Antibiotic Residues on Plant Tissues

Antibiotics have been used to control plant diseases for over 50 years (McManus et al., 2002). The quantity of antibiotics used for crop protection is small relative to livestock production and potential human exposure from antibiotics used on plants is miniscule relative to therapeutic use by patients. Nonetheless, questions persist about the fate of these materials when applied in agricultural environments, the potential for human exposure to antibiotics, and the risk of selecting for antibiotic resistance in human pathogens. The discussion below highlights the research done on antibiotic residues on treated plant tissues.

Many studies have examined streptomycin residues on apple and pear trees, while fewer have addressed residues of oxytetracyline. For streptomycin, the residue tolerance level on tree fruit crops is 0.25 ppm (USEPA, 2006b). Shaffer and Goodman (1969) published the first evaluation of residues of streptomycin on apple leaves and fruit. They sprayed trees up to ten times from flowering in April to early fruit development in mid-June. They detected residues on leaves during the season (detection limit of 0.1μg/ml) and on developing fruit, but residues on fruit were below the residue tolerance within a month after the last spray (about 70 days before harvest). At harvest, residues were not detected on apple fruit, even on trees sprayed ten times with streptomycin (Goodman, 1961; Shaffer and Goodman, 1969). Subsequent studies by numerous independent investigators have corroborated their results – fruit from trees treated with streptomycin for fire blight management does not have residues near the tolerance levels permitted by governmental agencies (see, for example, Gardan and Manceau, 1984). The Environmental Protection Agency (2006b) concluded that anticipated dietary residues of streptomycin from plant agriculture were extremely low: even in worst-case scenarios with contaminated water sources and food, the dietary exposure dose would be 3,000 to 21,000 times lower than a typical therapeutic dose.

Recently, a study (published as two papers) reported that streptomycin may be detected in a portion of apple fruit harvested from orchards exposed to multiple applications of the antibiotic. One paper presented the methods used to quantify antibiotics in apples (Bohm et al., 2010) and the second presented preliminary data on antibiotic detection in apples (Mayerhofer et al., 2009) in Austria. The latter study was reported in a research letter and assessed apples collected from orchards that were untreated or orchards treated one to three times with streptomycin during bloom to late bloom. Apple fruit were collected about three months later and tested for streptomycin. The level of detection was 2 μg/kg (0.002 ppm or 2 ppb) and the limit of quantification was identified as 7 μg/kg (0.007 ppm or 7 ppb).

From non-treated orchards, none of the 14 apples tested had detectable levels of streptomycin. From orchards treated once, twice or three times, 4 of 11 fruit, 1 of 5 fruit, and 15 of 25 fruit, respectively, had detectable levels of streptomycin (presumably between 2 to 7 μg/kg), but the concentration was too low to quantify the antibiotic. An unspecified number of apples from orchard(s) treated three times with streptomycin had concentrations of streptomycin that could be quantified (≥7 μg/kg). They reported that the highest concentration of streptomycin detected was 18 μg/kg (0.018 ppm), well below the EPA tolerance of 250 μg/kg (0.25 ppm).

The amount of antibiotic in positive apples was below the minimum inhibitory concentration (MIC) for gut flora, strongly suggesting that any antibiotics transferred to the gut would have negligible impacts on gut flora. There is no universally agreed upon value for acceptable daily intake (ADI) for streptomycin. The Austrian ADI for streptomycin is 0.03 mg per kg of body mass per day (0.03 ppm). Thus, for a 100 kg person, 3 mg streptomycin consumed each day would be expected to have no appreciable effects on health over their lifetime. Assuming that all apple fruits contained the maximum streptomycin residue detected in the study (18 µg per kg fruit or 0.018 mg per kg fruit), a person would need to eat 166 kg of fruit or >1,000 apples (fruit weight of 150 g) each day. Furthermore, in rare situations when streptomycin is administered to humans, it is delivered intravenously. Streptomycin is ineffective when taken orally by humans. All together, this means that the selection of streptomycin-resistant human gut flora due to consumption of apples containing trace amounts of streptomycin is extremely unlikely.

Even though antibiotics may be detected on plant surfaces for up to a month after application using sensitive analytical chemistry methods, antibiotics lose activity rapidly and their capacity to inhibit bacterial growth is lost within a week after application. Thus, although antibiotics may be detected on plant surfaces with analytical methods, they may no longer be active as agents to select for antibiotic resistant bacteria. In a laboratory experiment, streptomycin no longer prevented fire blight on flowers inoculated with *Erwinia amylovora* (the causal agent of fire blight) five days after spraying the antibiotic on apple flowers (Vanneste, 1996). Stockwell et al. (2008) treated trees in a screenhouse with streptomycin and/or oxytetracycline. Under conditions where trees were protected from rain and ultraviolet irradiation from sunlight, growth of *E. amylovora* was suppressed for only 4 days after antibiotic treatment (Stockwell et al. 2008). The persistence of antibiotics is probably even lower under fully exposed conditions (Brink et al., 1945). In a study of potential plant uptake, no oxytetracycline uptake was found using soil drench or foliar spray on coconut palm (McCoy, 1976). Direct injection of oxytetracycline into the trunk resulted in detectable levels of the antibiotic in leaves (up to 20 μg/g) with a half-life of two weeks.

