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A field spray drift study to determine the downwind effects of isoxaflutole herbicide to nontarget plants

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Abstract

Spray drift buffers are often required on herbicide labels to prevent potential drift effects to nontarget plants. Buffers are typically derived by determining the distance at which predicted exposure from spray drift equals the ecotoxicology threshold for sensitive plant species determined in greenhouse tests. Field studies performed under realistic conditions have demonstrated, however, that this approach is far more conservative than necessary. In 2016, the US Environmental Protection Agency estimated that isoxaflutole (IFT), a herbicide used to control grass and broadleaf weeds, could adversely affect downwind nontarget dicot plants at distances of ≥304 m from the edge of the treated field due to spray drift. This prediction implies that a buffer of at least 304 m is required to protect nontarget plants. To refine the predicted buffer distance for IFT, we conducted a field study in which sensitive nontarget plants (lettuce and navy bean, two to four leaf stage) were placed at various distances downwind from previously harvested soybean fields sprayed with Balance® Flexx Herbicide. The test plants were then transported to a greenhouse for grow out following the standard vegetative vigor test protocol. There were three trials. One had vegetation in the downwind deposition area (i.e., test plants placed in mowed grass; typical exposure scenario) and two had bare ground deposition areas (worst-case exposure scenario). For both plant species in bare ground deposition areas, effects on shoot height and weight were observed at 1.52 m but not at downwind distances of ≥9.14 m from the edge of the treated area. No effects were observed at any distance for plants placed in the vegetated deposition area. The field study demonstrated that a buffer of 9.14 m protects nontarget terrestrial plants exposed to IFT via spray drift even under worst-case conditions. *Integr Environ Assess Manag* 2022;18:757–769. © 2021 Bayer. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Buffers, Field study, Herbicide, Nontarget terrestrial plants, Spray drift

INTRODUCTION

In the US Environmental Protection Agency's (USEPA) standard risk assessment framework for nontarget plants, seedling emergence (OCSPP 850.4100) and vegetative vigor (OCSPP 850.4150) studies are required for the registration of pesticide active ingredients (USEPA, 2004). Typically, the most sensitive endpoints from the plant studies are combined with predictions from a conservative spray drift model (i.e., AgDRIFT or AgDISP) to estimate a no-spray buffer distance protective of downwind off-field nontarget plants. As discussed by Brain et al. (2017) and Brain et al. (2019), this approach assumes that nontarget off-field plants experience exposure analogous to in-field target weeds, that is, an overhead spray application that provides even saturation coverage on the foliage. However, nontarget plants do not receive even herbicide coverage on the foliage because spray drift is more likely to contact the upwind (via lateral interception) and top (via deposition) portions of

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the foliage. In addition, numerous properties of the downwind plant community (e.g., plant density and height, drag characteristics of the foliage, plant architecture, collection efficiency, etc.) influence exposure of nontarget plants to herbicide drift (Marrs & Frost, 1997).

The models typically used by the USEPA to estimate exposure of nontarget plants to spray drift with distance from treated areas are highly conservative. For ground spray applications, the Tier 1 AgDRIFT model is generally used (USEPA, 2021). This model is based on empirical data collected in the early 1990s using application nozzles and equipment now considered outdated. In addition, the model combined "fine to medium/coarse" droplets into one category. These factors lead to over predictions of spray drift, particularly at large distances from the application area.

Isoxaflutole (IFT) is a herbicide used to control a wide range of grass and broadleaf weeds. It may be applied via ground equipment at pre plant, pre emergence, and post emergence of the crop. In the United States, IFT is currently registered for use on corn at a maximum application rate of 0.11 kg a.i./ha. This registration is currently limited to 26 states (USEPA, 2016a). IFT is also registered for use in the European Union and elsewhere.

IFT is rapidly transformed to the diketonitrile degradate, IFT-DKN, or RPA 202248. IFT-DKN is also herbicidally active. The molecular target is the enzyme 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD). This mode of action prevents the biosynthesis of carotenoid pigments that protect chlorophyll from decomposition by sunlight. Without carotenoid pigments, chlorophyll pigments are photo-oxidized and chloroplasts break down. The typical symptom of IFT activity is bleaching in newly developed leaves during the vegetative growth of susceptible species. Available effects data indicate that dicot species are more sensitive than grasses (USEPA, 2016a).

The USEPA (2016a) estimated that spray drift from an application of the Balance® formulation of IFT could adversely affect sensitive, downwind nontarget dicot plants at distances of 304 m, which is the prediction limit for the AgDRIFT ground model. This prediction implies that a buffer of approximately 304 m, perhaps more, is required for this product to protect nontarget plants. The USEPA used their standard approach to determine the downwind distance at which nontarget plants may be affected by IFT drift, that is, comparison of Tier 1 AgDRIFT exposure predictions at varying distances to sensitive effects metrics from sensitive plant species in greenhouse studies (including lettuce and navy bean, the species used in this study). The USEPA also used the results of a field spray drift study (Hanzas et al., 2014) and concluded predicted effects to sensitive nontarget dicot plants at distances of ≥304 m (see fig. 10 in USEPA, 2016a).

The objectives of the present study were to: (1) measure the spray drift deposition and flux at varying distances downwind of bare fields treated with Balance® Flexx, and (2) determine the downwind distance at which sensitive nontarget plants are no longer affected by the IFT formulation under typical (plants placed in vegetation that may intercept drift) and worst-case (plants placed on bare soil with no vegetation to intercept drift) exposure conditions. The results of this study may be used to develop a safe and realistic buffer for IFT to ensure the protection of downwind nontarget plants. More broadly speaking, this study will be useful in further developing the methodology for spray drift bioassays conducted in the field and demonstrating their utility for deriving realistic buffer distances that are protective of nontarget plants.

The spray drift and plant toxicity data sets and supporting information (e.g., methodological details, environmental conditions during spray drift and greenhouse portions of the study, etc.) are included as Supporting Information.

