Uptake of the Pharmaceutical Triclosan in Vegetables Fertilized with a Triclosan-Containing Biosolids

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Biosolids produced by municipal sewage treatment facilities may contain various pharmaceuticals, such as Triclosan, a commonly-used antibacterial and antifungal agent contained in soaps, deodorants, toothpastes, mouthwashes, and cleansing supplies. Milorganite® is a biosolids-based fertilizer that has been produced by the Milwaukee Metropolitan Sewerage District for over 85 years. It is widely distributed and sold for lawns and gardens, including vegetable production. It contains detectable amounts of Triclosan. A greenhouse study was conducted to evaluate Triclosan concentrations in vegetables fertilized with Milorganite 5-2-0. Milorganite containing Triclosan at 2mg kg^{-1} was mixed at the rate of 1.6 g L^{-1} with quartz sand containing sphagnum peat at 100 ml L^{-1}. A second Milorganite application was surface applied six-weeks later at the same rate. Lettuce (*Lactuca sativa*), sweet corn (*Zea mays*), carrots (*Daucus carota*), and tomatoes (*Solanum lycopersicum*) were grown to provide the edible parts, which were harvested and analyzed for Triclosan. The sweet corn had the highest concentration of Triclosan, at 29.7µg kg^{-1}, followed by tomatoes (8.2µg kg^{-1}), carrots (1.1µg kg^{-1}), and lettuce (0.8µg kg^{-1}), on a fresh-weight basis. Based on an EPA Triclosan reference dose of 0.26 mg kg^{-1} body weight d^{-1}, it would be impossible to consume sufficient quantities of any of the vegetables to create adverse health effects.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)- phenol) is an antibacterial and antifungal agent that is widely used in personal care and cosmetic products such as soaps, deodorants, tooth paste, and mouth washes. It also is present in various household cleaning supplies, and in veterinary and therapeutic products. Following many of these uses, it enters public sewage systems, and is frequently observed in biosolids resulting from sewage treatment processes (USEPA, 2009). Biosolids often are land applied as fertilizers, and Triclosan has been observed in various plants, including food crops, grown in soils fertilized with biosolids (eg. Pannu, et al., 2012). However, biosolids can vary widely in their contents of Triclosan because of the variety of sewage treatment processes (Austrialian Government, 2009).

Milorganite® is a biosolids fertilizer that has been produced by the Milwaukee WI Metropolitan Sewerage District for over 85 years. It is marketed for homeowner use on lawns and gardens, including vegetable gardens. Since previous studies on Triclosan involved high application rates of biosolids that are not sold for homeowner use, a greenhouse study was conducted to quantify Triclosan uptake in the edible parts of vegetables fertilized with Milorganite.

Methods and Materials

The experiment was conducted in pots of various sizes (Table 1), using a 90/10 (sand/sphagnum peat, by volume) mined United States Golf Association specification sand provided by Golf Agronomics, Sarasota, FL, from their Ortona, FL, mine. The sand was selected as the growing media to avoid Triclosan contamination. Since the sand was very infertile, it was amended with K_2SO_4 (0.16 g L^{-1}), triple superphosphate (0.24 g L^{-1}), dolomite (0.82 g L^{-1}), and Scotts Micromax Plus® (1.0 g L^{-1}).

The Milorganite web page (http://milorganite.com/Gardening-and-Landscaping/Vegetables-and-Fruit/Vegetable-Application-Rates.aspx) calls for incorporating Milorganite at 0.24 kg m^{-2} (5 lb per 100 ft^2). Assuming an incorporation depth of 15 cm (6 inches), the rate is 1.6 g L^{-1} (6 g gal^{-1}) The sand was mixed with Milorganite (packaged in 5 lb bags by Sunniland Corp., Sanford FL) in 38 L (10 gal) batches in a cement mixer. In addition, the web site calls for a second Milorganite application at the same rate (0.24 kg m^{-2}), especially for tomatoes (*Solanum lycopersicum*) , which was mixed into the soil surface for all vegetables, at rates depending on the pot size (Table 1).

After filling three replications of black plastic pots, the lettuce (*Lactuca sativa*) and tomatoes (Table 1) were planted as small sets. The sets were commercially produced in a peat rooting media, which was mostly, though not completely, removed prior to transplanting. Sweet corn (*Zea mays*) and carrots (*Daucus carota*) were planted as seed (Table 1), and later thinned to one sweet corn and three carrots per pot. Planting occurred on September 14, 2012. The pots were placed in a greenhouse at the University of Florida Ft. Lauderdale Research and Education Center and irrigated to maintain adequate soil moisture. The second Milorganite fertilization occurred October 30, 2012.

