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Supplemental Material

Levels and Determinants of DDT and DDE Exposure in the VHEMBE Cohort

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1. Quantification of o,p' and p,p'-DDT and DDE Isomers

To quantify *o,p'* and *p,p'* isomers of DDT and DDE, 1 mL aliquots of serum were spiked with isotopically labeled internal standards and vortex mixed to homogenize. Five percent Na_2SO_4 (in water): propanol (85:15 v/v) solution was added to denature serum proteins. Denatured serum samples were extracted using C18 solid phase extraction (SPE) cartridges with a water/propanol wash and elution with hexane/ethyl acetate (1:1 v/v). The eluates were dried over anhydrous Na_2SO_4 and concentrated to 2 mL. Concentrates were passed through a Florisil® SPE cartridge to remove residual biogenic material. SPE cartridges were eluated with hexane:ethyl acetate (1:1 v/v) and concentrated to dryness. Residues were reconstituted in 100 µL isooctane and placed in GC vials for analysis. 2 µL of the reconstituted residues were injected into a gas chromatography and separated using a DB-5MS (5%-Phenyl)-methylpolysiloxane, 0.25 µ film thickness, 0.25 mm ID x 30 m) capillary column. The target analytes were analyzed using tandem mass spectrometry. Two precursor-product ion transitions (one for quantification, one for confirmation) were monitored for each target analyte and one for each internal standard. In order to be positively identified as the target analyte, the following criteria had to be met: (1) both quantification and confirmation ions must be present; (2) the ratio of the quantification to confirmation ion areas had to be within ± 20% of the theoretical Cl isotope ratio; (3) co-elution of native analyte with its respective 13C-labelled internal standard or elution 4-6 seconds after its respective 2H-labeled internal standard. The concentration of each analyte was calculated from its individual matrix-matched linear isotope dilution calibration.

2. Quality Control Results for VHEMBE Serum Analysis

Laboratory quality control procedures included matrix-matched calibrants and serum and reagent blanks prepared and analyzed concurrently with unknown samples in each analytic run. Across the 9 calibration standards (n = 64), the average recovery ranged from 97 to 103% for all DDT/E isomers and the coefficient of variation of the standard measurements ranged from 7.7% to 13.7% for all DDT/E isomers. The average (SD) laboratory blank concentration (n = 18) was 0.01 (0.02) ng/mL for *p,p'*-DDT, 0.01 (0.02) ng/mL for *o,p'*-DDT, 0.20 (0.18) ng/mL for *p,p'*-DDE, and 0.02 (0.02) ng/mL for *o,p'*-DDE. In addition to laboratory-prepared quality control samples, three field blanks, sealed blanks, and spiked samples were prepared by the field staff in South Africa to test for contamination and accuracy of DDT/E serum concentration measurements. Pesticide-grade isopropanol (Fisher Chemical OptimaTM LC/MS) was used as the field and sealed blanks and National Institute of Standards and Technology's Standard Reference Material 1958 (Organic Contaminants in Fortified Human Serum) was used as the blood spikes. For field blanks, all steps of the blood collection procedure were mimicked (pulled into vacutainer, spun in centrifuge, aliquoted into cryovials, etc.) to determine if any contamination occurred during either the blood collection or analysis. Sealed blanks were directly aliquoted into cryovials to determine if any contamination occurred during the analysis. All field and sealed blanks (n = 6) were below the LOD, indicating little contamination during sample collection, processing, and analysis (results not shown).

Field spikes indicated that our *p,p*'-DDE concentrations were approximately three times lower than expected. New *p,p*'-DDE standards were quantified, which confirmed that our previously used standards were scaled incorrectly. The ratio of the average percent recovery across nine

standards for *p,p*'-DDE concentrations from the new and old standards was used to scale our *p,p*'-DDE measurements (scaling factor = 3.30). To further test our scaling approach, we compared 11 *p,p*' and *o,p*'-DDE concentrations using our old calibration curve after applying the scaling factor to those quantified using the new calibration curve and found strong agreement between these replicate measurements. For example, the average relative percent difference (RPD) between the measurements was 4.7% for *p,p*'-DDE (RPD range: 0.8-11.1%) and 5.4% for *o,p*'-DDE (RPD range: 0.6-10.9%).

3. Additional Results

Table S1. Propensity score distributions for estimating $E(A|W)$ for assessing positivity violations.