METHODS

Experimental design

A field study following Good Laboratory Practices (GLP) was conducted to determine the quantity of downwind drift and the toxicity of Balance® Flexx Herbicide to sensitive, nontarget terrestrial plants located 1.52, 9.14, 15.2, and 30.5 m downwind of the application area (Figure 1). Control plants were placed 4.57 m upwind of the application area. The experimental design of this study was conceptually similar to that employed by Brain et al. (2019). Lettuce and navy bean were chosen for this study because they are among the most sensitive species in vegetative vigor testing conducted to date with IFT (see tab. 3 in USEPA, 2016a). Three independent spray trials were conducted. One trial had typical near field vegetation (30.5 cm mowed grass) in the downwind deposition area (typical exposure scenario) and two trials had bare ground deposition areas (worst-case exposure scenario). Three sets of two types of field collectors (horizontal solvent pads and rod towers) were placed in the downwind deposition areas at 1.52, 9.14, 15.2, 22.9, and 30.5 m downwind of the application area and analyzed for IFT concentration to quantify off-target area drift. The collectors were placed at the ends and center of each row of test plants (Figure 1) and were 5.18 m apart. Collectors were also placed 4.57 m upwind of the application area. Upwind collectors were 10.4 m apart because of the increased number of upwind control plants. Verification samples were located beneath each side of the spray boom in each swath (Figure 1).

Shortly before each application, potted navy bean and lettuce plants were transported from the pre-exposure cultivation site to the field. The placements of pots and collectors at each distance were staggered to ensure that they did not interfere with spray drift movement to the more distant downwind collectors and test plants (Figure 1).

Twenty-five pots (replicates) per species with two plants each were placed at each specified downwind distance and plant species alternated (i.e., navy bean, lettuce, navy bean, lettuce, etc.). The same setup was used for upwind controls except that there were 50 pots of each species. For the

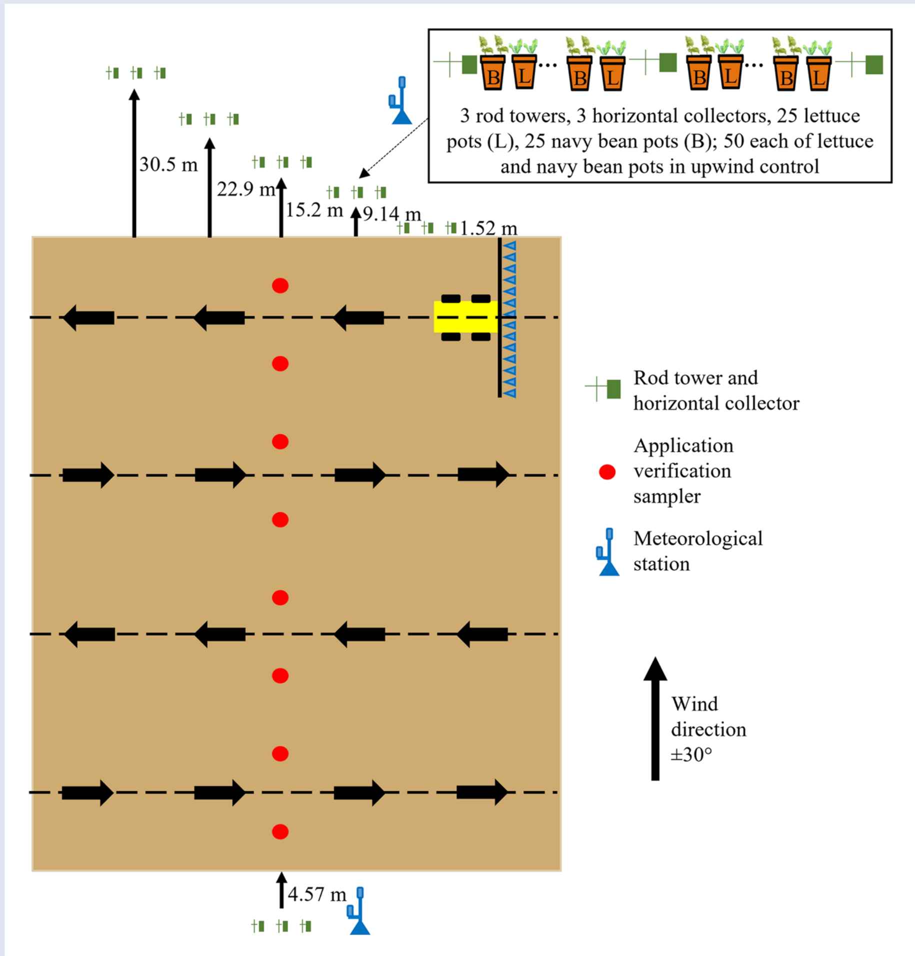

FIGURE 1 Experimental design for a field study to determine effects of spray drift of IFT on downwind nontarget plants. No test plants were located at the off-field downwind distance of 22.9 m. The horizontal arrows indicate the directions traveled by the tractor during application and the vertical arrows indicate target wind direction. Drawing not to scale

"in-vegetation" plot, vegetation surrounding the potted plants was mowed preapplication to match the height of the pots + test plants (i.e., 30.5 cm). Control plants were handled in the same manner as treated plants.

Two trials were conducted on 21 October 2018 (one bare ground and one in-vegetation) and the third trial was conducted on 22 October 2018 (bare ground). Following exposure in the field, plants remained in place for at least 5 min to allow for drying of the spray drift deposit on the leaves before being transferred to a greenhouse for the vegetative vigor phase of the study. The postapplication greenhouse phase of the study lasted 21 days for navy bean and 28 days for lettuce. Plant survival, growth stage, phytotoxicity, and height were assessed at 7, 14, and 21 days after treatment (DAT) and additionally at 28 DAT for lettuce. On completion of the final height and phytotoxicity assessments, all plants were cut at soil level and dried in an oven to determine shoot dry weight.

The spray drift portion of the study was conducted on three plots at two agricultural fields near Gardner in Johnson County, Kansas, USA. At the time of applications, there was no crop on the fields (i.e., the soybean crop had been harvested 24 h earlier). The plots had less than a 2% slope and measured at least 157 by 102 m each, including both the application and deposition areas (Figure 1). The plant exposure areas were adjacent to the application areas on the downwind side. Two weather stations located upwind and downwind of the application area were used during the study. The weather stations recorded wind speed, wind direction, temperature, and relative humidity at two heights (1.83 and 4.57 m). Wind speed and direction were monitored in real time at 1-s intervals before, during, and after each application using an HP laptop computer connected to a Gill WindMaster (Part 1590-PK-020) 3D ultrasonic anemometer located at the 1.83 m height on the upwind weather station. Data from the 3D ultrasonic anemometer were used to determine when applications commenced, that is, once the instantaneous and 2-min running averages for wind direction and wind speed were within 30° of perpendicular to the spray swaths and within approximately 11.3–16.1 kph, respectively. Gill Windsonic 2D anemometers (Part 1405-PK-021) were located at the 4.57 m height on the upwind weather station and both heights on the downwind weather station. Wind, temperature, and relative humidity

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were recorded at the same heights as the anemometers on both weather stations.

