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Captan residue reduction in apples as a result of rinsing and peeling

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Abstract

Apples, treated with captan for disease control in a commercial orchard in Quebec, Canada, were collected and sorted into post-harvest preparation types (no preparation; rinse; rinse and peel). Captan residues were greatest (25.5–5100 ng/g) in apples with no post-harvest preparation and lowest (0.146–136 ng/g) in apples that had been rinsed and peeled prior to extraction and analysis. Residues were significantly lower ($p = 0.003$) in apples that had been rinsed prior to extraction than in apples with no post-harvest preparation. Similarly, apples subjected to rinsing and peeling had significantly lower captan residues than had apples that had been rinsed alone ($p < 0.0001$). Although captan residues in rinsed apples were approximately 50% lower than those in apples that received no post-harvest preparation, the reduction associated with peeling of apples was much greater (98%). Estimated mean captan intakes resulting from consumption of raw apples were established and single day intakes, based on apples with no preparation, ranged from 2.58 μg/kg in females >70 years to 9.48 μg/kg for individuals aged three years (at this age no distinction is made between males and females). Mean intakes estimated using rinsed and peeled apples were approximately two orders of magnitude lower than intakes estimated using apples with no post-harvest preparation, demonstrating the effect of post-harvest preparation on captan intakes. Mean captan intake estimates from all post-harvest preparation types were well below the World Health Organization acceptable daily intake of 100 μg/kg/day, based on raw apple consumption.

Keywords: Captan; Residue reduction; Rinsing; Peeling; Intake

1. Introduction

Captan (1,2,3,6-tetrahydro-*N*-(trichloromethylthio) phthalimide) is used to control fungal disease on a wide variety of crops and seeds in Canada. It also has a broader industrial application for control of mould in paints, lacquers and wallpaper pastes. Captan acts through inhibition of a fungal process of respiration and metabolism through a non-specific thiol reactant (Barreda et al., 2006). Although it is not a systemic fungicide, adjuvants can enhance transport of captan through a plant cuticle (Bondada, Sams, Deyton, & Cummins, 2007).

Captan is detected in fruit and vegetables during market basket monitoring and total diet studies (Rawn et al., 2004; Sadlo, Szpyrka, Jazwa, & Zawislak, 2007; US FDA, 2006). Captan is the most frequently detected fungicide in some market basket studies and average concentrations were reported to be relatively high (98 ng/g) (Krol, Arsenault, Pylypiw, & Incorvia Mattina, 2000). Compliance testing has also shown that captan levels in fruit samples, particularly berries, can be extremely high and may exceed maximum residue limit (MRL) concentrations (e.g., Canadian MRL 5000 ng/g) (CFIA, 2004, 2005; Rawn et al., 2004).

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Captan is used extensively to control diseases such as grey mould fungus (*Botrytis cinerea*), which is found in both pre-harvest and post-harvest strawberries (Blacharski, Bartz, Xiao, & Legard, 2001; Ritcey, Frank, McEwen, & Braun, 1987). Research has determined captan dissipation rates, levels in fruit and absorption rates by field workers (Krieger & Dinoff, 2000; Ritcey et al., 1987). Captan residue levels in other fruit and vegetables also have been measured (Burchat et al., 1998; Fernández-Cruz et al., 2006; Frank, Braun, & Stanek, 1983; Rawn et al., 2007).

Unlike the organophosphate insecticides, captan is effectively removed from fruit and vegetables with water. Krol and coworkers (2000) reported that captan residues were reduced dramatically when fruit and vegetables were rinsed under tap water. Burchat et al. (1998) similarly observed that captan was effectively removed from both carrots and tomatoes following washing/hand rubbing under water, while pesticides belonging to other classes were not as easily removed from tomatoes because of their waxy surface and the greater characteristic absorbency of the other classes.

Frank et al. (1983) studied the reduction of captan residues in apples associated with a variety of post-harvest preparations and reported that residues were reduced by 43–91% when apples were washed, wiped and/or boiled. The study, however, was performed using apples that were dipped into a captan solution immediately prior to being prepared (washed, wiped), rather than exposed under field conditions.