One replication of each vegetable also was planted in pots that did not received Milorganite, but which received the same amounts of the various nutrient chemicals as was used previously. Six days after planting, the non-Milorganite pots were fertilized with MiracleGro® “24-8-6” at a rate designed to provide the same amount of N (0.08 g L^{-1}) as was provided by the Milorganite. A second application was made October

30, 2012. On October 4th, stakes were placed in the corn and tomato pots to help support the plants, which were secured to the stakes with plastic twist ties.

All lettuce leaves were harvested on November 12, 2012 and the plants were cut back to about 5 cm above ground level. The carrots (3 to 4 per pot) were harvested on December 6, 2012. Sweet corn (one ear per plant) was harvested on December 13th. The grains were cut from the cob. The tomatoes were harvested on December 24th, with two or more available per plant. A second harvest of lettuce and carrots was made on April 5, 2013, for reasons discussed below.

A sample of Milorganite was obtained from the Milwaukee WI factory production line in July, 2012, prior to the greenhouse study, and submitted to Exova Inc., Santa Fe Springs, CA for Triclosan analysis. They extracted an aliquot by sonication with methylene chloride. The extract was concentrated using a steam bath, spiked with internal standard and analyzed using GC-MS. A sample of the Milorganite used in the greenhouse study also was analyzed by Exova Inc., using the same methodology. The harvested vegetable parts were analyzed for Triclosan by Jupiter Analytix, Jupiter FL, using dispersive solid phase extraction. The samples were extracted with acetonitrile and centrifuged. The supernatants were appropriately diluted and analyzed for Triclosan by HPLC Triple quad (ABSciex 5500 QTrap).

Results and Discussion

The Milorganite sample obtained from the production line contained 4.3 mg kg^{-1} Triclosan, so the experiment was conducted with the expectation that Milorganite contains Triclosan. However, the Milorganite used in the study was determined to contain 2.0 mg kg^{-1}.

The analysis of the first harvest of lettuce yielded Triclosan concentration values below 50 ng kg^{-1} , and the carrots below 100 ng kg^{-1}in all samples; both those fertilized with Milorganite and those not receiving Milorganite. By contrast, the corn and tomatoes had substantially greater amounts of triclosan (Table 2). Because of the difference, a second, composite sample of the Milorganite-fertilized lettuce and carrots remaining in the three pots was made on April 5, 2013 and submitted to the same laboratory. These samples were found to contain more Triclosan than the first set (Table 2). There was insufficient sample in the pots not receiving Milorganite to provide a second sample for analysis.

The sweet corn fertilized with Milorganite had the highest concentration of Triclosan, averaging 29.7 µg kg^{-1}, with 0.9 µg kg^{-1} being determined in the sweet corn that did not receive Milorganite fertilization (Table 2). The oral reference dose (RfD) is an estimate by the USEPA, with uncertainty spanning one to two orders of magnitude on the conservative side, of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. For Triclosan, the RfD is 0.26 mg kg^{-1} body weight d^{-1} (http://www.health.state.mn.us/divs/eh/risk/guidance/gw/triclosan.pdf). Based on this value, a person weighing 70 kg (154 lb) could consume 18.2 mg Triclosan on a daily basis for a lifetime, without deleterious effects. To ingest 18.2 mg Triclosan every day for a lifetime by eating sweet corn containing the amount obtained in the current study would require eating 613 kg sweet corn (1,349 lbs) daily for a lifetime. Larger amounts would be required of the other vegetable crops. Based on this work, it is unlikely that any deleterious effects of Triclosan would be realized from eating vegetables fertilized with the biosolids Milorganite.

Literature Cited

Australian Government. 2009. Triclosan. Priority Existing Chemical Assessment Report, No. 30. http://www.nicnas.gov.au/publications/car/pec/pec30/pec_30_full_report_pdf.pdf

Pannu, M.W., G.S. Toor, G.A. O'Connor, and P.C. Wilson. 2012. Toxicity and bioaccumulation of biosolids-born Triclosan in food crops. Environ. Tox. Chem. 31:2130-2137.

USEPA. 2009. Targeted national sewage sludge survey sampling and analysis technical report 2009. EPA 822/R-08/016. Technical Report. Washington, D.C.

Table 1. Information on vegetables and pots used in the study

		Pot diameter	**Pot depth**	**Pot volume**
Vegetable	**Source**	**cm**	**cm**	**L**
Sweet corn	Burpee Silver Queen hybrid	13	11	23
Tomato	Bonnie Plants Better Boy hybrid	10	8	10
Carrot	Burpee Scarlet Nantes	9	7	7
Lettuce	Bonnie Plants Red Sails red lettuce	8	7	6

Table 2. Fresh-weight concentration of Triclosan in the vegetable edible parts.

Vegetable	Triclosan (µg kg^{-1})	CV (%)
Lettuce[1]	0.8	- -
Carrots[1]	1.1	- -
Corn	29.7	38
Tomatoes	8.2	9
Corn w/o Milorganite	0.9	- -
Tomatoes w/o Milorganite	1.1	- -

1. Data from second harvest, April 5, 2013.