Test substance, tank mix, and sprayer calibration

Applications were performed following typical procedures using the maximum rate on the herbicide label. The test substance was Balance® Flexx Herbicide, which consists of the active ingredient IFT (20.2%), the safener cyprosulfamide (20.1%) to increase the tolerance of corn plants to IFT, and other inert ingredients. To prepare the tank mixes, well water was added to a clean, empty tank followed by the test substance. Tank mixes were agitated by the sprayer prior to and during applications. No additives intended to reduce drift were used in the tank mix.

AIXR11002 coarse spray nozzles were installed on the tractor sprayer and calibrated shortly before applications. These nozzles were selected because they had been used in a previous IFT spray drift study, thus enabling comparability of results with the present study (Hanzas et al., 2014). The AIXR11002 nozzles are also designed to reduce spray drift and are being increasingly adopted by the agricultural sector in the United States and elsewhere. Nozzles were verified at a pressure of 275 kPa, the pressure required to achieve the desired coarse spray quality. Verification samples taken immediately after application in the application areas demonstrated that the target application rate was achieved (i.e., 99%–101% of the target application rate of 0.11 kg a.i./ha across the three trials).

Test substance applications

All applications were made with a RoGator 854 equipped with 60 AIXR11002 nozzles at 50.8 cm spacing and at the maximum permitted application rate of 0.11 kg a.i./ha, a spray rate of 93.6 L/ha, and a boom height of 61 cm above the ground. Applications consisted of four spray swaths and each swath was 102 m long and 30.5 m wide. All applications began with the swath closest to the downwind deposition area (Figure 1).

Applications were not begun if the 2-min running average for wind direction deviated by more than ±30° from the perpendicular to the spray swaths or was outside of the target wind speed range (11.3–16.1 kph). Under ideal conditions, the wind direction would be perpendicular to the spray swaths, but some variation occurred as expected under natural conditions.

Drift deposition and interception sampling

For each of the three trials, spray drift deposition and interception stations were located 1.52, 9.14, 15.2, 22.9, and 30.5 m downwind of the downwind edge of the application area (Figures 1 and S5). Each of the five downwind sampling distances consisted of three sets of evenly spaced, paired deposition collectors. One horizontal collector (13.7 by 22.0 cm Pall Life Sciences solvent pad collectors) and one vertical interception rod tower collector (30.5 cm tall stainless-steel rod containing eight vertically spaced 3 mm diameter by 100 mm long horizontal rods, half of the total surface area of eight rod cylinders was used as collecting area) were placed at each sampling station. Solvent pad collectors were selected because they have a long history of use including in previous IFT field studies (Hanzas et al., 2014; Toth et al., 2016). Rod towers were used to enable a direct comparison between past studies conducted under different conditions. The simplified vertical structure and known capture efficiency enable modeling of the deposition and capture data to be linked to application parameters. The horizontal deposition samplers were placed on a platform slightly above the height of field roughness—the height of the surrounding vegetation for the in-vegetation trial and 15.2 cm above ground for the bare ground trials.

After collection, samples were immediately placed in coolers containing dry ice before being taken to SynTech Research Laboratory Services, LLC., where they were stored in walk-in freezers prior to being shipped to the analytical laboratory via an A.C.D.S. Research, Inc. freezer truck. At the time of the study, the SynTech facility was 17.7 km from the field sites and temperatures in the walk-in freezer were ≤−18 °C.

Verification sampling

Spray applications were verified by eight horizontal solvent pad collectors located randomly at ground level throughout the application area to capture various portions of the spray boom. Two application verification samples were collected from each swath pass (Figure S13). To measure upwind spray, a control sample station was placed 4.57 m upwind of the windward edge of the application area.

Tank mix samples were collected prior to and following the addition of the test substance.

Vegetative vigor test system

Potting soil. The soil used for this study was a mixture of a natural pulverized topsoil and sand. The soil was collected and mixed by Johnson County Topsoil to produce a sandy loam type soil. The GLP characterization indicated an organic matter content of 0.79% (0.46% organic carbon), which is within the required specifications of the OCSPP 850.4150 Vegetative Vigor test study guidance (USEPA, 2012).

Test species. The two plant species in this study (i.e., lettuce and navy bean) have differing structures during the early growth stages (rosette and erect, respectively), which was expected to lead to different spray drift interception rates. Each species is readily cultivated and widely used in research. Seeds were not treated with fungicides, insecticides, or repellents prior to test initiation.

Planting and grow out. Pots were 15.2 cm in diameter and 13 cm tall. For both species, pots were filled to 12 cm deep with natural sandy loam soil. Lettuce seeds were planted into 288-cell plug trays, then later transplanted into the prepared pots. Navy bean seeds were planted directly into the soil-filled pots to a depth of approximately 1.27 cm. For both species, extra seeds were planted in each pot, and extra pots were prepared and planted to allow for the selection of pots with uniform plant sizes for the study. Lettuce was sown approximately 4 weeks prior to experimental initiation and navy bean approximately 3 weeks prior to ensure that the plants were at the two to four leaf stage during the spray applications. Prior to pot selection for the study, seedlings were thinned down to two plants per pot.

On the day prior to each spray application, pots with plants that were the most uniform in size and developmental stage were selected for inclusion in the study. The selected pots were randomly assigned to a treatment distance and replicate number. The shoot height and developmental stage (number of true leaves) of selected test plants were recorded after selection and prior to spray application. In total, 450 pots each of both lettuce and navy bean (900 pots total) were selected for use in the study.

Analysis of residues

The analytical method for the determination of IFT and its metabolites, IFT-DKN and IFT-benzoic acid, on solvent pads was developed at Bayer Crop Science, Research Triangle Park, North Carolina, USA (now located in Chesterfield, Missouri, USA; Netzband, 2014).

Field fortifications were not conducted in this study, but the results of a previous IFT spray drift study (Hanzas et al., 2014) proved that sample concentrations are stable over time while stored frozen.

Inputs to the regression analyses conducted to derive the spray drift curves were the analytically determined residues collected from downwind samples and the distances from the edge of the field at which the samples were obtained. The proportion of applied IFT was calculated from total residues, including IFT and IFT-DKN per sample, the sampling device surface area, and the target application rate of 0.11 kg a.i./ha.