Intervention	Minimum	25th %ile	Median	Mean	75th %ile	Maximum
Water piped into home	0.07	0.33	0.64	0.55	0.77	0.94
Wet mopping home ≥ 7 times per week	0.32	0.46	0.53	0.52	0.6	0.63
Washing bed sheets ≥ 2 times per month	0.48	0.56	0.57	0.57	0.59	0.64
Not eating a high fat diet	0.56	0.73	0.75	0.75	0.77	0.84
Not consuming local animal products during pregnancy	0.17	0.63	0.73	0.69	0.78	0.85

Table S2. Algorithm weights used by SuperLearner to estimate $E(Y|A,W)$.

Outcome	Intervention	GLM	GAM	Bayes GLM	GLMNET	randomForest	RPART	NNET	POLYMARS	SVM
p,p'-DDT	Water piped into home	0	0	0.21	0.47	0.29	0	0	0.02	0
	Wet mopping home ≥ 7 times per week	0	0	0.48	0	0.48	0	0.03	0.01	0
	Washing bed sheets ≥ 2 times per month	0	0.07	0.08	0.26	0.21	0	0	0.19	0.18
	Not eating a high fat diet	0	0	0.48	0	0.46	0.03	0.02	0	0
	Not consuming local animal products during pregnancy	0	0	0.24	0.16	0.34	0.05	0.01	0.2	0
p,p'-DDE	Water piped into home	0	0	0.51	0	0.49	0	0	0	0
	Wet mopping home ≥ 7 times per week	0	0	0.44	0.22	0.16	0.15	0	0.04	0
	Washing bed sheets ≥ 2 times per month	0	0	0.51	0	0.46	0	0.03	0	0
	Not eating a high fat diet	0	0	0.48	0.13	0.24	0.12	0.04	0	0
	Not consuming local animal products during pregnancy	0	0	0.77	0	0.04	0	0.06	0.14	0

Table S3. Algorithm weights used by SuperLearner to estimate $E(A|W)$.

Intervention	GLM	stepGLM	bayesGLM	GLMNET	randomForest	RPART	POLYMARS
Water piped into home	0.4	0	0	0	0.6	0	0
Wet mopping home ≥ 7 times per week	0	0	0	0	0.09	0.07	0.85
Washing bed sheets ≥ 2 times per month	0.18	0.08	0	0	0	0	0.74
Not eating a high fat diet	0	0	0.43	0	0	0	0.57
Not consuming local animal products during pregnancy	0	0	0	0.42	0	0.02	0.56

Table S4. *p,p'*-DDT and *p,p'*-DDE concentrations (ng/g-lipid) by selected characteristics in VHEMBE participants, Limpopo, South Africa.

Exposure Characteristic	n	(%)[a]	*p,p'*-DDT Median	*p,p'*-DDT (IQR)[b]	*p,p'*-DDT p-value[c]	*p,p'*-DDE Median	*p,p'*-DDE (IQR)[b]	*p,p'*-DDE p-value[c]
Time living in home sprayed for malaria control					<0.01			<0.01
0 years	405	(53.9)	32.6	(14.1-93.5)		152.7	(67.1-392.0)	
> 0 to ≤ 2 years	224	(29.8)	162.4	(44.1-577.6)		649.2	(168.9-1827.5)	
> 2 years	90	(12.0)	233.9	(44.0-734.2)		722.7	(235.9-2354.9)	
Don't know	32	(4.3)	26.9	(9.7-84.7)		146.8	(63.2-516.4)	
Maternal education					0.18			0.40
< 12th Grade	412	(54.9)	56.9	(19.3-251.0)		231.6	(84.4-796.3)	
Completed grade 12	229	(30.5)	70.7	(22.1-329.6)		312.9	(105.3-1130.6)	
Further studies started	50	(6.7)	43.8	(12.8-119.8)		223.9	(94.0-590.7)	
Diploma or further degree	60	(8.0)	41.1	(16.8-176.4)		206.3	(84.3-870.5)	
Maternal age (years)					0.59			0.23
18-24	377	(50.2)	51.6	(18.3-257.6)		277.3	(91.8-993.3)	
25-30	172	(22.9)	75.0	(24.4-258.1)		281.2	(101.8-818.2)	
30-35	111	(14.8)	42.4	(16.6-146.9)		177.9	(82.7-443.5)	
> 35	91	(12.1)	82.6	(19.2-382.1)		293.8	(87.5-817.8)	
Maternal BMI post-delivery (kg/m^2)					0.76			0.18
≤ 24.8	244	(32.5)	45.2	(18.5-263.9)		239.4	(92.3-1256.0)	
> 24.8 to ≤ 29.1	244	(32.5)	65.1	(18.5-285.5)		265.2	(91.8-893.2)	
> 29.1	244	(32.5)	54.7	(20.3-221.1)		208.8	(87.1-758.1)	
Lost to follow-up	19	(2.5)	67.0	(37.4-139.3)		294.3	(193.8-386.0)	
Poverty[d]					0.05			0.45
Above food poverty line	310	(41.3)	43.0	(16.8-234.1)		215.2	(81.3-810.8)	
Below food poverty line	438	(58.3)	65.7	(21.4-279.7)		271.2	(94.0-972.3)	
Don't know	3	(0.4)	22.1	(17.0-117.0)		110.9	(89.1-262.1)	