The relative pesticide reduction resulting from post-harvest preparations may be related to time following application of captan. Increased residue reductions have been observed in tomatoes washed with water 4 h after pesticide application (90%), relative to those that were washed 14 days after captan treatments (84%) (Cengiz, Certel, KarakaÞ, & G'Hmen, 2007). Fernández-Cruz et al. (2006) reported that captan residues were reduced by 30% in some cauliflower samples immersed in water for 10–15 min, seven days post-application. Similar reductions, resulting from this post-harvest preparation method, were not observed in cauliflower samples collected 3 h post-application.

Because captan is a non-systemic pesticide, removal of peel is anticipated to have a greater impact on residue levels than washing alone. In a Turkish study, peeled tomatoes were indeed consistently found to have lower residue levels (93–95% reduction) than those samples that were washed (84–90% reduction) (Cengiz et al., 2007).

In the present study, apples treated with captan in response to pest pressures were collected by hand just prior to commercial harvesting at 104 d post-treatment. Residue analysis for captan was conducted to determine the relative impact of rinsing (i.e., washing with water) or rinsing and peeling on the reduction of captan residues via comparison of residues in apples with these post-harvest preparations relative to those obtained in apples that received no preparation.

2. Materials and methods

2.1. Field application

During the 2003 agricultural season, three rows of apple trees were treated with captan, using an air blast sprayer to control fungal disease in an orchard in Quebec, Canada. Each row comprised 10 trees and pesticide application was consistent with the recommended rates (Crop Captan 80 WP, 3.75 kg/ha) and followed label directions (Rawn et al., 2007). Apples were collected 104 d following application which exceeded the required Canadian pre-harvest interval (7 d) for captan.

2.2. Experimental design and harvest

The design protocol sampled apples from experimental orchard rows, McIntosh trees in a row and zones in a tree, with appropriate randomization (harvest plans, trees, zones within tree, and apples within zone) and randomized harvested apples to preparations according to an incomplete block design: a $3 \times 3 \times 2$ factorial design (preparations, heights, and faces) arranged into blocks (of 6 trees per row of 10) by partially confounding the 3-way interaction with differences among blocks (trees) and further blocked into chemical analysis sets by partially confounding higher order interactions with differences among blocks (chemical analysis sets).

2.3. Chemicals

The captan analytical standard was received as a gift from Agriculture Canada (Ottawa, ON). $^{13}C_{12}$ PCB 101, used as a performance standard, was purchased from Cambridge Isotope Laboratories, Andover, MA. High purity solvents (acetone, hexane, dichloromethane, and cyclohexane), suitable for LC, GC and residue analysis, that were used in the extraction and clean up, were purchased from EMD Biosciences Inc. (Mississauga, ON). Florisil (60–100 mesh), used in the clean up of samples, was purchased from Fisher Scientific (Ottawa, ON). Additionally, reagent grade sodium sulphate and sodium chloride were purchased from EMD Biosciences Inc. (Mississauga, ON).

2.4. Post-harvest preparation of apples

While the apples were sorted into preparation group (no preparation, rinse only and rinse and peel) and prepared, they were stored at 4 °C. Rinsed apples were held under running de-ionized water for 10–15 s with continuous rubbing by hand. Apples requiring peeling were initially rinsed, as described above, and then peeled with a paring knife. Following preparation, apples were cored and sliced into 10 equal segments, using a domestic coring and slicing tool. The apple slices were chopped manually using a knife and placed in a plastic bag for storage at −80 °C until extraction. The corer/slicers, knives and cutting boards

used in apple preparation were thoroughly washed with detergent and rinsed with de-ionized water between apple and post-harvest preparation types.