Biological assessment

The duration of the in-life phase following the spray application was 21 days for navy bean plants and 28 days for lettuce plants. The postexposure growth duration for lettuce was extended 7 days due to phytotoxic symptoms first occurring in lettuce between Days 14 and 21 postapplication. The plants were evaluated in accordance with a typical vegetative vigor trial (USEPA, 2012) during which growth parameters, including weekly survival, shoot height, and termination dry weights were measured. Additionally, at each of the weekly evaluations, developmental stage and phytotoxicity symptoms and ratings were recorded for each plant.

Following shoot height measurements at termination of the in-life portion of the test, surviving plants were clipped at soil level, dried, and weighed.

Statistical analyses for biological effects

A two-factor analysis of covariance (ANCOVA) was conducted for each of the following parameters: 21- and 28-day shoot dry weight for navy bean and lettuce, respectively; and 7-, 14-, 21-, and 28-day (lettuce only) shoot height for each test species. Each replicate consisted of the average value of the two plants in each pot. The two factors were trial number and downwind distance from the edge of the application area. The covariate was pretreatment (Day 1) shoot height.

If the two-factor ANCOVA indicated a significant trial × distance interaction (i.e., the slopes for the relationship between the dependent variable and downwind distance differed between trials), a Type III Sum of Squares (SS) ANCOVA was used for the analysis. Otherwise, a Type I SS ANCOVA was used because it assumes that the slopes for the relationship between the dependent variable and downwind distance do not differ between trials. When the trial factor was significant, the analysis proceeded to a one-factor ANCOVA for each trial.

To determine the choice of multiple comparison test, model assumptions (i.e., homogeneity of variance, errors are normally distributed) were checked with a variety of graphical plots (e.g., residuals histograms for different model terms, probability density functions for residuals compared to a normal distribution) and statistical tests (for normality, Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises, and Anderson–Darling tests). When the normality assumption was met or the deviations from normality were minor, the lowest observed effect distance (LOED) and no observed effect distance (NOED) were determined using the parametric Dunnett–Hsu multiple comparison test. Otherwise, the nonparametric exact Wilcoxon two-sample test was used with a Holm–Bonferroni correction for the number of comparisons.

The statistical power of each test was determined a posteriori by calculating the minimum detectable difference (MDD). The MDD defines the difference between the mean of treatment and the control that must exist to detect a statistically significant effect. MDDs were determined for each combination of species, trial, and biological endpoint, except when the ANCOVA determined that trial was not a significant factor (this occurred for the 7-, 14-, and 21-day height endpoints for navy bean). In the latter case, the data were combined across trials for the biological endpoint of interest.

RESULTS

Spray drift modeling

Wind direction for all trials was within 30° of the target range (Table S2), with average speeds during applications ranging from 15.6 to 21.9 kph. Mean temperature and relative humidity for the three trials ranged from 14.2 to 18.2 °C and 39.2%–56.6%, respectively.

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Residue data expressed in units of proportion of applied target application rate were log transformed and a Morgan-Mercer-Flodin model fit to each of six data sets (3 trials × 2 drift collector types). The model accounts for the number and distance of the multiple swaths used for application at each site:

$$y = \sum_{i=0}^{3} \frac{B}{(1 + C(d + 100 \times i))^D}$$

where y is the proportion of applied IFT on the treated field; d is the distance from the edge of the field (feet); B is the value of y at $d = 0$ (i.e., 1); C is the slope; D is the curvature of the function; and i is the swath number.

Model fitting and statistical analyses were conducted using R (R Core Team, 2017). For each trial and sample type, nonlinear ordinary least squares analysis was used to determine model parameters (Table S6). R^2 values (range = 0.916–0.980) and visual inspection (Figures S14–S19) indicated excellent model fit for all data sets. For all fitted models, the D coefficient (curvature) was statistically significant ($p < 0.05$).

The fitted spray drift models showed much lower off-field spray drift in the in-vegetation exposure trial than in the bare ground trials (Figures 2 and 3). Even in the worst-case scenario (bare ground exposure, Trial 1) airborne residue intercepted by the rod tower collectors was 10.5% of the application rate at 1.52 m downwind and less than 1% at 10.7 m and beyond. By comparison, airborne residue intercepted by the rod tower collectors in the in-vegetation trial was 0.6% of the field application rate at 1.52 m downwind and less than 0.072% at 10.7 m and beyond.

There was greater spray drift in the first bare ground trial than in the second trial, particularly closer to the treated area (Figures 2 and 3). This result was likely due to higher wind speeds in the first trial (average wind speed = 21.9 kph) compared to the second trial (average wind speed = 15.6 kph) as the topography of the fields and surrounding areas was similar.

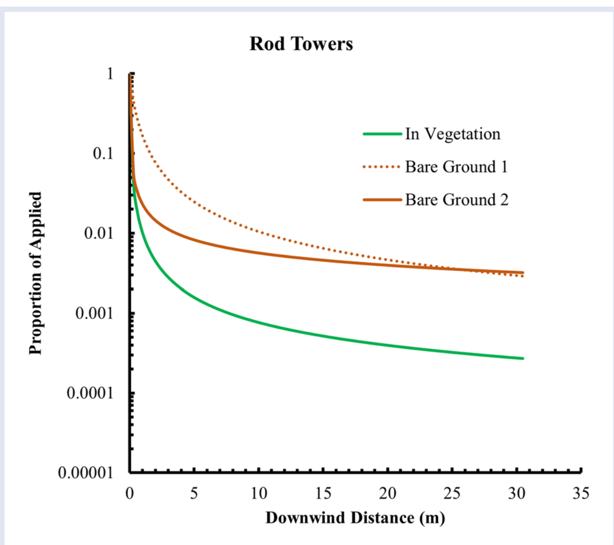

FIGURE 2 Fitted spray drift models for rod tower collectors

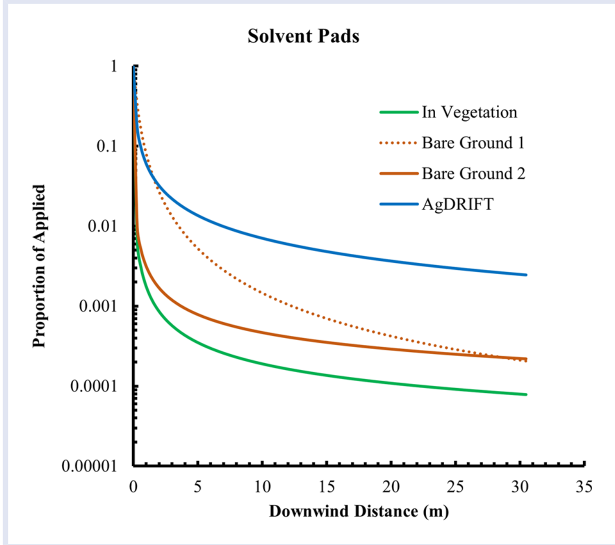

FIGURE 3 Fitted spray drift models for solvent pad collectors. Corresponding Tier 1 AgDRIFT Model is also shown