Table S4 continued. *p,p'*-DDT and *p,p'*-DDE concentrations (ng/g-lipid) by selected characteristics in VHEMBE participants, Limpopo, South Africa.

Exposure Characteristic			*p,p'*-DDT			*p,p'*-DDE		
	n	(%)[a]	Median	(IQR)[b]	p-value[c]	Median	(IQR)[b]	p-value[c]
Parity					0.91			0.02
0	325	(43.3)	50.5	(18.1-280.1)		308.1	(97.1-1280.3)	
1	201	(26.8)	56.3	(20.5-189.3)		219.8	(82.3-610.3)	
≥ 2	225	(30.0)	58.8	(20.2-244.6)		197.3	(86.2-749.2)	
Breastfeeding history (months)					0.61			0.01
≤ 3	337	(44.9)	50.0	(18.0-272.0)		294.1	(97.0-1246.8)	
>3 to ≤ 25	203	(27.0)	61.8	(20.5-217.9)		235.2	(80.4-741.5)	
> 25	211	(28.1)	56.8	(20.7-246.8)		197.3	(87.1-599.5)	
Distance to body of water (meters)					<0.01			<0.01
≤ 1400	241	(32.1)	74.5	(22.4-314.1)		308.8	(99.5-1248.9)	
> 1400 to ≤ 2760	240	(32.0)	67.7	(21.8-372.1)		298.6	(119.2-1099.6)	
> 2760	240	(32.0)	41.8	(12.7-128.7)		179.5	(68.3-552.4)	
Lost to follow-up/ GPS error	30	(4.0)	53.0	(31.9-230.1)		218.4	(68.4-915.9)	
Pregnancy Home Building Type[e]					0.14			0.04
Mkhuku	10	(1.3)	44.1	(24.6-111.8)		107.0	(72.3-259.4)	
Rondavel	33	(4.4)	163.3	(30.4-710.3)		490.7	(195.4-2132.2)	
Western Structure	676	(90.0)	52.7	(18.4-257.2)		239.7	(92.1-856.8)	
Other	3	(0.4)	120.8	(92.8-149.9)		239.8	(190.2-425.6)	
Lost to follow-up	29	(3.9)	48.9	(30.9-162.5)		197.1	(67.1-938.1)	
Presence of rondavel on homestead					<0.01			0.01
No	577	(76.8)	48.7	(17.4-223.5)		231.2	(85.7-784.8)	
Yes	145	(19.3)	99.4	(30.4-440.7)		376.4	(125.7-1681.4)	
Lost to follow-up	29	(3.9)	48.9	(30.9-162.5)		197.1	(67.1-938.1)	

Table S4 continued. *p,p'*-DDT and *p,p'*-DDE concentrations (ng/g-lipid) by selected characteristics in VHEMBE participants, Limpopo, South Africa.

Exposure Characteristic			*p,p'*-DDT			*p,p'*-DDE		
	n	(%)[a]	Median	(IQR)[b]	p-value[c]	Median	(IQR)[b]	p-value[c]
Household owned livestock during pregnancy					<0.01			0.01
No	560	(74.6)	47.2	(17.6-221.1)		219.6	(80.8-759.5)	
Yes	190	(25.3)	80.0	(26.8-434.4)		347.7	(124.2-1573.1)	
Lost to follow-up	1	(0.1)	725.4	(725.4-725.4)		763.2	(763.2-763.2)	
Structure density- 250 m radius (#/hectare)					0.06			0.53
≤ 8.1	241	(32.1)	53.7	(21.2-305.9)		217.4	(87.2-1234.8)	
> 8.1 to ≤ 13.4	240	(32.0)	66.5	(20.1-267.5)		284.0	(96.0-760.6)	
> 13.4	240	(32.0)	49.1	(16.0-216.3)		243.2	(96.1-806.1)	
Lost to follow-up	30	(4.0)	53.0	(31.9-230.1)		218.4	(68.4-915.9)	
Structure density- 1000 m radius (#/hectare)					0.21			0.83
≤ 6.5	241	(32.1)	60.7	(21.0-220.6)		231.2	(98.1-816.3)	
> 6.5 to ≥ 9.4	240	(32.0)	52.7	(16.5-308.2)		190.3	(76.3-889.6)	
> 9.4	240	(32.0)	56.2	(19.1-244.8)		283.7	(111.4-896.9)	
Lost to follow-up	30	(4.0)	53.0	(31.9-230.1)		218.4	(68.4-915.9)	