2.5. Extraction and clean up

Extraction and clean up of samples were performed as described by Rawn et al. (2006). Briefly, 25 g apple aliquots were weighed into a 500 ml Erlenmeyer flask and homogenized with 250 ml of acetone: 50 ml of hexane. Crude extracts were then filtered through glass wool into a separatory funnel and 100 ml of saturated NaCl were added. The aqueous layer was removed, following gentle shaking, and re-extracted with 50 ml hexane. The organic phases were combined, dried over anhydrous Na_2SO_4, and evaporated to near dryness using a rotary evaporator. Extracts then were dissolved in 8 ml of (1:1) dichloromethane (DCM):cyclohexane, filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter and subjected to gel permeation chromatography (GPC) with 200–400 mesh SX-3 biobeads (O-I Analytical, College Station, TX) to remove high molecular weight impurities (e.g., pigments). Following GPC, sample volumes were reduced to 2 ml and eluted from a 6 g Florisil (2% deactivated) column with 70 ml of 60% DCM: hexane, followed by 100 ml of 15% acetone:hexane. The performance standard ($^{13}C_{12}$ PCB 101) was added to final extracts prior to concentrating the samples to a 1 ml final volume in *iso*-octane. Extracts were stored at −80 °C prior to analysis.

2.6. Instrumental conditions

All analyses were performed using a Micromass Autospec-Ultima mass spectrometer (Manchester, UK) coupled to an Agilent 6890 gas chromatograph (Mississauga, ON, Canada). The system was equipped with an on-column injector set to track oven temperatures. The analytical column (30 m DB-5 fused silica column with 0.25 mm i.d. × 0.25 μm film thickness) (J&W Scientific, Folsom, CA) was coupled to a 3 m × 0.53 mm retention gap (Chromatographic Specialties, Brockville, ON, Canada). The oven temperature was initially set to 80 °C and increased at a rate of 8 °C/min to 240 °C, followed by a temperature increase to 280 °C at a rate of 5 °C/min. The oven temperature was maintained at 280 °C for 5 min. Injection volumes were 1 μl for all analyses and helium was used as the carrier gas which was set to a constant pressure of 150 kPa. Samples were analyzed using selected ion monitoring ($m/z = 264$ and 266).

The electron energy was 70 eV, the photomultiplier voltage was set to 350 V. The trap current was 600 μA and the source temperature was 250 °C. The re-entrant temperature and capillary line temperature were maintained at 280 °C and perfluorokerosene-L (PFK) was used as the reference substance. The mass resolution was set to between 3000 and 4000.

2.7. Quality assurance/quality control

Additional apples were collected from an orchard where captan was not applied and were prepared in the same manner as the rinsed and peeled apples for use as blank apple matrix in quality assurance testing. Two 25 g aliquots of blank apple were extracted with each set of samples analyzed. One aliquot was used as a blank while the other was spiked with captan (8.95 ng/g) prior to initiating extraction. The spiked apple was allowed to sit for 30 min prior to initiating extraction. The blank and spiked samples were extracted, cleaned up and analyzed using the method for unknown samples. Captan was observed at trace levels in some blank matrix samples and background levels were subtracted from spiked matrix only in the determination of recovery. Concentrations in unknown samples were not blank-corrected because captan was not detected in any of the reagent blank samples analysed. The mean captan recovery was 81% from spiked blank matrix ($n = 31$).

The average method detection limit (MDL) established for captan was 0.414 pg injected (0.026 pg/g), based on a 3:1 signal to baseline noise ratio. The MDL was determined by averaging the captan MDL obtained in all individual chromatograms.

2.8. Statistical methods

A mixed effects model described the variability among individual apple captan residue concentrations:

$$(Y) : \ln(Y) = P + H + F + HF + \{T(R) + R + S + \text{Error}\} \quad (1)$$

where preparation [P] and tree zone (height [H], face/side [F]) from which an apple was sampled are fixed effects, and where tree within row [$T(R)$], replicate row [R] and the chemical analysis set in which an apple was analyzed [S], are random effects. Other 2-way (PH, PF) and estimable 3-way interactions were not significant at the 5% level and were excluded from this reduced model.

The model was fitted using SAS® 8.02 PROC MIXED – restricted maximum likelihood estimation; Satterthwaite approximation for degrees of freedom; Wald-type confidence intervals for parameters; Bonferroni adjustment for multiple comparisons. Least squares means (population marginal means) rather than simple means are reported and Type III tests were performed to assess the significance of model effects since the data were unbalanced once the experimental design and missing (3) observations were taken into account.