Biological results

Greenhouse environmental conditions during the grow out portion of the study met or came close to the targets specified in the USEPA (2012) protocol for vegetative vigor tests. The average day length was 16.6 h with light intensity ranging from 3312 and 51 200 lux. Average temperature was 18.4 °C (target = 23.3 °C), and the overall mean relative humidity was 63.5% (target = 70%).

Plant survival, shoot height, and dry weight. The biological results for the final measurements are summarized in Table 1. For lettuce and navy bean, no significant adverse effects were observed for shoot height or weight at any distance for plants placed in vegetation during spraying (Table 2). For lettuce plants placed on the bare ground during the first trial (worst-case scenario), significant adverse effects were observed downwind at 1.52 m for 14-, 21-, and 28-day height, and 28-day weight. At further downwind distances (≥9.14 m), no adverse effects were observed for lettuce plants in the first bare ground exposure trial. In the second bare ground exposure trial for lettuce, no adverse effects were observed at any distance. For navy bean, the only adverse effects observed were in the first and second bare ground exposure trials at 1.52 m downwind for 21-day height and weight. Thus, the overall NOED for this study is 9.14 m and the corresponding LOED is 1.52 m (Table 2).

The covariate (Day-1 shoot height) was a significant factor ($p < 0.05$) in every analysis except for the 28-day height of lettuce in the first bare ground exposure trial. Thus, this

TABLE 1 Plant dry weight, shoot height, and survival after exposure of lettuce and navy bean to Balance® Flexx Herbicide formulation

Species	Trial	Treatment distance (feet)	Day 28 (lettuce) or 21 (navy bean) endpoint				Survival (%)
			Weight (g)	Inhibition (%)	Height (cm)	Inhibition (%)	
Lettuce	In-vegetation exposure	Control	0.344	NA	5.8	NA	100
		100	0.355	−3.2	6.1	−4.5	100
		50	0.395	−14.7	6.4	−8.9	100
		30	0.321	6.7	5.9	−1.7	100
		5	0.387	−12.6	6.2	−6.8	100
	Bare ground exposure 1	Control	0.368	NA	6.6	NA	100
		100	0.362	1.8	6.5	1.5	100
		50	0.323	12.3	6.3	4.0	100
		30	0.366	0.59	6.5	1.5	100
		5	0.127	65.5	4.6	30.5	98
	Bare ground exposure 2	Control	0.342	NA	6.0	NA	100
		100	0.521	−52.2	7.4	−23.5	100
		50	0.405	−18.5	6.6	−10.1	100
		30	0.418	−22.1	6.7	−12.1	100
		5	0.333	2.8	6.2	−3.8	98
Navy bean	In-vegetation exposure	Control	1.59	NA	18.6	NA	100
		100	1.62	−2.0	18.5	0.6	100
		50	1.60	−1.0	18.8	−0.6	100
		30	1.64	−3.3	18.4	1.3	100
		5	1.49	6.1	18.6	0.4	100
	Bare ground exposure 1	Control	1.42	NA	17.8	NA	100
		100	1.47	−3.4	18.0	−0.8	100
		50	1.48	−4.4	18.1	−1.4	100
		30	1.30	8.4	18.4	−3.4	100
		5	1.02	27.9	18.2	−2.1	100
	Bare ground exposure 2	Control	1.59	NA	18.4	NA	100
		100	1.46	8.5	17.6	4.7	100
		50	1.43	10.0	18.2	1.2	100
		30	1.42	11.0	17.9	2.9	100
		5	1.49	6.3	19.2	−3.9	100

Abbreviation: NA, not applicable.

aspect of the study design was successful in removing a significant portion of the non-treatment-related variability in plant endpoints.

The results of the MDD analyses are shown in Figures 4 and 5 and Table 3. The MDD was <15% for 18/21 data sets. Generally, MDDs were lowest for earlier measurements (e.g., 7-day height) and increased with later measurements. Navy bean had lower MDDs than did lettuce for comparable endpoints (Figures 4 and 5).

Developmental stage and phytotoxicity. No relationship was observed between developmental stage and downwind distance in the three trials. Symptoms of phytotoxicity (e.g., bleaching, necrosis) were observed in some lettuce and

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TABLE 2 Results of statistical analyses for lettuce and navy bean exposed to Balance® Flexx Herbicide

Species	Endpoint	Trials significantly different?	Trial	Covariate significant?	LOED (m)	NOED (m)	Notes
Lettuce	7-day height	Yes	Bare ground 1	Yes	<1.52	≤1.52	
			Bare ground 2	Yes	<1.52	≤1.52	1.52 m distance was close to being significantly better than control
			In vegetation	Yes	<1.52	≤1.52	1.52 m significantly better than control
	14-day height	Yes	Bare ground 1	Yes	1.52	9.14	
			Bare ground 2	Yes	<1.52	≤1.52	30.5 m significantly better than control
			In vegetation	Yes	<1.52	≤1.52	
	21-day height	Yes	Bare ground 1	Yes	1.52	9.14	
			Bare ground 2	Yes	<1.52	≤1.52	9.14 and 30.5 m significantly better than control
			In vegetation	Yes	<1.52	≤1.52	
	28-day height	Yes	Bare ground 1	No	1.52	30	
			Bare ground 2	Yes	<1.52	≤1.52	9.14 and 30.5 m significantly better than control
			In vegetation	Yes	<1.52	≤1.52	
	28-day weight	Yes	Bare ground 1	Yes	1.52	9.14	
			Bare ground 2	Yes	<1.52	≤1.52	15.2 and 30.5 m significantly better than control
			In vegetation	Yes	<1.52	≤1.52	
Navy bean	7-day height	No	Bare ground 1	Yes	<1.52	≤1.52	
			Bare ground 2				
			In vegetation				
	14-day height	No	Bare ground 1	Yes	<1.52	≤1.52	
			Bare ground 2				
			In vegetation				
	21-day height	No	Bare ground 1	Yes	<1.52	≤1.52	30.5 m significantly reduced but effect was slight and not treatment-related
			Bare ground 2				
			In vegetation				

