

**Analysis of Metals and Polychlorinated Biphenyl (PCB) Residues in
Honeybees, Honey and Pollen Samples Collected from the
Paducah Gaseous Diffusion Plant and Other Areas**

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INTRODUCTION

During a 24-month period, the Kentucky Agreement in Principle (AIP) and Division of Waste Management utilized honeybees (*Apis mellifera L.*) as biological monitors of contamination at the Paducah Gaseous Diffusion Plant (PGDP). Honeybees are proven biomonitoring tools and have been recommended by U.S. EPA and the National Research Council (Maybriar, 1998). Five sites were established by AIP (BBC, Curtis, Ballard, GY site, TS site), from which bees, honey and pollen samples were collected. No specific locations were given as to the sampling sites. Therefore, concerning distinctions between PGDP property and other areas, no clear comparisons could be made. In this respect, sample analysis constituted a “blind” study. Such samples were received by our lab for analysis of three Aroclors (*i.e.* 1248, 1254, and 1260) and 9 metals (*i.e.* Ag, Be, Cd, Cr, Cu, Fe, Ni, Pb and Zn). Bees collected in November 11, 2000 included a total of 12 bees from BBC, 10 from Ballard, 21 from GY, and 10 from TS. No bee samples were received for the Curtis site. Four honey samples collected August 25 – October 11, 2000 included sites BBC, Ballard, GY, and TS. No honey was received for the Curtis site. A total of 8 pollen samples were collected. Stations BBC-01, Curtis, GY-01, and TS-01 were collected August 17-25, 2000, whereas sites BBC-02, Ballard, GY-02, and TS-02 were collected September 27 – October 11, 2000.

METHODS

Digestions for Metal Assays

A. Honeybees

Honeybees selected for analysis were prepared according to modified procedures described by Hogstrand *et al.* (1996), and U.S. EPA (1997). All chemicals used were ACS grade or better and all acids were TraceMetal grade. Whole-body samples were weighed and placed in 50-mL Hot-Block® digestion tubes. The samples were digested with 2.0 mL TraceMetal grade HNO₃, allowed to digest for 24 h and then heated to 95° C for 10 min. The samples were allowed to cool to room temperature and 1.0 mL of 30% H₂O₂ was added to each sample followed by heat-instilling. The samples were then heated until dry and reconstituted to a final volume of 10.0 mL with 0.5 % HNO₃. The samples were then analyzed by AAS.

B. Honey

Honey was digested according to modified procedures described by Jones (1987). A 10 g sample of honey was weighed out and 10-20 mL 0.5 % HNO₃ were added and mixed well. The samples were then taken to a final volume of 100.0 mL with 0.5 % HNO₃. The samples were then analyzed by AAS.

C. Pollen

Pollen samples followed the same digestion procedure as described above for bees. A 1.0-2.0 g sample of pollen was weighed out and digested. In order to remove suspended particulates, the samples were filtered through a Gelman Sciences Type A/E glass fiber filter. The filters were rinsed with 0.5 % HNO₃ prior to use and filtrates were taken to a final volume of 10.0 mL with 0.5 % HNO₃. The samples were then analyzed

by AAS.

Metal Determinations

Nine metals, including silver (Ag), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), and zinc (Zn), were analyzed by atomic absorption spectrophotometry (AAS), using either graphite furnace or flame atomization techniques. The instruments were a Varian AAS (Model Spectra AA-20), equipped with a GTA-96 graphite furnace and a Perkin-Elmer (Model 603) for flame analysis. All gases used were ultra pure carrier grade. Calibration curves were based on five standards. The instrument was programmed to take three readings per sample and average the absorbance. Instrument blanks (0.5 % HNO₃) and check standards were processed with all samples. Sample concentrations were then corrected for deviations from the standards and sample weights were factored into the calculation of final values.