[a] Percentages may not add to 100% due to rounding.
[b] IQR = inter-quartile range, NC = not calculated
[c] p-values from Kruskall-Wallis tests for all determinants except for maternal age and BMI, distance to river and lake/reservoir, structure density, and fat consumption (p-values from Spearman's correlation test)
[d] Food poverty line developed by Statistics South Africa (370 Rands or $25 monthly income per household member)
[e] The pregnancy home is the building the mother lived and slept during her pregnancy. Mhkuku are buildings typically made out of corrugated iron, rondavels are earth/thatch structures, and western buildings are typically concrete walls with corrugated/tile iron roofs

Table S5. Comparison of VHEMBE lipid-adjusted serum concentrations with previously reported blood concentrations in adults living in IRS communities and pregnant women living in the United States

				p,p'-DDT (ng/g-lipid)			*p,p'*-DDE (ng/g-lipid)		
Study[a]	**Collected**[b]	**Exposure classification**	**N**	**25th %ile**	**Median**	**75th %ile**	**25th %ile**	**Median**	**75th %ile**
VHEMBE	2012-2013	Home sprayed with DDT during pregnancy	23	161.8	736.9	1726.7	840.4	2129.0	3238.1
VHEMBE	2012-2013	Home not sprayed with DDT during pregnancy	720	18.6	50.0	236.9	87.2	230.9	803.3
VHEMBE	2012-2013	Home ever sprayed with DDT	254	55.5	225.5	733.4	238.3	803.6	2189.2
VHEMBE	2012-2013	Home never sprayed with DDT	478	14.8	33.5	106.5	68.7	155.8	439.4
VHEMBE	2012-2013	All	751	19.0	55.3	259.3	91.8	242.2	878.7
SOWB	2010-2011	Home probably sprayed with DDT	100	296.1	750.5	1565.6	566.7	2411.4	4396.1
SOWB	2010-2011	Home probably sprayed with pyrethroids	106	155.6	395.1	737.8	973.9	2164.9	3633.3
SOWB	2010-2011	Unsprayed village	175	30.7	84.1	243.0	187.2	493.0	1688.5
NSA-BRC	2008	High-risk malaria area	91	1279.0	2788.0	4525.0	1986.0	4092.0	7341.0
NSA-BRC	2008	Low-risk malaria area	47	7.0	27.0	165.0	54.0	184.0	908.0
NSA-BRC	2008	Non-malaria area	117	6.0	7.0	8.0	18.0	26.0	49.0
THES	2008	Home sprayed with DDT 2 months prior	19	NA[c]	NA[c]	NA[c]	1200.0	4700.0	23000.0
LMSQ[e]	2003-2005	Home sprayed with DDT within year	249	41149.5	71200.2	126495.7	103878.0	180655.1	298455.6
LMSQ[e]	2003-2005	Home not sprayed	48	6100.8	14261.2	34700.2	32252.5	62871.0	122518.0
NHANES	1999-2004	Non-IRS population	277[d]	< 5.1[f]	< 5.1[f]	8.0	78.2	131.0	291.0
CHAMACOS	1999-2000	Non-IRS population	426	6.9	12.5	35.6	568.0	1052.0	2668.0
CHDS	1959-1967	Non-IRS population	283	1000.0	1400.0	1900.0	3800.0	5200.0	6900.0
CPP	1959-1966	Non-IRS population	1393	780.0	1100.0	1730.0	2090.0	3000.0	4490.0

[a] Study key: VHEMBE = this study, SOWB = Study of Women and Babies (Whitworth et al. 2014), NSA-BRC = Norway-South Africa Bilateral Research Collaborative (Channa et al. 2012), THES = Total Homestead Environment Study (Van Dyk et al. 2010), LMSQ = Limpopo men's serum quality study (Aneck-Hahn et al. 2007), NHANES = National Health and Nutrition Examination Survey (Center for Disease Control 2000, 2002, 2004), CHAMACOS = Center for the Health Assessment of Mother and Children of Salinas study (Bradman et al. 2007) ,CHDS = Child Health and Development Study (Bhatia et al. 2004), CPP = Collaborative Perinatal Project (Jusko et al. 2012)

[b] Year(s) blood collected.

