Examining Dicamba Vapor Concentrations and Plant Response

TOM ORR / OFF-TARGET ASSESSMENT LEAD, MONSANTO

The volatility assessment conducted by the United States Environmental Protection Agency (U.S. EPA) confirmed that a downwind buffer is protective of adverse effects on plant height to soybean, the most sensitive species to dicamba exposure. In order to understand the potential effects of off-target movement of dicamba vapor, it is important to not only understand dicamba concentrations in air that could move off-target but also the corresponding air concentrations that could result in an adverse effect on plant height and possible yield reduction. To establish the relationship between dicamba air concentrations and plant effects, the potential for dicamba to cause adverse effects to soybean plants from vapor-phase exposure was investigated in a study that was conducted in a growth chamber under controlled environmental conditions.

The purpose of this study in examining the relationship between dicamba vapor concentration and plant response was to identify a no observed effect concentration (NOEC) using a modification of a previously described closed dome (humidome) based system[1]. The NOEC value builds on and further supports the risk assessment conducted by U.S. EPA for dicamba use on dicamba-tolerant crops. Specifically, this work refines previous research conducted by Walter Gavlick*, former Analytical Chemistry Lead with Monsanto, in which there was a 30x difference between the NOEC of 17.1 ng/m^3 and the next highest concentration tested (539 ng/m^3) that resulted in plant height reductions (i.e., the lowest observed effect concentration [LOEC]). This testing refined the closed laboratory system results from Gavlick (2016) by testing air concentrations over a narrower air concentration range between NOEC and LOEC previously reported by Gavlick (2016) to determine a refined NOEC over a narrower concentration range.

The ability to correlate laboratory plant effects when exposed to a known air concentration within a closed system over time could then be used to estimate maximum potential for plant exposure in "real world" field environments. The laboratory system did not allow for any dilution or dissipation of concentration of vapor from applications to exposed susceptible soybean plants. The resulting correlations were used to assess how measured field flux measurements and potential off-target dicamba air concentrations may impact exposed off-target susceptible plants.

This results from this study 1) provide increased certainty in the plant effects endpoint used in the EPA's risk assessment, 2) further define the relationship between dicamba air concentrations and sensitive plant response, and 3) provide additional margin of safety and support the EPA's previous conclusion that off-field air concentrations from volatility are well below the NOEC.

METHODS

Petri dishes sprayed with dicamba solutions served as the source of dicamba to be volatilized in the humidome. A track sprayer was used to dose Petri dishes with various combinations of M1691 (formulation containing dicamba diglycolamine (DGA) salt) and Banvel® (formulation containing dicamba dimethylamine (DMA) salt) solutions. Older, more volatile dicamba formulations such as Banvel were used in this study because it was not possible to increase volatility to levels across the concentration range using the lower volatility formulation M1691 alone. The Petri dishes were placed inside a humidome containing four soybean indicator plants. The humidome was then sealed and placed inside a growth chamber where air was collected using a polyurethane foam (PUF) based dicamba collection system for 24 hours. The dicamba was extracted from the PUF and the resultant extract solution was analyzed for dicamba by liquid chromatography - mass spectrometry (LC-MS/MS). After exposure, the plants were removed from the closed dome and placed in a greenhouse where they were rated for visual response at 14 and 20 days after treatment (DAT) and plant height at 20 DAT.

PETRI DISH DOSING

Two, four, or six Petri dishes were sprayed at 10 GPA with dicamba solutions as indicated in the following table. In addition, deionized water was sprayed onto Petri dishes (Treatment 1) to serve as a control.

Treatment Number	(w/w) Composition	Number of Petri Dishes
1	DEIONIZED WATER	6
2	100% M1691 (1.2% AE)	6
3	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE)	2
4	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE)	4
5	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE)	6
6	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE)	2
7	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE)	4
8	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE)	6

Three replicates were performed for each treatment. The spray solutions were analyzed by liquid chromatography (HPLC) to confirm that dicamba acid concentrations for Treatments 2 – 8 were each 1.2% dicamba acid.

CLOSED DOME SYSTEM ASSEMBLY

Each closed dome system was prepared by placing a labeled soybean indicator plant in each corner of the tray bottom, for a total of four plants. A commercially available variety of soybean (AG2632) was used in the study. Soybean plants at the beginning of the study were approximately 4 to 5 inches tall and near V1 growth stage with the first trifold starting to open, which allowed for the plants to fit in the humidome. The soybean plants did not touch the sides of the tray bottom, the petri dishes, or each other. The sprayed petri dishes were placed in the center of a plastic tray bottom. The previously prepared closed dome lid and tubing assembly was secured onto the tray bottom with eight metal binder clamps along edges and ends of the tray.

HUMIDOME SETUP

The humidome was placed in a growth chamber maintained at 85°F for 16 hours and 70°F for 8 hours with 40% relative humidity for 24 hours. A vacuum line was used to allow air to flow through the dome and on to the PUF. The vacuum system consisted of a 12-port vacuum manifold with mass flow controllers and displays which allowed for 12 closed domes to be used simultaneously. A vacuum pump was connected to the manifold and exhausted outside the growth chamber. The closed dome remained undisturbed in the growth chamber for 24 hours with air drawn through it at a flow rate of 2 standard liters per minute (SLPM).