(Continued)

TABLE 2 *(Continued)*

Species	Endpoint	Trials significantly different?	Trial	Covariate significant?	LOED (m)	NOED (m)	Notes
	21-day weight	Yes	Bare ground 1	Yes	1.52	9.14	
			Bare ground 2	Yes	1.52	9.14	
			In vegetation	Yes	<1.52	≤1.52	

Abbreviations: LOED, lowest observed effect distance; NOED, no observed effect distance.

navy bean plants in the bare ground exposure trials, particularly for navy bean at the closest distance of 1.52 m. At greater downwind distances, symptoms of phytotoxicity were not observed in lettuce plants, and only infrequently observed in navy bean plants in bare ground exposures.

DISCUSSION

For lettuce and navy bean in bare ground deposition areas, effects on shoot height and weight were observed at 1.52 m but not at downwind distances of 9.14 m and greater from the edge of the treated area. No effects were observed at any distance for plants placed in the vegetated deposition area, which is a much more realistic exposure scenario as IFT is typically applied to fields that are surrounded by vegetation. Thus, our field study demonstrated that a buffer of 9.1 m, at least 33-fold less than the ≥304 m distance originally predicted by the USEPA using a screening-level assessment, would be protective of crops and other nontarget terrestrial plants exposed to IFT via spray drift even under worst-case conditions.

The approach used by the USEPA (2016a) to derive a spray deposition distance for IFT was conservative for the following reasons:

- Tier 1 Ground AgDRIFT is an empirical model based on outdated data sets that relied on old nozzle technology and were from studies conducted at wind speeds above current label guidelines (Brain et al., 2019). Many current nozzles (e.g., air induction nozzles for ground application) and other application technologies (e.g., spray shields, drift-reducing adjuvant chemicals added to formulations or tank mixes) are designed to reduce spray drift and have been increasingly adopted in recent years (Reimer & Prokopy, 2012).
- The Tier 1 Ground AgDRIFT modeling for IFT assumed fine to medium/coarse droplets. The Balance labels for IFT, however, require nozzles that deliver a coarse or larger spray droplet size. Spray drift increases with wind speed and when small droplets comprise a larger proportion of the spray (Ferguson et al., 2015). Thus, Tier 1 Ground AgDRIFT overestimates spray drift for IFT, as was shown in our study (Figure 3).
- The predictions from Tier 1 Ground AgDRIFT are upper-bound estimates for spray drift because the model relies on generic default assumptions, such as the use of 90th centile wind speed from the calibrating Spray Drift Task Force data sets (Brain et al., 2019).
- The data sets used to generate the Tier 1 Ground AgDRIFT spray drift predictions were from studies conducted on bare ground with minimal surface roughness, as was done in our bare ground treatments. In natural plant communities adjacent to treated areas, spray drift would be considerably reduced with increasing distance by the presence of wind breaks and intercepting vegetation (Brown et al., 2004; Felsot et al., 2011; Marrs et al., 1991a). This point was confirmed in our study as spray drift declined much more rapidly with distance in the

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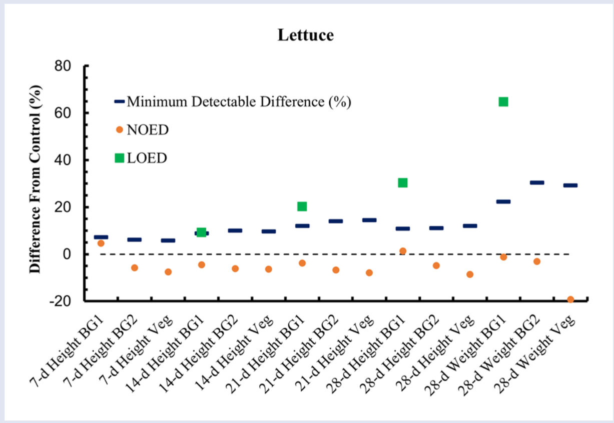

FIGURE 4 Minimum detectable differences, NOEDs and LOEDs versus percent difference from control for lettuce. NOEDs without corresponding LOEDs are unbounded. BG1, bare ground exposure, Trial 1; BG2, bare ground exposure, Trial 2; LOED, lowest observed effect distances; NOED, no observed effect distances; Veg, in-vegetation exposure

vegetated treatment than in the bare ground treatments (Figures 2 and 3).

- Although the use of Tier 1 Ground AgDRIFT is inherently conservative, it is likely the effects metrics used by the USEPA (i.e., most sensitive endpoints from vegetative vigor studies conducted in the greenhouse) that have the most influence on buffer size derivation (Brain et al., 2017; Brain et al., 2019). Guideline vegetative vigor studies simulate worst-case exposure by saturating the foliage of test plants from an overhead sprayer, analogous to how weeds are exposed under a ground boom in treated fields. Downwind nontarget plants, however, are exposed via sedimenting deposition from above and lateral airborne interception. Thus, the upper and leading edges of plants are likely to be more exposed than are other plant parts. Our spray drift results for the horizontal deposition samplers (Figure 3) indicate that effects to sensitive dicot species from greenhouse studies (e.g., ER25 of 0.0000115 lb a.i./A for shoot length of navy bean; USEPA, 2016a) would be expected at distances ≥30.5 m in all treatments, including the vegetated treatment. However, no effects were observed in the vegetated treatment at any distance at or beyond 1.52 m in the bare ground treatments for navy bean and lettuce (Table 2). This result suggests that the greenhouse effects metrics are the more important driver for buffer derivation than is the AgDrift model.

