 17		0.7105	-0.0491 ± 0.2027
41	Spiromesifen	2.75E + 19		0.7279	0.0625 ± 0.2210
42	Glyphosate	4.62E + 34		0.8161	0.0359 ± 0.2902

[a] Commercial name (formulation) and Manufacturer: 1. Bidrin 8 EC by AMVAC Chemical Co.; 2. Centric 40 WG by Syngenta; 3. Denim 0.16 EC by Syngenta; 4. Belay 50 WDG by Valent; 5. Epi-Mek (Agri-Mek 0.15EC) by Syngenta; 6. Endigo 2.06ZC by Syngenta; 7. Bracket97 by Winfield Solutions LLC; 8. Mustang Max/Respect by FMC; 9. Lorsban 4E by Dow AgroSciences; 10. Dimethoate 4 E by Cheminova; 11. Lannate 2.4 LV by DuPont; 12. Tombstone 2 EC by Loveland; 13. Athena by FMC; 14. Arctic 3.2EC by Winfield Solutions LLC; 15. Leverage 360EC by Bayer CropScience; 16. Vydate 3.77 CLV by DuPont; 17. Transform 5G by Dow AgroSciences; 18. Brigade 2 EC by Agrisolutions; 19. Tracer 4 SC by Dow AgroSciences; 20. Baythroid XL 1 EC by Bayer; 21. Holster by Agrisolutions; Loveland; 22. Hero 1.24 by FMC; 23. Advise 2 F (Couraze 1.6 F) by Winfield Solutions, LLC; 24. Declare by Cheminova; 25. Karate Z 2.08 CS by Syngenta; 26. Intrepid Edge by Dow AgroSciences; 27. Sevin XLR Plus by Bayer CropScience; 28. Steward EC by DuPont; 29. Asana XL 0.66 EC by Bayer; 30. Larvin 3.2 F by Bayer; 31. Besiege by Syngenta; 32. Domark 230 ME by Valent; 33. Portal 0.4 EC by Nichino America Inc; 34. Intruder 70 WP by Gowan; 35. Carbine 50 WG by FMC; 36. Zeal by Valent; 37. Diamond 0.83 EC by Mana/Chemtura; 38. Comite II by Chemtura; 39. Belt 4SC by Bayer; 40. Prevathon 0.43 SC by DuPont; 41. Oberon 2 SC by Bayer; 42. Roundup PowerMAX by Monsanto.

[b] Because the chi-square is small ($P > 0.1000$), some fiducial limits were not calculated by SAS.

high risk of acute toxicity to foraging bees because these chemicals may kill more than 99% of bees at the field use rate. The remaining 13 chemicals may be considered to have intermediate toxicity risk to bees. They may kill 1 to 99% foraging honey bees at field use rate, and more bees are expected to be killed by chemicals that have higher ratios of field-used concentration to LC_{99} (Table 3).

Discussion

Southern row crops, especially the cotton with longer blooming period, are frequently sprayed (Gore et al. 2012) from July to early September to control a complex of sucking insects and a few lepidopterans. Acephate and neonicotinoids are often used to control sucking insects because these insecticides may have both contact and systemic toxicities. To better protect honey bees and other pollinators, it is important to understand that 1) field sprays of pesticides may inevitably pose a risk to foraging honey bees and 2) the risk to honey bees could be minimized through the careful selection of pesticides having lower toxicity. To achieve this goal, one strategy is to screen commonly used pesticides and to determine which pesticides have low toxicity to honey bees.

Table 2. Toxicity of 42 pesticides, expressed as lethal concentration (LC_{50}: mg/liter) and lethal dose (LD_{50}: μg/bee) of formulation (F) and active ingredient (AI)