2.9. Single day captan intakes

The effect of preparation on the captan residue consumed with raw apple eaten alone, not as an ingredient in a recipe or preparation and not processed (e.g., cooked,

frozen, dehydrated), was considered as it changes with preparation. A simple model for the amount of captan consumed with raw apple is the product: [amount (g) of apple eaten] × [captan concentration (ng/g) in/on an individual apple]/[body weight (kg)].

Summary characteristics for population-level variability in single-day captan residue intake per kilogram body weight were calculated from approximately 450,000 simulated single-day captan intakes, generated using: individual respondents' food recalls to account for among-individuals apple intake and body weight variation, resampling using the consumption surveys' sample weights as resampling weights, and individual apples' captan residue concentration variation (modelled fixed and random effects), with assumed 1/6 of apples from each of the 6 tree zones. Simulations were replicated 500 times to account for parameter uncertainty in captan residue concentrations (mean log concentrations were sampled from Gaussian distributions and variance log concentration components sampled from Gamma distributions) to capture the effect on population characteristic estimates. The share of consumption under different preparation methods is unknown; the results are calculated *as if* all apples had no preparation, *as if* all apples were rinsed only and *as if* all apples rinsed and peeled are compared.

Apple intakes were estimated using raw apple consumption reported on 24 h recall data from existing nutrition surveys (a series of Canadian Federal-Provincial Nutrition Surveys, 1991–1999, for individuals $\geqslant$6 years old; the US Department of Agriculture Continuing Survey of Food Intakes of Individuals (CFSII) (1994–1996), the Supplemental Children's survey (1998) and the Diet and Health Knowledge survey (1994–1996), for 1–5 year olds) US Department of Agriculture (2000). Among age-sex groups, an estimated 11–31% of the population consumes raw apple as defined here on $\geqslant$1 occasion on a day at random, and estimated mean single-day apple consumption (eaters only) varies from 72 g to 258 g.

3. Results and discussion

3.1. Residues

Captan residues were detected in all 212 samples analyzed, regardless of post-harvest preparation (none, rinse, rinse and peel), apple location within a tree or the row from which an apple was collected. Residue levels ranged from 0.146 ng/g to 5100 ng/g. Only one apple had captan concentrations exceeding the Canadian maximum residue limit (MRL) (5000 ng/g) for this compound. The high captan level was observed in an apple that did not receive any post-harvest preparation (i.e., not rinsed or peeled).

Because captan was applied to all experimental rows, residue distributions from rows were expected to be similar. A greater range in residue concentrations was observed in apples from Rows 1 (0.146–5100 ng/g) and 2 (0.387–4919 ng/g), while the distribution in residues observed in apples from Row 3 was much smaller (0.556–962 ng/g). In all rows, the maximum captan concentration was observed in apples that did not receive any post-harvest preparation while the minimum level was observed in apples that had been subjected to both rinsing and peeling. Captan residue concentrations were significantly higher in apples from the bottom and middle of the tree than from the top of the tree and in apples that faced the sprayer directly than in apples that did not face direct pesticide spray (Fig. 1), consistent with Rawn et al. (2007).

Of the apples that did not receive any post-harvest treatment, captan residues ranged from 25.8 ng/g to 5100 ng/g and least squares means estimates for apples facing direct spray were 714 ng/g, 723 ng/g and 164 ng/g for apples collected from the bottom, middle and top, respectively (Table 1). Rinsing of apples under de-ionized water while rubbing with hands resulted in residue levels ranging from 24.5 ng/g to 1180 ng/g and least squares means concentrations for apples facing direct spray at three tree heights were 363 ng/g, 368 ng/g and 83 ng/g for bottom, middle