PCB Tissue Extraction

A. Honeybees

PCBs in whole-body bee samples were extracted and analyzed using standard U.S. EPA methods (Watts, 1980; U.S. EPA, 1997; Erickson, 1997; Morse *et al.*, 1987). The weighed samples were ground with 5.0 g anhydrous sodium sulfate and the powder extracted with hexane in a Soxhlet apparatus for 5-h. The extracts were concentrated to near dryness in a Roto-evaporator (Buchi Model RE121). Reconstituted samples (2.0 mL in hexane) were then cleaned of interferences as described below and then analyzed by gas chromatography. Lipid and pesticide clean-up was performed by eluting the 2.0 mL

sample through a micro-column of 2.0 g activated 100-200 mesh Florisil® (100 °C/24 h) with 10 mL 6% ethyl ether in hexane and evaporated to 2.0 mL (Erickson, 1997; U.S. EPA, 1997, SW-846 Method 3620B, Florisil cleanup). Elemental sulfur was then removed by shaking with 2-propanol (2 mL) and tetrabutylammonium sulfite (2 mL), adding ultra-pure water (8 mL), and reshaking. The organic extract was removed and mixed with 2.0-mL concentrated sulfuric acid (Jensen *et al.*, 1977; U.S. EPA, 1997, SW-846 Method 3660B, sulfur cleanup). A 4 µL sub-sample was then analyzed by gas chromatography.

B. Honey

Honey was extracted according to modified procedures described by Jan and Cerne (1993), Fernandez and Lorenzen (1991), and Morse *et al.* (1987). The honey samples were weighed (15.0 g) and were then mixed with 50 mL nanopure water. The mixture was liquid-liquid extracted with hexane three times (50, 25, and 25 mL). The extracts were concentrated to near dryness and reconstituted to 2.0 mL with hexane. The samples then were cleaned of interferences as described above and analyzed by gas chromatography.

C. Pollen

Pollen samples followed the same extraction procedure as described above for bees. A 5.0-10.0 g sample of pollen was weighed out and extracted.

PCB Determinations

Samples were analyzed for Aroclors 1248, 1254, and 1260 according to SW-846 Method 8082 (Polychlorinated biphenyls by gas chromatography, U.S. EPA, 1997). Analyses were performed using a Hewlett-Packard (HP) Model 5890A gas

chromatograph equipped with an electron capture detector and an HP Model 7673A Automatic Sampler. Samples were analyzed using a 60m X 0.53mm ID SPB-5 (0.5 μ m film) fused silica megabore column (Supelco, Inc.) with ultra-high purity helium and nitrogen as carrier and makeup gases, respectively. The temperature program was set at 160 °C (6 min)-10 °C/min-235 °C (0 min)-0.9 °C/min-260 °C (10 min); Injector temperature, 280 °C; Detector temperature, 300 °C. PCB peak heights were quantified using an HP Model 3396A integrator. Aroclor concentrations were calculated from heights of 6 to 9 peaks for Aroclors 1248 and 1260 and 4-6 peaks for Aroclor 1254. Five external standards were used for calibration curves and for every tenth sample either a solvent blank or a standard was analyzed. Statistical quantitation of peak heights was determined by multiple-peak linear regression analysis with Lotus-123® software. The Lotus program regresses data from PCB standards to the sample being analyzed. Each peak selected for each Aroclor class was statistically analyzed (e.g., standard deviation; standard error; relative deviation). Chromatographs and bench records for all PCB assays will be maintained as given below under quality assurance.

Quality Assurance

Permanent bench records were kept of all assays and annotated as required under Good Laboratory Practices (*Federal Register*, 40 CFR, Part 160, August 17, 1989). All printouts and graphic recordings were filed and are open for inspection. These bench records will be archived within two years after the close of the project but retrievable upon request. Chain of Custody was maintained for the samples collected.

RESULTS AND CONCLUSIONS

Metals

The results for metal assays are presented in Table 1 for honeybees; Table 2 for pollen; and Table 3 for honey. Overall, metal concentrations most often were higher in pollen samples, next highest in honeybees, and lower in honey. It should be noted, however, that there was considerable variation from station to station, as well as among different metals. As compared with honeybee samples, assays of pollen revealed higher concentrations across stations for Ag, Cd, Cr, Cu, Ni, Pb and Zn. This may indicate that pollen is a better “biomarker system” for metal pollution. As compared with honey samples, bees generally contained higher concentrations of Be, Cr, Cu, Ni, and Zn. It is noteworthy that silver (Ag) usually was detected at significant concentrations in pollen and honeybees but not in honey. However, two toxic nutritionally non-essential metals, Cd and Pb, occurred at part-per-million concentrations in honey from several sampling stations.