[c] NA = not available. Majority of samples below limit of detection (LOD) and lipid-adjusted LOD not presented.

[d] Number of *p,p'*-DDT measurements = 263.

[e] Distributions estimated from the reported mean and standard deviation (see text).

[f] LOD for *p,p'*-DDT was approximately 5.1 ng/g-lipid across the three sampling periods (1999-2000, 2001-2002, and 2003-2004).

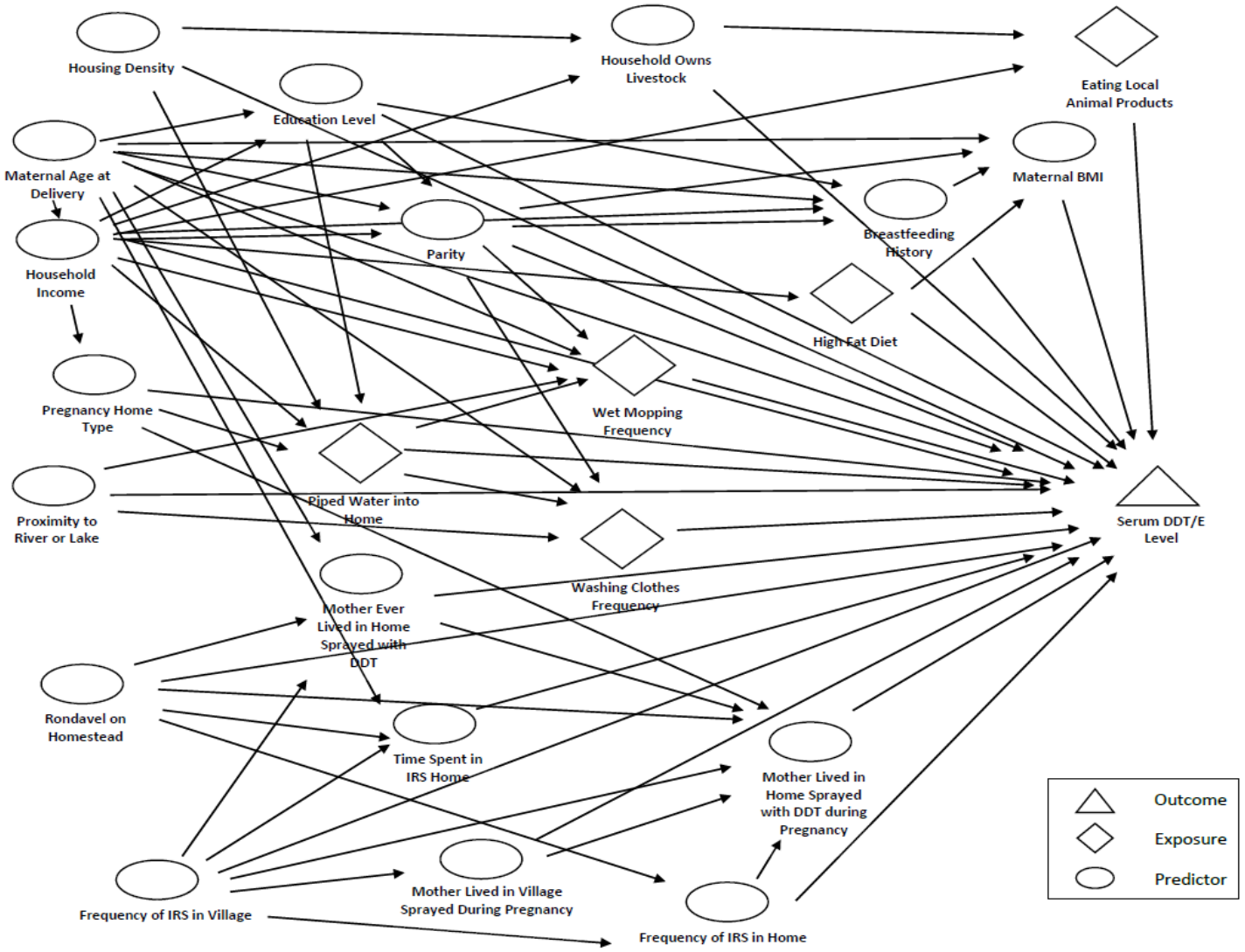

Figure S1. Directed Acyclical Graph (DAG) used to conceptualize the relationship between exposures and outcomes.