Christiano et al. (2010) conducted an extensive study of the stability of oxytetracycline (applied at 300 μg/ml a.i.) on peach leaves. At least 50 ppm oxytetracycline (0.06 μg/cm leaf surface) on leaves was required to control bacterial spot of stone fruits. Oxytetracycline was thermostable on leaves, but rapidly degraded when exposed to natural sunlight, with 44% degradation within 1 day and 92% within 4 days, and to levels near the detection limit (0.05 ppm) by a week after application (Christiano et al., 2010). Oxytetracycline was not rainfast on leaves: 2 minutes of simulated rain (44 mm/h) reduced residual concentrations of oxytetracycline by 67%, and after an hour of simulated rain the material was near the detection limit. The authors concluded that the oxytetracycline concentrations on trees in orchards would be insufficient to suppress the pathogen *X. arboricola* pv. *pruni* after 2 days under full sunlight, 4 days under overcast skies, or 2 minutes during a heavy rainstorm (Christiano et al., 2010).

Residue data for oxytetracycline were reported by the US EPA (2005) as part of the process to allow the material to be used on apples. Field trials were conducted by the registrant in apple orchards in various regions of the country. Oxytetracycline (as the formulated material Mycoshield) was applied six times; the first five sprays were applied at 3 to 6 day intervals and the sixth spray was applied 49 to 50 days before harvest. Among 15 trials, the total amount of Mycoshield applied within a season varied from 0.765 to 7.65 pounds a.i./acre, or 0.5 to 11 times the proposed seasonal rate of 1.53 lb a.i./acre. Apples were harvested, frozen, and residues from samples were extracted with water. The limit of quantification of oxytetracycline was 0.013 ppm. Most samples were at or below the limit of detection. , while the highest residue level detected was 0.25 ppm in two fruits of the 128 fruits tested. None of the samples had residues at the permitted rate of 0.35 ppm. Several dose rates were tested in the field trials and a correlation between amount of Mycoshield applied and detectable residues was not observed. No data were reported for trees treated only once or twice during bloom, which is the most common use pattern in the western US (Stockwell and Duffy 2012).

Governmental regulations established by the US Environmental Protection Agency restrict the level of permissible pesticide residues on conventional managed crops. For oxytetracycline, the residue tolerance level on tree fruit crops is 0.35 ppm or 0.35 mg oxytetracycline per kilogram fruit. (USEPA, 2006a; USEPA, 2008). In a risk assessment study (USEPA, 2008), the Environmental Protection Agency states that typical pharmaceutical oxytetracycline exposure to humans would be 50,000 to 200,000 times greater than the theoretical dietary exposure (i.e. combined food and potentially contaminated water sources) associated with the application of oxytetracycline in plant agriculture. The agency concluded that the potential dietary exposure of humans to oxytetracycline used in plant agriculture would result in no harm compared with its pharmaceutical usage (USEPA, 2008).

The following puts the permitted residue level of oxytetracycline on fruit in context of oral therapeutic doses of tetracyclines for humans. For humans, tetracyclines are administered at doses between 1000 mg to 2000 mg daily for at least a week (http://www.drugs.com/dosage/tetracycline.html) or a minimal exposure of 7,000 to 14,000 mg during a prescribed cycle. To date, there are no reports of fruit with residues at or above the permitted tolerance for oxytetracycline at 0.35 mg/kg fruit. Nonetheless, even if fruit contained the permitted tolerance for oxytetracycline, a person would need to consume about 2,857 kg of fruit in a single day (or 28 times their body weight for a 100 kg person) to be exposed to the minimal daily therapeutic dose of tetracycline. The U.S. Apple Association estimated that the average U.S. consumer eats about 16.5 pounds (7 kg) of fresh-market apples and 33.3 pounds (15 kg) of processed apples, for a total of 49.8 pounds (22 kg) of fresh apples and processed apple products in a year (http://www.usapple.org/consumers/all-about-apples). The annual consumption of apples and apple products by the average consumer is 130-fold less than the amount of apples with oxytetracycline residues that would need to be consumed in a single day to approach a minimal oral therapeutic dose of tetracycline.

In the Code of Federal Regulations (21CFR556, Sec. 556.720: Tetracycline), the acceptable daily intake (ADI) for total tetracycline residues (chlortetracycline, oxytetracycline, and tetracycline) is 25 μg/kg of body weight per day. The ADI is an estimate of the amount of a substance which can be ingested daily over a lifetime by humans without appreciable health risk. For a 100 kg person, the ADI for tetracyclines is 2.5 mg. If a person ate fruit with oxytetracycline residues of 0.35 mg/kg fruit, then they would need to consume 7 kg of fruit daily or 47 apples (150 g) each day to reach the ADI.

Overall, the potential that daily consumption of apples from trees treated with oxytetracycline during bloom, even if fruit had the maximum permissible levels of residues of the antibiotic, would exert negative impacts on health or lead to selection of tetracycline-resistant bacteria in humans is unlikely.

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