Chemical name	LC_{50} mg/liter (F)	LC_{50} mg/liter[a,b] (AI)	Toxicity rank by LC_{50} (F)	Toxicity Rank by LC_{50} (AI)	LD_{50} μg/bee[c] (F)	LD_{50} μg/bee[a,c] (AI)	LD_{50} μg/g[a,d] (AI)	LD_{50} μg/g[e]
Dicrotophos	24.92	20.43	1	7	0.04	0.03	0.26	1.72(0.41–3.05)
Thiamethoxam	62.56	25.02	2	8	0.10	0.04	0.32	0.202(0.067–0.299)
Emamectin Benzoate	65.51	1.41	3	2	0.10	0.00	0.02	
Clothianidin	67.27	15.88	4	5	0.11	0.03	0.20	
Abamectin	68.32	1.37	5	1	0.11	0.00	0.02	
Thiamethoxam + l-cyhalothrin	107.32	13.52	6	4	0.17	0.02	0.17	
Acephate	126.43	122.64	7	23	0.20	0.19	1.55	
Zeta-cypermethrin	138.31	13.28	8	3	0.22	0.02	0.17	
Chlorpyrifos	141.10	67.73	9	16	0.22	0.11	0.85	0.847(0.59–1.14)
Dimethoate	142.78	62.11	10	14	0.22	0.10	0.78	1.62(1–2.47)
Methomyl	179.38	52.02	11	12	0.28	0.08	0.66	
Cyfluthrin	182.54	45.16	12	11	0.29	0.07	0.57	0.677(N/A)
Bifenthrin + avermectin	197.21	17.43	13	6	0.31	0.03	0.22	
Permethrin	198.25	72.96	14	17	0.31	0.11	0.92	1.18(0.172–2.03)
Imidacloprid + b-cyfluthrin	213.12	44.76	15	10	0.34	0.07	0.56	
Oxamyl	214.03	89.89	16	19	0.34	0.14	1.13	
Sulfoxaflor	229.50	114.75	17	21	0.36	0.18	1.45	
Bifenthrin	258.30	64.83	18	15	0.41	0.10	0.82	0.1(N/A)
Spinosad	302.26	133.60	19	26	0.48	0.21	1.68	0.402(0.025–0.78)
Beta-cyfluthrin	312.15	39.64	20	9	0.49	0.06	0.50	
Cypermethrin	332.55	101.76	21	20	0.52	0.16	1.28	1.18(0.2–3.7)
Bifenthrin + Zeta-cypermethrin	498.31	56.06	22	13	0.78	0.09	0.71	
Imidacloprid	552.20	118.17	23	22	0.87	0.19	1.49	0.403(0.128–0.75
Gamma-Cyhalothrin	575.31	82.84	24	18	0.91	0.13	1.04	0.2770
Lambda-Cyhalothrin	575.41	131.19	25	25	0.91	0.21	1.65	
Methoxyfenozide + spinetoram	712.21	201.56	26	29	1.12	0.32	2.54	
Carbaryl	895.21	394.79	27	30	1.41	0.62	4.97	7.8700
Indoxacarb	1140	171.00	28	27	1.80	0.27	2.15	
Esfenvalerate	1500	126.00	29	24	2.36	0.20	1.59	
Thiodicarb	1774	603.16	30	31	2.79	0.95	7.60	
l-cyhalothrin + chlorantraniliprole	2059	190.66	31	28	3.24	0.30	2.40	
Tetraconazole	8105	1661.53	32	33	12.77	2.62	20.94	
Fenpyroximate	1.46E+04	732.25	33	32	23.07	1.15	9.23	
Acetamiprid	2.51E+05	1.76E+05	34	34	395.50	276.85	2214.80	
Flonicamid	9.76E+05	4.88E+05	35	35	1537.34	768.67	6149.35	
Etoxazole	1.30E+06	9.33E+05	36	36	2041.96	1470.21	11761.69	
Novaluron	4.03E+07	3.75E+06	37	37	63524.34	5907.76	47262.11	
Propargite	8.06E+07	5.61E+07	38	38	126866.99	88299.42	706395.38	
Flubendiamide	5.96E+08	2.32E+08	39	39	938523.93	366024.33	2928194.66	
Chlorantraniliprole	2.93E+17	1.46E+16	40	40	4.6092E+14	2.3046E+13	1.8437E+14	
Spiromesifen	2.75E+19	6.36E+18	41	41	4.3331E+16	1.0009E+16	8.0076E+16	
Glyphosate	4.62E+34	2.25E+34	42	42	7.2843E+31	3.5474E+31	2.8380E+32	

[a] Percentage of the active ingredient was obtained from pesticide label.
[b] The active ingredient and toxicity rank in five insecticide mixtures were calculated based on one component with higher percentage.
[c] LD_{50} μg/bee was calculated by multiplying LC_{50} value by the volume of pesticide solution deposited on each worker bee (1.575 μl/bee).
[d] LD_{50} μg/g (or LD_{50} mg/kg) is the lethal dose (μg) at per gram bee weight base, calculated by dividing LD_{50} μg/bee by average weight of worker bee (0.125 g/bee).
[e] Data were from Hardstone and Scott (2010).