In a study by Vinas *et al.* (1997) results of metal analysis for honey considered “free” of pollution, the values ($\mu\text{g}/\text{Kg}$) for Cu, Cd, Pb, and Zn ranged from 90 to 1032; 1.2 to 5.4; 20 to 270; and 1280 to 3200, respectively. By comparison, all honey samples analyzed in this study were contaminated with Cu and Cd and most contained elevated concentrations of Pb (Table 3). Leita *et al.* (1996) analyzed honey and pollen for Cd, Pb, and Zn in areas where the bee hives were located in or near areas of metal pollution. The values for honey were much higher than those reported by Vinas *et al.* (1997). Mean values ($\mu\text{g}/\text{Kg}$) for Cd, Pb, and Zn in honey were 1900, 1800, and 29400, respectively. Given in the same order and units, the mean values for pollen were 2100, 3900, and 99000. Rowarth (1990) also reported Pb in honey at 2600 $\mu\text{g}/\text{Kg}$ where bees

and plants were subject to roadside Pb contamination. These values for metal contamination of honey and pollen from polluted areas mostly fall within the range or well below values given in Tables 2 and 3. This provides support for the premise that most pollen and honey samples analyzed in this report were contaminated with metals. The studies by Leita *et al.* (1996) further support the prospect that pollen is a good indicator of metal pollution.

PCBs

Results for PCB levels in whole-body honeybees, pollen, and honey are presented in Tables 4, 5, and 6, respectively. Detection of PCBs were most frequent for pollen, infrequent in honeybees and undetected in honey. PCB concentrations detected in pollen ranged from 90 µg/Kg (*i.e.* ppb) at station TS to 33 at BBC-01. Morse *et al.* (1987) analyzed pollen from impacted areas for PCBs and reported values of 100 to 300 µg/Kg (ppb). The detectable concentrations reported in Table 5 for Aroclor 1248 approach this range for several sites. The only bee found to contain detectable PCB (*i.e.* Aroclor 1248) was from station GY.

There was some variation among the different sample matrices as to the sensitivity of PCB assays. Detection limits generally were higher for whole-body bees and lowest for honey. Increased sample size for whole-body bees is recommended to lower the detection limits. Recoveries for PCB-spiked honey ranged from 77% to 86% (Table 7). A literature search was undertaken for this study and the references collected are given in the Addendum.

Table 1. Metal concentrations in whole-body honeybee samples.

Station	Date	Sample ^a	Wt. of Sample (g) ^b	Metal Conc. (µg/Kg)							(µg/g)	
				Ag	Be	Cd	Cr	Cu	Ni	Pb	Zn	Fe
BLANK ^c	1/2/01	BEEBlk	---	<0.25	<1.00	<1.00	1.13	<1.00	<6.00	<1.0	N.A.	<100.0
BBC	10/11/00	BEE1	0.239	<10.46	64.52	52.30	42.34	4686.2	422.18	399.2	23.01	<4184.1
BBC	10/11/00	BEE2	0.240	<10.42	55.75	77.50	<41.67	4666.7	677.50	421.3	34.58	<4166.7
Ballard	10/11/00	BEE1	0.214	<11.68	72.06	156.07	<46.73	7289.7	690.19	452.3	25.23	<4672.9
Ballard	10/11/00	BEE2	0.214	<11.68	71.59	233.18	<46.73	5233.6	425.70	440.7	32.24	<4672.9
GY	10/11/00	BEE1	0.169	<14.79	72.90	<59.17	<59.17	6627.2	411.83	517.2	35.50	<5917.2
GY	10/11/00	BEE2	0.227	<11.01	62.69	<44.05	<44.05	<4405.3	696.92	357.7	24.67	<4405.3
TS	10/11/00	BEE1	0.173	<14.45	66.82	134.68	<57.80	<5780.4	614.45	368.2	33.53	<5780.4
TS	10/11/00	BEE2	0.248	<10.08	62.02	80.65	46.33	4516.1	314.92	382.7	21.77	<1032.3

^a Samples designated BEE1 and BEE2 are duplicates from the same site.