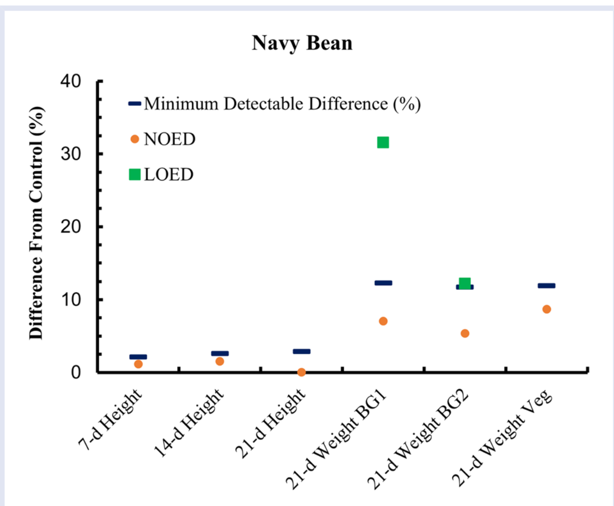

FIGURE 5 Minimum detectable differences, NOEDs and LOEDs versus percent difference from control for the navy bean. NOEDs without corresponding LOEDs are unbounded. BG1, bare ground exposure, Trial 1; BG2, bare ground exposure, Trial 2; LOED, lowest observed effect distances; NOED, no observed effect distances; Veg, in-vegetation exposure

The design of our study was based on an earlier study conducted by Brain et al. (2017) for the herbicide, mesotrione, which has the same mode of action as IFT. Using a worst-case exposure methodology (i.e., sensitive lettuce and tomato plants placed in a bare ground deposition area), they found a NOED of 9.1 m. The field-based buffer for mesotrione was well below the theoretical buffer of ≥305 m predicted using USEPA's standard methodology (Brain et al., 2017). In a subsequent study with atrazine, Brain et al. (2019) found a NOED of 4.6 m, again well below the buffer of 91.4 to 183 m predicted by the USEPA (2016b) using their standard methodology. Field studies of the design employed in the current study, and by Brain et al. (2017, 2019), and others (e.g., De Jong & de Haes, 2001; Marrs & Frost, 1997; Marrs et al., 1989, 1991a, 1991b) are influenced by natural variation in wind speed and direction, humidity and other factors that affect the movement of spray drift. Nevertheless, there is an emerging consensus across multiple studies showing that herbicide drift is unlikely to affect nontarget plants beyond distances of roughly 10 m downwind of the application area. The realism arising from conducting field studies under natural conditions is what makes them higher-tier studies. Such studies should supersede the screening-level approach currently employed by the USEPA when such studies are available to derive buffers protective of downwind nontarget plants.

To ensure that the IFT spray drift study produced results that were statistically robust, we included several modifications of previous study designs that determined the effects of spray drift on downwind nontarget plants. The first was to increase the sample size for each treatment distance to 25 pots per species, up from the 10 pots per species at each treatment distance in the Brain et al. (2017, 2019) studies. In addition, we doubled the number of upwind control replicates to 50 pots per species.

The second major modification to the design of the IFT drift study was to include a preapplication covariate in the statistical analyses, that is, pretreatment height. The covariate was a significant factor ($p < 0.05$) in nearly every analysis (Table 2). Thus, inclusion of pretreatment height was successful in removing a significant portion of the non-treatment-related variability in plant endpoints. As a result

TABLE 3 Results of MDD analyses for lettuce and navy bean exposed to Balance® Flexx Herbicide

Species	Endpoint	Trial	Control least squares mean	MDD	MDD (%)	LOED (m)	LOED least squares mean	LOED % difference from control	NOED (m)	NOED least squares mean	NOED % difference from control
Lettuce	7-day height	Bare ground 1	4.11	0.295	7.18	NA	NA	NA	≤1.52	3.92	4.63
		Bare ground 2	4.23	0.260	6.14	NA	NA	NA	≤1.52	4.47	−5.77
		In vegetation	4.04	0.230	5.70	NA	NA	NA	≤1.52	4.35	−7.50
	14-day height	Bare ground 1	4.03	0.353	8.76	1.52	3.66	9.16	9.14	4.22	−4.55
		Bare ground 2	4.12	0.410	9.97	NA	NA	NA	≤1.52	4.37	−6.18
		In vegetation	4.06	0.390	9.61	NA	NA	NA	≤1.52	4.31	−6.32
	21-day height	Bare ground 1	5.12	0.610	11.9	1.52	4.08	20.2	9.14	5.31	−3.85
		Bare ground 2	4.93	0.687	13.9	NA	NA	NA	≤1.52	5.26	−6.77
		In vegetation	4.98	0.716	14.4	NA	NA	NA	≤1.52	5.38	−7.94
	28-day height	Bare ground 1	6.54	0.705	10.8	1.52	4.56	30.3	9.14	6.46	1.30
		Bare ground 2	6.00	0.663	11.0	NA	NA	NA	≤1.52	6.29	−4.82
		In vegetation	5.83	0.694	11.9	NA	NA	NA	≤1.52	6.33	−8.58
	28-day weight	Bare ground 1	0.360	0.0803	22.3	1.52	0.127	64.7	9.14	0.365	−1.24
		Bare ground 2	0.340	0.103	30.4	NA	NA	NA	≤1.52	0.350	−3.07
		In vegetation	0.341	0.0994	29.1	NA	NA	NA	≤1.52	0.407	−19.3
Navy bean	7-day height	All	16.0	0.345	2.15	NA	NA	NA	≤1.52	15.9	1.14
	14-day height	All	17.8	0.470	2.64	NA	NA	NA	≤1.52	17.6	1.56
	21-day height	All	18.4	0.523	2.84	NA	NA	NA	≤1.52	18.4	0.0689
	21-day weight	Bare ground 1	1.44	0.177	12.3	1.52	0.987	31.6	9.14	1.34	7.05
		Bare ground 2	1.59	0.186	11.7	1.52	1.39	12.2	9.14	1.50	5.36
		In vegetation	1.63	0.195	12.0	NA	NA	NA	≤1.52	1.49	8.67

Abbreviations: LOED, lowest observed effect distance; MDD, minimum detectable difference; NA, not applicable; NOED, no observed effect distance.

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of the modifications to the study design, the study MDDs were at the very low end of the range observed in standard vegetative vigor studies conducted entirely in the greenhouse (Staveley et al., 2018). Thus, this IFT spray drift study had excellent statistical power.

The final major modification to the design of the IFT spray drift study was to include a trial wherein potted test plants were placed in existing vegetation to replicate how nontarget plants are exposed in the real world. In this trial, spray drift decreased more rapidly with distance than in the bare ground trials. Effects to test plants were also reduced in the vegetation trial compared to the bare ground trials. We recommend the inclusion of vegetation trials in future spray drift studies to provide a more typical exposure scenario rather than just the worst-case bare ground exposure scenario.