In this study, we evaluated formulated pesticides with a spray tower application, to realistically simulate field spray situations rather than using a topical application of technical grade pesticides to measure contact toxicity. At a constant spray pressure and standard spray distance and spray volume, the spray tower delivers a stream of pesticide mist uniformly which covers the whole bodies of target honey bees and all the inner sides of the cage. Therefore, the spray tower application overcomes the disadvantages of the popular topical application which lacks continuous exposure to pesticide residues by bees through tarsal contact and oral licking, a real situation present in fields after they are sprayed. In addition, spray tower application takes advantage of the ability to generate pesticide vapor that may enter respiratory tracts to incur inhalation toxicity (Gerolt 1970). The second novelty of the techniques used in this study was the selection of commercially formulated pesticides instead of technical grade (relatively pure) active ingredients. Using formulation or active ingredient may substantially change the toxicity ranking for many pesticides among the 42 pesticides tested in this study, due to the toxicity

Table 3. Risk assessment of 42 pesticides commonly used for spray treatment of row crop pests, determined using formulated pesticides and spray tower

No[a]	Chemical name	Target pests[b]	Field use rate oz/acre	Field use concentration (FUC)[c] mg/liter	Ratios of FUC:LC_1[d]	Ratios of FUC:LC_{99}[e]	Potential honey bee mortality
1	Dicrotophos	TPB/Thrip/BSB/GSB	6.5	4,868	524.00	72.66	>99%
2	Thiamethoxam	TPB	2.1	1,573	152.25	4.15	>99%
3	Emamectin Benzoate	BW/TBW	10	7,489	451.43	28.92	>99%
4	Clothianidin	TPB	4.5	3,370	219.12	11.46	>99%
5	Abamectin	Mite/WF	5	3,745	204.51	14.68	>99%
6	Thiamethoxam + l-cyhalothrin	Many	5	3,745	297.19	4.10	>99%
7	Acephate	TPB/Thrip/BSB/GSB	11	8,238	162.20	26.15	>99%
8	Zeta-cypermethrin	GSB	3.12	2,337	78.86	3.62	>99%
9	Chlorpyrifos	TPB	8	5,991	90.11	20.04	>99%
10	Dimethoate	Thrip	6.4	4,793	81.67	13.81	>99%
11	Methomyl	BW/TBW	1.88	1,408	26.83	2.30	>99%
12	Cyfluthrin	BW/GSB	2.1	1,573	50.96	1.46	>99%
13	Bifenthrin + avermectin	Many	13.7	10,260	185.27	14.62	>99%
14	Permethrin	ECB/CEW	3	2,247	44.62	2.88	>99%
15	Imidacloprid + b-cyfluthrin	Many	3	2,247	52.98	2.10	>99%
16	Oxamyl	TPB/GSB	13.75	10,298	104.68	22.10	>99%
17	Sulfoxaflor	TPB/Aphid	1.88	1,408	15.89	2.37	>99%
18	Bifenthrin	BW/BSB/GSB	4.48	3,355	85.46	1.97	>99%
19	Spinosad	BW/TBW	2.51	1,880	28.17	1.37	>99%
20	Beta-cyfluthrin	BW/GSB	2.1	1,573	42.27	1.13	>99%
21	Cypermethrin	BW	3.5	2,621	37.53	1.66	>99%
22	Bifenthrin+Zeta-cypermethrin	BW/FAW/Mite/TPB	10.3	7,714	47.70	5.03	>99%
23	Imidacloprid	TPB	4	2,996	37.48	0.79	98%
24	Gamma-Cyhalothrin	BW	1.67	1,251	7.32	0.65	93%
25	Lambda-Cyhalothrin	BW/GSB	2.08	1,558	21.29	0.34	86%
26	Methoxyfenozide+spinetoram	BW/TBW/FAW	6	4,493	23.26	1.71	>99%
27	Carbaryl	ECB/FAW/CEW	32	23,965	98.09	7.31	>99%
28	Indoxacarb	BW/TBW	10.25	7,676	36.30	1.25	>99%
29	Esfenvalerate	BW/GSB	7.7	5,767	14.85	1.00	99%
30	Thiodicarb	BW/TBW	2	1,498	3.72	0.19	40%
31	l-cyhalothrin+chlorantraniliprole	Many	9.5	7,115	8.55	1.40	>99%
32	Tetraconazole	Rust	4.5	3,370	1.28	0.13	3%
33	Fenpyroximate	Mite/WF	16	11,983	4.49	0.15	39%
34	Acetamiprid	Aphid	0.95	711	0.77	0.00	<1%
35	Flonicamid	TPB/Aphid	2.3	1,723	2.24	0.00	2%
36	Etoxazole	Mite	0.83	622	0.70	0.00	<1%
37	Novaluron	TPB/SB/BW	10.5	7,864	1.33	0.00	1%
38	Propargite	Mite	28	20,970	14.53	0.00	4%
39	Flubendiamide	BW/TBW	2.5	1,872	2.13	0.00	1%
40	Chlorantraniliprole	BW/TBW	20.5	15,353	1.37	0.00	1%
41	Spiromesifen	Mite/WF	8	5,991	3.25	0.00	1%
42	Glyphosate	Weed/Grass	125	93,614	0.03	0.00	<1%