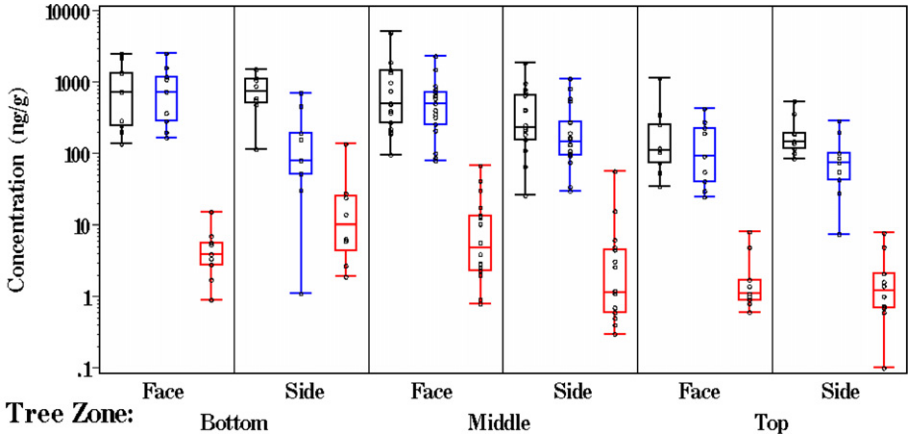

Fig. 1. Captan residue levels (ng/g) in apples in each tree zone, residue distribution from left to right corresponds to apples with no preparation, rinse only, rinse and peel. Boxes mark 25th percentile, median, 75th percentile. Whiskers mark 5th percentile, 95th percentile.

Table 1
Captan least squares means (ng/g) [95% Wald-type confidence interval] for apples from different parts of a tree

Height	Face	Preparation	Estimate
Bottom	Direct spray	None	714 [378, 1350]
		Rinse only	363 [194, 682]
		Rinse and peel	5.95 [3.18, 11.1]
	Side	None	455 [239, 865]
		Rinse only	231 [121, 441]
		Rinse and peel	3.79 [2.00, 7.19]
Middle	Direct spray	None	723 [409, 1279]
		Rinse only	368 [210, 645]
		Rinse and peel	6.03 [3.45,10.5]
	Side	None	263 [150, 459]
		Rinse only	134 [76.0, 235]
		Rinse and peel	2.19 [1.26, 3.81]
Top	Direct spray	None	164 [87.0, 309]
		Rinse only	83 [44.0, 156]
		Rinse and peel	1.36 [0.73, 2.55]
	Side	None	150 [80.0, 282]
		Rinse only	76 [40.0, 144]
		Rinse and peel	1.25 [0.67, 2.35]

Model: $\ln Y \sim P + H + F + HF + \{T(R) + R + S + \text{Error}\}$.

and top, respectively. The captan levels observed in apples that were rinsed prior to extraction and analysis were significantly lower than concentrations observed in apples with no post-harvest preparation ($p = 0.003$).

Captan concentrations in rinsed and peeled apples were much lower (0.146–136 ng/g) than those observed in apples that received either no post-harvest preparation or were rinsed prior to extraction and analysis (Table 1). Rinsed and peeled apples that faced direct spray during the captan treatments were found to have higher least squares means concentrations than those collected from the sides of the tree (direct: 5.95 ng/g, 6.03 ng/g and 1.36 ng/g; side: 3.79 ng/g, 2.19 ng/g and 1.25 ng/g, for bottom, middle and top, respectively). The reduction in captan levels was found to be highly significant ($p < 0.0001$) when apples were rinsed, followed by peeling, relative to apples with no post-harvest treatment.

Previously, our group found that OP insecticide concentrations in apples were lowered by rinsing relative to those that received no post-harvest preparation, similar to the results of the present study. The rate of reduction, however, was found to be not significant for the OP insecticides, while captan reduction resulting from only rinsing of apples was found to be significant ($p = 0.003$) in this work. In the present study, captan levels were further reduced via peeling following rinsing. Apples that were rinsed only were found to have significantly higher captan concentrations ($p < 0.0001$) than had apples that were rinsed followed by peeling, prior to extraction.

Although captan residues in rinsed apples were approximately 50% lower than those in apples that received no post-harvest preparation, the reduction associated with peeling of apples was much greater (98%) (Fig. 2). The rates of reduction observed were independent of initial concentration on unprepared apples. Results of the present study are consistent with data in the literature reporting captan reduction resulting from post-harvest preparation in different commodities. Burchat and coworkers (1998) reported captan residue reduction in both carrots (33–100%) and tomatoes (70–85%) upon washing with water. Similarly, Cengiz et al. (2007) observed a decrease in captan levels of 84–90% if tomatoes were washed and 93–95% if peeled.