^b Due to small sample size, only 2 bees per site were digested.

^c The BLANK sample was 2.0 mL of HNO₃ digested as a regular sample.

Table 2. Metal concentrations in pollen samples.

Station	Date	Sample ^a	Wt. of Sample (g)	Metal Conc. ($\mu\text{g}/\text{Kg}$)							($\mu\text{g}/\text{g}$)	
				Ag	Be	Cd	Cr	Cu	Ni	Pb	Zn	Fe
BLANK ^b	1/3/01	POLBlk	---	<0.25	<1.00	<1.00	1.13	2.44	<6.00	<1.00	N.A.	<100.0
BBC-01	8/25/00	POL1	2.039	4.87	14.61	<9.81	22.55	4963.22	790.78	352.82	22.71	<490.4
BBC-01	8/25/00	POL2	1.977	5.39	10.18	118.77	28.13	5341.43	1239.15	263.58	35.20	<505.8
BBC-02	10/11/00	POL1	1.686	12.41	9.16	273.75	59.42	6002.37	1091.64	571.77	35.53	<593.1
BBC-02	10/11/00	POL2	1.634	9.80	23.71	461.69	48.84	4449.20	858.94	474.24	32.25	<612.0
CURTIS	8/25/00	POL1	2.160	29.25	17.11	280.83	15.75	4175.93	921.48	781.20	19.49	<463.0
CURTIS	8/25/00	POL2	1.589	6.43	26.51	93.71	17.61	4298.30	840.09	250.47	26.62	<629.3
Ballard	10/11/00	POL1	1.981	13.06	27.29	380.59	89.62	5552.75	1046.34	384.50	39.93	<504.8
Ballard	10/11/00	POL2	1.642	13.53	47.31	194.34	89.48	5627.28	896.77	328.68	38.86	<609.0
GY-01	8/25/00	POL1	1.583	3.30	7.60	139.86	43.04	6253.95	647.44	375.30	73.28	<631.7
GY-02	10/11/00	POL1	1.776	15.68	14.58	356.05	63.94	5945.95	1400.17	787.95	31.64	<563.1
TS	8/25/00	POL1	2.045	7.15	9.72	159.76	49.09	7740.83	396.09	375.65	41.22	<489.0
TS	10/11/00	POL1	1.609	6.99	21.63	443.29	99.04	5879.43	758.42	336.11	53.88	<621.5
TS	10/11/00	POL2	1.543	6.82	21.96	406.93	125.15	5988.33	553.73	423.85	76.34	<648.1

^a Samples designated POL1 and POL2 are duplicates from the same site.

^b The BLANK sample was 2.0 mL of HNO₃ digested as a regular sample.

Table 3. Metal concentrations in honey samples.

Station	Date	Sample ^a	Wt. of Sample (g)	Metal Conc. (µg/Kg)								(µg/g)
				Ag	Be	Cd	Cr	Cu	Ni	Pb	Zn	Fe
MIDWAY ^b	11/29/00	HON1	10.041	3.71	<9.96	1083.6	<9.96	1273.58	<99.59	1521.1	833.3	<995.9
MIDWAY	11/29/00	HON2	10.109	3.21	<9.89	1026.8	<9.89	1294.98	139.53	1534.8	916.6	<989.2
BBC	10/11/00	HON1	10.086	2.63	<9.91	997.4	<9.91	128.40	<99.15	1153.7	496.2	<991.5
BBC	10/11/00	HON2	10.036	<2.49	<9.96	1585.4	<9.96	117.05	<99.64	<996.4	1273.4	<996.4
Ballard	10/11/00	HON1	10.004	4.71	<10.00	704.2	<10.00	201.72	<99.96	<999.6	1097.8	<999.6
Ballard	10/11/00	HON2	10.064	4.53	<9.94	1356.5	<9.94	205.09	135.23	993.6	574.3	<993.6
GY	10/11/00	HON1	10.037	5.42	<9.96	829.9	<9.96	122.75	<99.63	<996.3	668.1	<996.3
GY	10/11/00	HON2	10.069	6.14	<9.93	1280.5	<9.93	167.94	<99.31	<993.2	809.1	<993.2
TS	10/11/00	HON1	10.098	<2.48	<9.90	932.9	<9.90	114.68	<99.03	<990.3	369.5	<990.3
TS	10/11/00	HON2	10.170	<2.46	<9.83	1270.4	<9.83	80.53	<98.33	<983.3	309.5	<983.3

^a Samples designated HON1 and HON2 are duplicates from the same site.