CONCLUSIONS

As has been observed in other recent herbicide drift studies, significant effects of IFT spray drift to two sensitive dicot species, two to four leaf stage lettuce and navy bean, were observed for only a short distance downwind of the treated area (i.e., 1.52 m in the worst-case bare ground treatment). No effects were observed downwind of the treated area in the more realistic vegetated treatment. The NOED for IFT was 9.14 m, far below the no-effect distance predicted by the USEPA using their standard approach of comparing a sensitive effect endpoint from a greenhouse vegetative vigor study to a Tier 1 AgDrift spray drift curve. Given the observed statistical power of the study, we are confident that the NOED is protective of both crop and other nontarget sensitive terrestrial plants that may inadvertently be exposed to IFT via spray drift.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

All raw data are provided in the Supporting Information. Questions regarding the raw data may be directed to author Dwayne Moore (dmoore@intrinsik.com).

SUPPORTING INFORMATION

Appendices, protocols and data files associated with the field study.

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REFERENCES

Brain, R., Goodwin, G., Abi-Akar, F., Lee, B., Rodgers, C., Flatt, B., Lynn, A., Kruger, G., & Perkins, D. (2019). Winds of change, developing a non-target plant bioassay employing field-based pesticide drift exposure: A case study with atrazine. *Science of the Total Environment*, *678*, 239–252.

Brain, R. A., Perine, J., Cooke, C., Ellis, C. B., Harrington, P., Lane, A., O'Sullivan, C., & Ledson, M. (2017). Evaluating the effects of herbicide drift on nontarget terrestrial plants: A case study with mesotrione. *Environmental Toxicology and Chemistry*, *36*, 2465–2475.

Brown, R. B., Carter, M. H., & Stephenson, G. R. (2004). Buffer zone and windbreak effects on spray drift deposition in a simulated wetland. *Pest Management Science*, *60*, 1085–1090.

De Jong, F. M. W., & de Haes, H. A. U. (2001). Development of a field bioassay for the side-effects of herbicides on vascular plants using *Brassica napus* and *Poa annua*. *Environmental Toxicology*, *16*, 397–407.

Felsot, A. S., Unsworth, J. B., Linders, J. B. H. J., Roberts, G., Rautman, D., Harris, C., & Carazo, E. (2011). Agrochemical spray drift; assessment and mitigation—A review. *Journal of Environmental Science and Health Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*, *46*, 1–23.

Ferguson, J. C., O'Donnell, C. C., Chauhan, B. S., Adkins, S. W., Kruger, G. R., Wang, R., Ferreira, P. H. U., & Hewitt, A. J. (2015). Determining the uniformity and consistency of droplet size across spray drift reducing nozzles in a wind tunnel. *Crop Protection*, *7*, 1–6.

Hanzas, J., Brayden, B., & Hofmann, C. (2014). *Spray drift field deposition testing for isoxaflutole: Final report. Study performed by Stone Environmental, Montpelier, VT* (Study No. 14-043.1). Submitted to Bayer CropScience, Research Triangle Park, NC. Study ID: MEISN015. [Study report may be requested by sending an email to cropscience-transparency@bayer.com].

Marrs, R. H., & Frost, A. J. (1997). A microcosm approach to the detection of the effects of herbicide spray drift in plant communities. *Journal of Environmental Management*, *50*, 369–388.

Marrs, R. H., Frost, A. J., & Plant, R. A. (1991a). Effects of herbicide spray drift on selected species of nature conservation interest: The effects of plant age and surrounding vegetation structure. *Environmental Pollution*, *69*, 223–235.

Marrs, R. H., Frost, A. J., & Plant, R. A. (1991b). Effect of mecoprop drift on some plant species of conservation interest when grown in standardized mixtures in mesocosms. *Environmental Pollution*, *73*, 25–42.

Marrs, R. H., Williams, C. T., Frost, A. J., & Plant, R. A. (1989). Assessment of the effects of herbicide spray drift on a range of plant species of conservation interest. *Environmental Pollution*, *59*, 71–86.

Netzband, D. J. (2014). *An analytical method for the determination of residues of isoxaflutole and its metabolites RPA 202248 and AE B197555 on dosimeter pads using LC-MS/MS* (Method Number IS-005-F14-01). Bayer CropScience, Research Triangle Park, NC. [Study report may be requested by sending an email to cropscience-transparency@bayer.com].

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Reimer, A. P., & Prokopy, L. S. (2012). Environmental attitudes and drift reduction behavior among commercial pesticide applicators in a U.S. agricultural landscape. *Journal of Environmental Management*, *113*, 361–369.

Staveley, J. P., Green, J. W., Nusz, J., Edwards, D., Henry, K., Kern, M., Deines, A. M., Brain, R., Glenn, B., Ehresman, N., Kung, T., Ralston-Hooper, K., Kee, F., & McMaster, S. (2018). Variability in nontarget terrestrial plant studies should inform endpoint selection. *Integrated Environmental Assessment and Management*, *14*, 639–648.

Toth, B. N., Brayden, B. B., Xu, T., Beguin, S. J., Arpino, M. R., & Rixon, C. A. (2016). *Ground sprayer drift deposition field study for isoxaflutole. Study performed by Stone Environmental, Montpelier, VT* (Study No. 16-029). Submitted to Bayer CropScience, Research Triangle Park, NC. Study ID: MEIS0003. [Study report may be requested by sending an email to cropscience-transparency@bayer.com].

United States Environmental Protection Agency (USEPA). (2004). *Overview of the Ecological Risk Assessment Process in the Office of Pesticide*

Programs, U.S. Environmental Protection Agency: Endangered and Threatened Species Effects Determinations. Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC. Retrieved July 13, 2020, from: http://www.epa.gov/espp/consultation/ecorisk-overview.pdf

United States Environmental Protection Agency (USEPA). (2012). *Series 850—Ecological effects test guidelines, OCSPP Number 850.4150: Vegetative Vigor*. US Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (USEPA). (2016a, April). *Addendum to ecological risk assessment for the isoxaflutole proposed section 3 registration for use on soybeans*. Office of Pesticide Products, US Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (USEPA). (2016b, July). *Refined ecological risk assessment for atrazine*. Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (USEPA). (2021). *Models for pesticide risk assessment*. US Environmental Protection Agency, Washington, DC. Retrieved May 17, 2021, from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment

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