3.2. Consumption/intake

Consumption of apple increases with age among children and youths, while consumption by adults remains approximately constant within each age/gender group (Table 2), based on food consumption surveys, as indicated above. A decrease in raw apple consumption is observed in elderly individuals (>70 years) relative to younger adults.

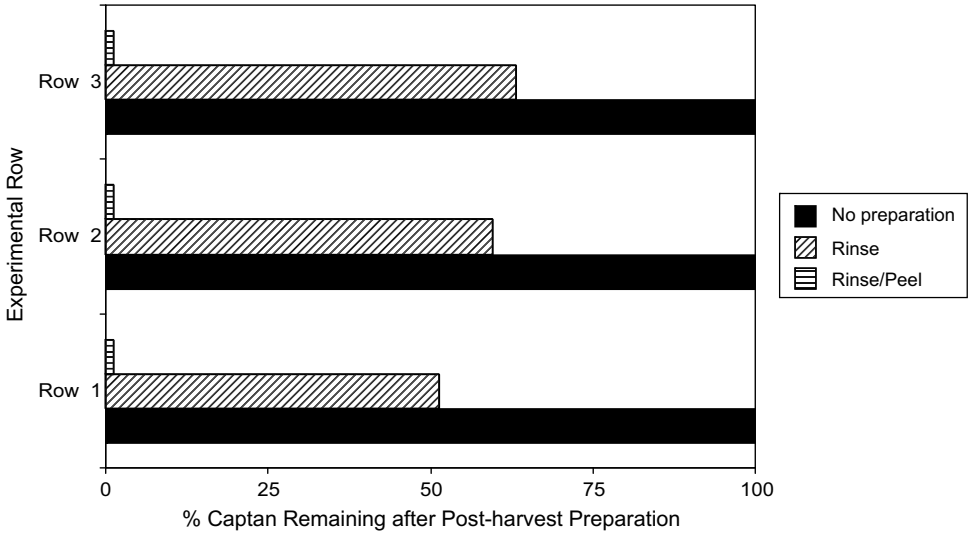

Fig. 2. Mean captan residue (%) in apples relative to apples receiving no post-harvest preparation.

Table 2
Estimated mean single day captan intakes (μg/kg) [simulation interval: 5%, 95%] based on 500 replications of the model using raw apple consumption data coupled to residue concentrations observed following post-harvest treatments (no preparation; rinse only; rinse and peel)

	Age/sex group (years)[a]	Single-day apple intake eaters only (g)	No preparation	Rinse	Rinse and peel
Male and Female	1	72	8.70 [5.68, 30.0]	4.34 [3.01, 15.7]	0.073 [0.048, 0.258]
	2	103	8.96 [6.02, 22.8]	4.52 [3.19, 12.4]	0.075 [0.196, 0.582]
	3	109	9.48 [6.26, 27.3]	4.78 [3.32, 15.0]	0.079 [0.054, 0.242]
	4	121	8.83 [5.80, 31.7]	4.41 [10.5, 28.9]	0.074 [0.049, 0.267]
	5	132	9.39 [5.98, 32.9]	4.73 [3.19, 18.3]	0.078 [0.052, 0.287]
Female	6–8	171	8.52 [5.93, 17.9]	4.37 [3.03, 9.33]	0.072 [0.050, 0.154]
	9–13	182	6.53 [4.34, 16.5]	3.35 [2.23, 8.84]	0.055 [0.038, 0.145]
	14–18	203	4.15 [2.88, 8.70]	2.13 [1.48, 4.72]	0.035 [0.024, 0.076]
	19–30	166	3.83 [2.65, 8.75]	1.97 [1.35, 4.57]	0.032 [0.022, 0.075]
	31–50	180	4.70 [3.19, 11.5]	2.41 [1.63, 6.31]	0.040 [0.027, 0.101]
	51–70	169	3.48 [2.37, 8.43]	1.79 [1.21, 4.42]	0.029 [0.020, 0.073]
	>70	122	2.58 [1.77, 5.68]	1.33 [0.916, 3.02]	0.022 [0.015, 0.049]
Male	6–8	159	7.85 [5.34, 17.1]	4.01 [2.77, 9.59]	0.066 [0.045, 0.149]
	9–13	190	5.81 [4.04, 13.1]	2.98 [2.05, 6.79]	0.049 [0.034, 0.110]
	14–18	258	5.64 [3.68, 16.0]	2.91 [1.89, 8.35]	0.048 [0.032, 0.138]
	19–30	221	4.13 [2.83, 9.60]	2.12 [1.44, 4.98]	0.035 [0.024, 0.083]
	31–50	226	4.14 [2.84, 9.91]	2.13 [1.44, 5.22]	0.035 [0.024, 0.086]
	51–70	180	3.40 [2.24, 8.96]	1.74 [1.15, 5.03]	0.029 [0.019, 0.079]
	>70	155	2.69 [1.82, 6.16]	1.38 [0.931, 3.26]	0.023 [0.016, 0.054]