TEST COMPLETION

After 24 hours, the vacuum pump was turned off, and the PUF was placed into a 20 mL glass vial and extracted with methanol. The soybean plants were removed from the humidome and transported to the greenhouse for monitoring and assessment of visual response and plant height over a 20-day period. The plants were set in a randomized placement along with the control plants, to be rated for visual response at 14 and 20 DAT and plant height at 20 DAT. In addition to the control plants that were placed in closed domes for 24 hours, 16 greenhouse control plants were placed in the greenhouse to serve as additional control plants.

RESULTS

SAMPLE ANALYSIS

Methanol was used to extract the dicamba from the PUF and the extract was analyzed. Twenty milliliters of methanol were added to the vial, and the dicamba was extracted from the PUF by repeatedly squeezing the PUF with a disposable pipet tip in an up and down motion. The resultant extract was analyzed by LC-MS/MS[1] for extracts in the range of 0.0005 to 0.2 ppm (0.5 to 200 ppb). The LC-MS/MS method used a Zorbax XDB-C8 (4.6 x 50 mm, 3.5 micron) column with a mobile phase gradient consisting of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The dicamba was quantitated using Q1 at 219 and Q2 at 175 daltons.

AIR CONCENTRATIONS WERE CALCULATED FROM CONCENTRATIONS ON THE PUF COLLECTORS FROM THE FOLLOWING EQUATION:

$$\text{Air Concentration } (ng/m^3) =$$

$$ng/PUF \div (2L/min \times 60min/hr \times 24hr \times m^3/1000L)$$

TABLE 1. MEASURED VAPOR-PHASE DICAMBA CONCENTRATIONS IN HUMIDOME.

Treatment Number	(w/w) Composition	Dicamba Acid (ng/PUF) Mean	Dicamba Acid (ng/PUF) Standard Deviation	Dicamba Acid (ng/m³) Mean
1	DEIONIZED WATER 6 PETRI DISHES	<10	-----	<3.5
2	100% M1691 (1.2% AE) 6 PETRI DISHES	89.9	29.9	31.2
3	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 2 PETRI DISHES	203	65	70.6
4	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 4 PETRI DISHES	344	97	120
5	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 6 PETRI DISHES	398	112	138
6	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 2 PETRI DISHES	684	127	238
7	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 4 PETRI DISHES	1394	113	484
8	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 6 PETRI DISHES	1546	638	537

Plant height measurements were made from the soil surface to the tip of the central, terminal bud and were recorded in inches (in). The average plant height measurements are given in Table 2. The average plant height for dicamba exposed plants ranged from 29.42 cm for the Treatment 3 treated plants to 19.88 cm for the plants exposed to Treatment 8. The plants in the internal control group (Treatment 1) had an average height of 28.08 cm. Treatments 6, 7, and 8 were statistically shorter than the control ($p < 0.0001$, $\alpha = 0.05$).

[1] Agilent 1200 series HPLC with Applied Biosystems API 3200 MS/MS

TABLE 2. PLANT HEIGHT MEASUREMENTS.

Treatment Number	(w/w) Composition	Plant Height (inches)		Significant
		Mean	Standard Deviation	
1	DEIONIZED WATER 6 PETRI DISHES	28.08	1.18	N/A
2	100% M1691 (1.2% AE) 6 PETRI DISHES	28.75	1.31	
3	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 2 PETRI DISHES	29.42	1.01	
4	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 4 PETRI DISHES	29.00	2.13	
5	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 6 PETRI DISHES	27.71	1.18	
6	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 2 PETRI DISHES	24.63	1.11	*
7	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 4 PETRI DISHES	21.08	2.27	*
8	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 6 PETRI DISHES	19.88	0.87	*

Plant response was assessed on a scale of 0 to 100 with 0 representing no visible plant response and 100 representing complete plant death. All of the Greenhouse Control plants were assessed as having no visible plant response (rating of 0) at 14 and 20 DAT. This indicated that there were no environmental factors which would have caused a plant response. Treatments 1 through 6 produced slight to no noticeable effects at 20 days. Treatments 7 and 8 produced moderate effects at 20 days. A summary of the plant response data is found in Table 3.

