

Appendix A

The metabolic fate of nectar nicotine in worker honey bees

Esther E. du Rand^{a,b}, Christian W. W. Pirk^b, Susan W. Nicolson^b, Zeno Apostolides^a

^aDepartment of Biochemistry, University of Pretoria, Private Bag x20, Hatfield, 0028, South Africa

^bDepartment of Zoology and Entomology, University of Pretoria, Private Bag x20, Hatfield, 0028, South Africa

Supplementary Table A.1 The distribution of nicotine and its metabolites in the honey crop, rectum, hindgut, midgut and haemolymph, during the 24 h after nicotine treatment.

Supplementary Fig A.1 PVC hoarding cage containing newly emerged worker bees.

Supplementary Table A.1 The distribution of nicotine and its metabolites in the honey crop, midgut, hindgut, rectum and haemolymph during the 24 h after nicotine treatment.

Nicotine and nicotine metabolites were present in all tissue samples from the digestive tract. The honey crop and rectum contained the highest levels of nicotine and nicotine metabolites at all time points. Data represent the mean of six experiments \pm SE and are expressed as ppb detected (n=6; averaged values of 10 bees were used for each colony) and as percentage of the total nicotine and nicotine metabolites detected (corrected for total dose of nicotine consumed).

HONEY CROP					
Compound		0 h	2 h	6 h	24 h
Nicotine	ppb	2797.56 \pm 739.09	1381.20 \pm 126.39	523.16 \pm 139.88	16.27 \pm 7.60
	%	78.14	42.60	32.54	1.91
Norcotinine	ppb	-	-	-	-
	%	-	-	-	-
Cotinine N-oxide	ppb	-	-	-	-
	%	-	-	-	-
3'-Hydroxy-cotinine	ppb	-	-	-	-
	%	-	-	-	-
4-Hydroxy-4-(3-pyridyl) butanoic acid	ppb	-	-	-	-
	%	-	-	-	-
Cotinine	ppb	-	-	-	-
	%	-	-	-	-
Nicotine N-oxide	ppb	-	-	-	-
	%	-	-	-	-
MIDGUT					
Nicotine	ppb	10.64 \pm 2.71	9.16 \pm 4.93	4.21 \pm 0.52	-
	%	0.30	0.28	0.26	-
Norcotinine	ppb	0.30 \pm 0.06	0.17 \pm 0.07	0.08 \pm 0.02	0.03 \pm 0.01
	%	0.01	0.01	0.005	0.00
Cotinine N-oxide	ppb	4.51 \pm 1.50	3.34 \pm 0.51	2.67 \pm 0.23	2.46 \pm 0.07
	%	0.13	0.10	0.17	0.29
3'-Hydroxy-cotinine	ppb	6.66 \pm 1.98	4.17 \pm 0.77	2.89 \pm 0.29	-
	%	0.19	0.13	0.18	-
4-Hydroxy-4-(3-pyridyl) butanoic acid	ppb	17.91 \pm 11.84	9.17 \pm 2.62	6.74 \pm 0.83	4.49 \pm 0.50
	%	0.50	0.28	0.42	0.53
Cotinine	ppb	12.46 \pm 4.47	9.27 \pm 2.91	4.44 \pm 0.53	3.69 \pm 0.36
	%	0.35	0.29	0.28	0.43
Nicotine N-oxide	ppb	2.63 \pm 0.25	2.95 \pm 0.76	-	-
	%	0.07	0.09	-	-
HINDGUT					
Nicotine	ppb	3.86 \pm 0.38	6.67 \pm 2.48	3.42 \pm 0.27	-
	%	0.11	0.21	0.21	-
Norcotinine	ppb	0.03 \pm 0.01	0.04 \pm 0.02	-	0.02 \pm 0.004
	%	0.07	0.001	-	0.002
Cotinine N-oxide	ppb	2.55 \pm 0.08	2.79 \pm 0.27	2.79 \pm 0.27	-
	%	0.07	0.09	0.17	-
3'-Hydroxy-cotinine	ppb	-	-	-	-
	%	-	-	-	-

HINDGUT (continued)					
Compound		0 h	2 h	6 h	24 h
4-Hydroxy-4-(3-pyridyl) butanoic acid	ppb	7.73 ± 1.48	7.56 ± 1.56	8.71 ± 2.99	3.78 ± 0.86
	%	0.22	0.23	0.54	0.44
Cotinine	ppb	3.72 ± 0.76	3.67 ± 0.93	3.15 ± 0.29	3.03 ± 0.02
	%	0.10	0.11	0.20	0.36
Nicotine N-oxide	ppb	-	-	-	-
	%	-	-	-	-
RECTUM					
Nicotine	ppb	7.60 ± 4.55	7.19 ± 0.86	5.99 ± 0.50	10.26 ± 2.13
	%	0.21	0.42	0.50	1.21
Norcotinine	ppb	0.83 ± 0.20	0.59 ± 0.15	0.46 ± 0.07	0.86 ± 0.27
	%	0.02	0.03	0.04	0.10
Cotinine N-oxide	ppb	46.95 ± 8.39	34.60 ± 4.37	31.32 ± 3.81	53.99 ± 13.88
	%	1.31	2.02	2.61	6.36
3'-Hydroxy-cotinine	ppb	33.36 ± 6.89	20.26 ± 3.14	22.44 ± 4.49	40.22 ± 11.76
	%	0.93	1.18	1.87	4.73
4-Hydroxy-4-(3-pyridyl) butanoic acid	ppb	488.54 ± 27.49	436.85 ± 37.00	456.17 ± 26.00	569.25 ± 35.85
	%	13.65	25.48	38.07	67.00
Cotinine	ppb	114.08 ± 23.52	93.48 ± 18.	85.34 ± 14.29	143.90 ± 27.38
	%	3.19	5.45	7.12	16.94
Nicotine N-oxide	ppb	12.10 ± 2.12	10.97 ± 0.86	12.43 ± 1.98	13.80 ± 1.66
	%	0.34	0.64	1.04	1.62
HAEMOLYMPH					
Nicotine	ppb	11.18 ± 2.55	8.87 ± 2.73	4.77 ± 0.39	3.02 ± 0.25
	%*	3.12	3.50	2.96	2.73
Norcotinine	ppb	-	-	-	-
	%*	-	-	-	-
Cotinine N-oxide	ppb	-	-	-	-
	%*	-	-	-	-
3'-Hydroxy-cotinine	ppb	-	-	-	-
	%*	-	-	-	-
4-Hydroxy-4-(3-pyridyl) butanoic acid	ppb	-	-	-	-
	%*	-	-	-	-
Cotinine	ppb	-	-	-	-
	%*	-	-	-	-
Nicotine N-oxide	ppb	-	-	-	-
	%*	-	-	-	-

- Not detected

* Value corrected for total haemolymph



Figure A.1 PVC hoarding cage containing newly emerged worker bees (Photo EE du Rand)