[a] No gender distinction was made for the 1–5 year age groups.

The increase in consumption by children and youths, however, is offset by their rapid increase in body weight.

Mean single day captan intakes per kg body weight resulting from consumption of raw apple with peel for young children ranged from 8.70 μg/kg to 9.48 μg/kg for individuals aged one year and three years of age, respectively, if no apple preparation occurred prior to consumption. Compared to no post-harvest preparation, a reduction in estimated intakes (approximately one-half lower) was observed if calculated using residues from rinsed apples and approximately two orders of magnitude lower for rinsed and peeled apples (Table 2). Mean intake estimates among females were found to be highest for those aged 6–8 years when no post-harvest preparation was considered and lowest from the greater than 70 year age group (Table 2). Similarly, males in these age groups (6–8 years and >70 years) had the largest (7.85 μg/kg) and smallest single day intakes (2.69 μg/kg), respectively, when residues from apples with no post-harvest preparation were used in the estimate determination.

The mean intake estimates were well below the World Health Organization (WHO) acceptable daily intake (ADI) value of 100 μg/kg (WHO, 2007); the model distribution of intake estimates developed indicates that a small fraction of raw apple eaters consuming apples that receive no post-harvest preparation (0.125–3.50%) or rinsing alone (0.043–0.959%) could exceed the ADI (Fig. 3). However, based on the simulations that were performed, individuals that consumed rinsed and peeled raw apples would have exceeded the ADI for captan zero percent of the time.

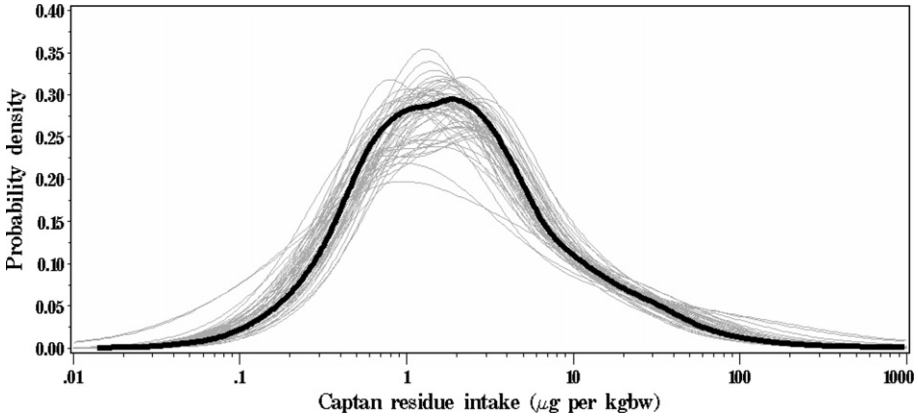

Fig. 3. Fifty uncertainty replicates of simulated single-day captan residue intake per kilogram body weight distribution from consumption of apples with no preparation (worst case scenario), males 6–8 years old. Heavy black line corresponds to simulation calculated at point estimates for captan residue concentrations.

4. Conclusions

Post-harvest preparation does result in a reduction in captan residues on apples, with rinsing alone. Rinsing, followed by peeling, resulted in nearly complete captan removal from apples (98% reduction) relative to no preparation. Captan intakes were reduced by approximately two orders of magnitude when residues from rinsed and peeled apples were used rather than those with no preparation.

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