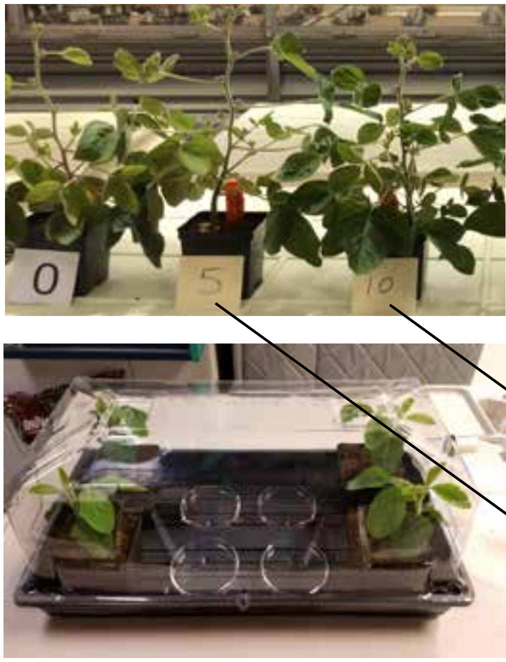
TABLE 3. PLANT RESPONSE ASSESSMENTS.

Treatment Number	(w/w) Composition	14 Day Visual Response (%)		20 Day Visual Response (%)	
		Mean	Standard Deviation	Mean	Standard Deviation
1	DEIONIZED WATER 6 PETRI DISHES	<1	-----	0	-----
2	100% M1691 (1.2% AE) 6 PETRI DISHES	3	6	<1	-----
3	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 2 PETRI DISHES	7	4	5	4
4	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 4 PETRI DISHES	11	5	10	8
5	95% M1691 (1.2% AE) & 5% BANVEL® (1.2% AE) 6 PETRI DISHES	19	10	11	8
6	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 2 PETRI DISHES	25	14	23	17
7	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 4 PETRI DISHES	42	13	38	11
8	75% M1691 (1.2% AE) & 25% BANVEL® (1.2% AE) 6 PETRI DISHES	52	12	43	14

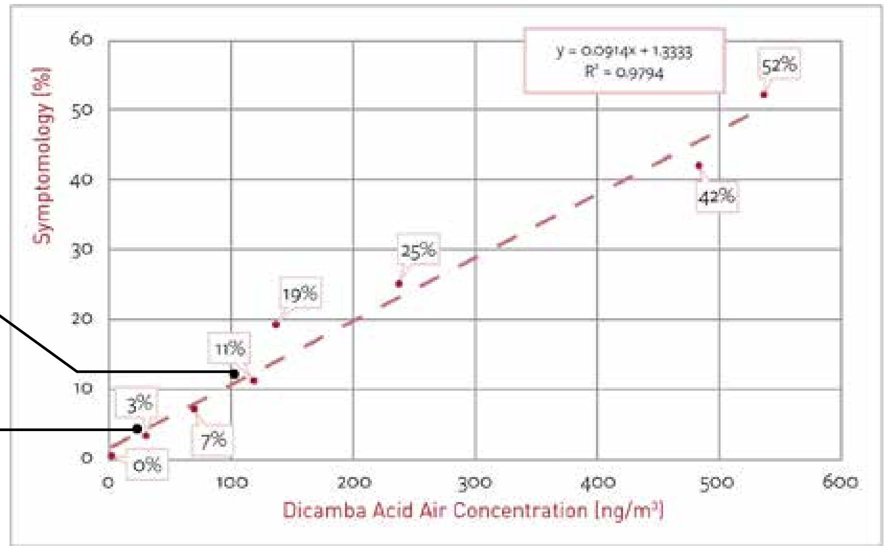
Minimal to moderate effects of dicamba vapors on plant height and morphological effects were observed in this study (Figure 1). Both the morphological observations and the reduced plant heights were consistent with dicamba exposures. No effects to soybean plant height were observed as a result of vapor-phase exposure to dicamba at concentrations of 138 ng/m^3 and below but effects were seen at concentrations of 238 ng/m^3 and above. Therefore, the NOEC was determined to be 138 ng/m^3.

This study provides increased certainty in the plant effects endpoint used in the EPA's risk assessment and further defines the relationship between dicamba air concentrations and sensitive plant response. Furthermore, this refined NOEC (138 ng/m^3) provides additional margin of safety and supports the EPA's previous conclusion that off-field air concentrations from volatility are well below the NOEC.

FIGURE 1.



SYMPTOMOLOGY MEASURED AT VARYING DICAMBA AIR CONCENTRATIONS IN HUMIDOME TO ENSURE CONSTANT EXPOSURE.



MR. TOM ORR

Mr. Tom Orr is the Off-Target Assessment Lead within Monsanto's Regulatory Sciences team. He has been with Monsanto for over 7 years and has been a key contributor for various off-target movement studies, including field spray drift and volatility studies, and wind tunnel studies to support product development and Regulatory data requirements. Prior to joining Monsanto, Tom spent 8 years as an Ecotoxicologist for Environmental Engineering firms conducting ecological risk assessments for various environmental remediation projects. Tom has a M.S. in Environmental Toxicology (Zoology) from Southern Illinois University-Carbondale and a B.A. in Zoology from Miami University in Oxford, Ohio.

* The NOEC of 17.1 ng/m³ was used by the EPA in its risk assessment of XtendiMax® Herbicide with VaporGrip® Technology prior to the November 2016 approval (MRID 49925703). This latest study was submitted to the EPA in April 2018 for review (MRID 50578901).