[a] Refer the footnote in Table 1 for Numbers, Commercial names (formulation), and Manufacturers.
[b] TPB: tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois); Thrip: western flower thrip *Frankliniella occidentalis* (Pergande); BSB: brown stink bug *Euschistus servus* (Say); GSB: green stink bug *Acrosternum hilare* (Say); Mite: two spotted spider mite *Tetranychus urticae* Koch; WF: whitefly *Bemisia tabaci* (Gennadius); BW: boll worm *Helicoverpa zea* (boddie); TBW: tobacco budworm *Heliothis virescens* (F.), ECB: European corn borer *Ostrinia nubilalis* (Hübner); CEW: corn earworm *Heliothis zea* (Boddie); SHB: small hive beetle *Aethina tumida* Murray; Aphid: cotton aphid *Aphis gossypii* Glover; FAW: fall armyworm *Spodoptera frugiperda* (J.E. Smith); Rust: soybean rust *Phakopsora pachyrhizi* Sydow; SB: stink bug complex; Many: multiple insect species.
[c] Field use concentration (FUC) was calculated by dividing field use rate by 10 gallon (field use volume).
[d] LC_1: lethal concentration that incurs 1% mortality in test bees; the ratios, <1 (without background in the column), indicate that those pesticides kill <1% of the test bees.
[e] LC_{99}: lethal concentration that incurs 99% mortality in test bees; the ratios, >1 (with gray background in the column), indicate that those pesticides kill >99% of the test bees.

of chemical itself, concentration in the formulated pesticides, and potential interaction between active ingredient and formulating materials. However, formulated pesticides, not technical grade chemicals, are the only choice for farmers to protect their crops when chemical control is necessary. When exposure to field sprays becomes a serious issue in foraging bees, therefore, measuring comparative toxicity of formulated pesticides instead of active ingredient would be more important to include total toxicities from the pesticide itself, the formulating agents, and potential additive and synergistic interactions.