^b The MIDWAY samples were provided by Jon Maybriar from Midway, KY as controls.

Table 4. PCB concentrations in whole-body honeybee samples.

Station	Date	Sample ^a	Wt. of Sample (g)	No. of Bees Anal.	Aroclor Conc. (µg/g)		
					1248	1254	1260
CONT ^b	1/10/01	C1	5.00		<0.016	<0.016	<0.016
BBC	10/11/00	BEE1	0.955	8	<0.084	<0.084	<0.084
Ballard	10/11/00	BEE1	0.548	6	<0.146	<0.146	<0.146
GY	10/11/00	BEE1	0.923	9	<0.087	<0.087	<0.087
GY	10/11/00	BEE2	0.765	8	0.294	<0.105	<0.105
TS	10/11/00	BEE1	0.535	6	<0.150	<0.150	<0.150

^a Samples designated BEE1 and BEE2 are duplicates from the same site.

^b The CONT sample was 5.0 g of sodium sulfate ground and extracted as a regular sample.

Table 5. PCB concentrations in pollen samples.

Station	Date	Sample ^a	Wt. of Sample (g)	Aroclor Conc. (µg/g)		
				1248	1254	1260
CONT ^b	1/9/01	C1	5.000	<0.040	<0.040	<0.040
CONT	1/9/01	C2	5.000	<0.040	<0.040	<0.040
BBC-01	8/25/00	POL1	10.012	0.033	<0.020	<0.020
BBC-02	10/11/00	POL1	10.007	0.048	<0.020	<0.020
BBC-02	10/11/00	POL2	10.026	0.056	<0.020	<0.020
CURTIS	8/25/00	POL1	10.002	0.038	<0.020	<0.020
Ballard	10/11/00	POL1	10.075	0.068	<0.020	<0.020
GY-01	8/25/00	POL1	10.053	<0.020	<0.020	<0.020
GY-02	10/11/00	POL1	10.009	0.069	<0.020	<0.020
GY-02	10/11/00	POL2	10.072	0.050	<0.020	<0.020
TS	8/25/00	POL1	10.042	0.046	<0.020	<0.020
TS	10/11/00	POL1	4.959	0.079	<0.040	<0.040
TS	10/11/00	POL2	4.889	0.091	<0.041	<0.041

^a Samples designated POL1 and POL2 are duplicates from the same site.

^b The CONT sample was 5.0 g of sodium sulfate ground and extracted as a regular sample.

Table 6. PCB concentrations of honey samples.

Station	Date	Sample ^a	Wt. of Sample (g)	Aroclor Conc. (µg/g)		
				1248	1254	1260
CONT ^b	1/23/01	CHON	---	<0.002	<0.002	<0.002
BBC	10/11/00	HON1	15.030	<0.005	<0.005	<0.005
Ballard	10/11/00	HON1	15.010	<0.005	<0.005	<0.005
GY	10/11/00	HON1	14.970	<0.005	<0.005	<0.005
GY	10/11/00	HON2	15.070	<0.005	<0.005	<0.005
TS	10/11/00	HON1	14.990	<0.005	<0.005	<0.005

^a Samples designated HON1 and HON2 are duplicates from the same site.

^b The CONT sample was 50 mL of nanopure water extracted as a regular sample.

Table 7. PCB concentrations in spiked recoveries^a for honey samples.

Site	Date	Name	Aroclor	Aroclor Conc. (μg)		
				Expected Conc.	Measured Conc.	Percent Recovery
MIDWAY	11/29/00	SPK1	---	0.000	<0.080	---
MIDWAY	11/29/00	SPK2	1248	1.000	0.774	77.4
MIDWAY	11/29/00	SPK3	1254	1.000	0.862	86.2
MIDWAY	11/29/00	SPK4	1260	1.000	0.774	77.4

^a A 15.0 g of control honey was spiked with the respective Aroclor, extracted and analyzed.

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