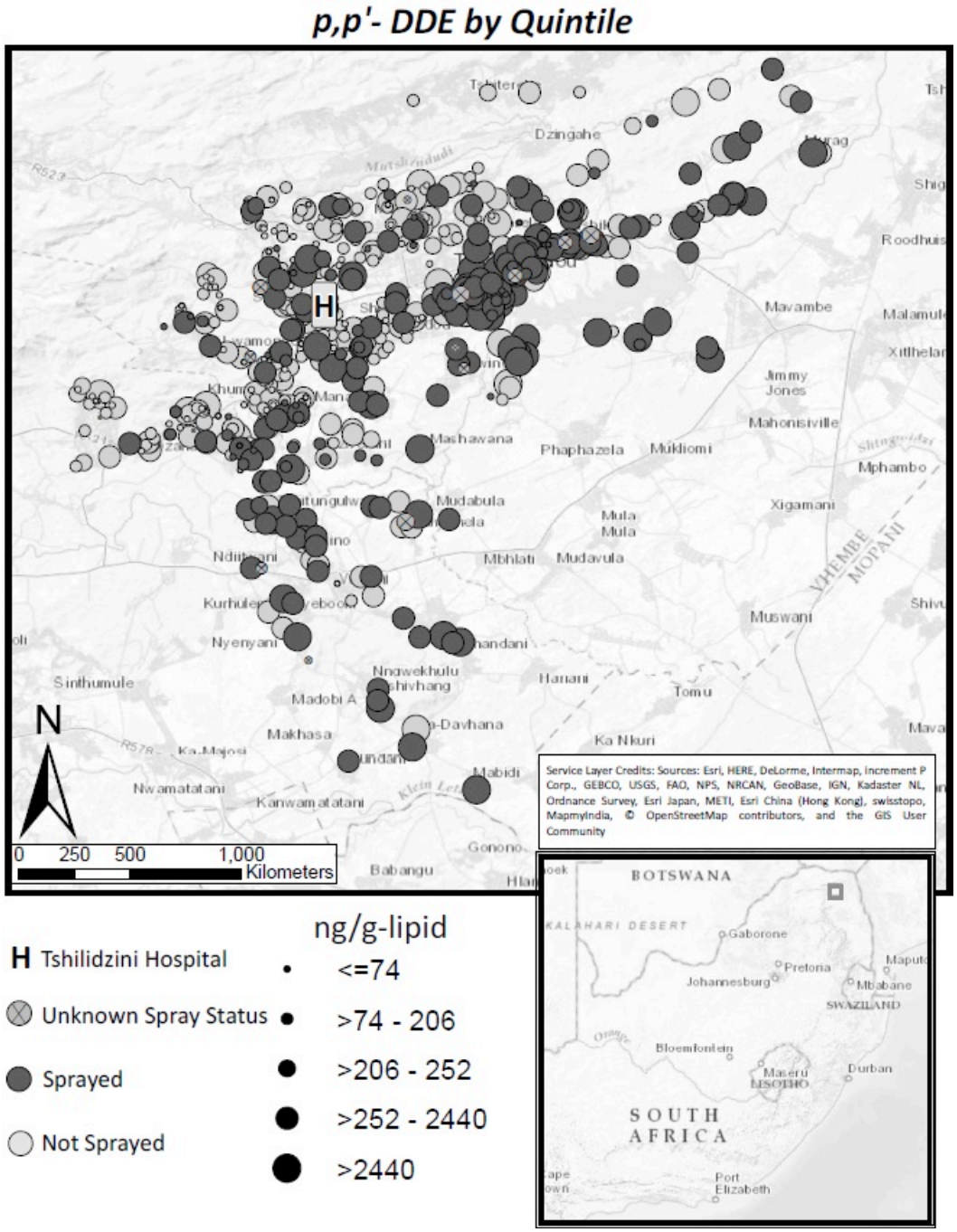

Figure S2. Spatial distribution of *p,p'*-DDE concentrations in relation to Tshilidzini Hospital

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