Our results make two major contributions to understanding variable acute toxicity to honey bees. First, the spray toxicity data for 42 commonly used pesticides in southern row crop systems help identify comparative toxicities to honey bees. These data provide valuable information for guiding the selection of chemicals in crop pest management to minimize risk to honey bees. The median lethal dose (LD_{50}) and median lethal concentration (LC_{50}) are commonly used parameters for measuring the toxicity of a substance (U.S. Environmental Protection Agency [EPA] 2004). We chose a spray tower method to treat honey bees to simulate the field exposure of formulated pesticides. Our data (Table 1) revealed a wide range of LC_{50} values among 42 commonly used pesticides, suggesting a possibility to minimize chemical risk to pollinators by choosing lower bee-toxicity pesticides for crop pest control. By referring to the classification standard of pesticide (World Health Organization [WHO] 2010), formulated dicrotophos may be classified as an extremely toxic insecticide to honey bees. Twenty insecticides (numbers 2 to 21 in Table 1) are highly toxic chemicals, including thiamethoxam, clothianidin, three organophosphates (acephate, chlorpyrifos, and dimethoate), and most pyrethroids tested. Ten pesticides (numbers 22 to 31) are moderately toxic, including imidacloprid and a few carbamate insecticides. The remaining 11 pesticides (numbers 32 to 42) are slightly toxic chemicals to honey bees, including acetamiprid, spiromesifan and novaluron. If LD_{50}s (μg/g of active ingredient) are used for the classification, 33 pesticides would fall into extremely toxic category. From this study, it is clear that tetraconazole (a fungicide), etoxazole (miticide), and glyphosate (a popular herbicide) have very minor or no acute toxicity to honey bees based on 48-h mortality data, with the results being supported by an additional week-long observation.

The second major contribution of this study is the risk assessment of the 42 pesticides to honey bees. Although the LC_{50} is an important parameter which reflects the acute toxicity of a chemical, different insecticides may be used at different rates. The recommended field use rates for row crop insect control are significantly different from 0.83 to 125 oz./acre (Table 2). Therefore, the risk of an insecticide to honey bees depends both on how toxic the chemical is and how much is used in field spray. Our risk assessment considered both acute spray toxicity (LC_1 and LC_{99}) of each pesticide to honey bee workers and recommended field use rate. The ratios of field application concentration to LC_1 or LC_{99} values (Table 3) gave clear indication of low-risk pesticide to bees if the ratio to LC_1 is less than 1, or high-risk pesticides if the ratio to LC_{99} is greater than 1. If the ratios were not included in these two categories, those pesticides are intermediate toxic to bees. Furthermore, our data provided a scale to measure the risk of each insecticide within each category, because the higher the ratio is, the higher risk to bees.

The risk is influenced by two factors, the field application concentration and the dose-response curve slope. In Table 1, 42 pesticides are listed from 1 to 42 according to their LC_{50} toxicity from the highest (number 1) to the lowest (number 42), whereas the corresponding risk (ratio to LC_{99}) did not follow the same order. While the first 22 pesticides (number 1-22) remained to be high-risk chemicals (killed >99% of test bees), four of the moderately toxic pesticides (number 26, 27, 28, and 31) from the moderately toxic group (number 23–31, classified according to LC_{50} ranges as described above) shifted to high-risk chemicals, because high dose-response curve slopes (number 26, 27, and 31), high field application concentrations (number 27 and 28), and both (number 31) increased the risk of these chemicals to honey bees with ratios to LC_{99} greater than 1. This is especially true for the higher use rate of carbaryl (number 27, Tables 1 and 3). The Gamma-Cyhalothrin (number 24) had relatively higher slope, but its low field use rate (1.67 oz./acre) still ranked this chemical as an intermediate-risk insecticide, suggesting the possibility to reduce bee mortality by decreasing field use rate.

In summary, an increased abundance of sucking insects, particularly on cotton, may trigger frequent foliar sprays that may pose a risk to foraging honey bees in the field and negatively impact developing brood in hives through contaminated pollen. This study was initiated to realistically simulate field sprays to assess the toxicity of 42 commonly used pesticides for row crop pest control. Our data, particularly the ratios of field application rates to lethal concentrations of each pesticide provide a quantifying scale to help extension specialists and farmers with pesticide selection to maintain effective control of target pests and minimize the risk to foraging honey bees as well. In addition, this study established a baseline and foundation for our future studies on the impact of sublethal doses of major concerning insecticides on honey bee physiology, including defense-, immunity-, stress-, and metabolic-related enzyme activities and gene regulations. This study will also facilitate our continuing research to understand whether pre-mixtures and tank-mixtures of major insecticides with other insecticide classes, fungicides, and herbicides synergize toxicity to honey bees and negatively interact with other mortality-causing factors.

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