*Supplementary Information*

# Comparison of Two Methods to Quantify 2,6-Dichlorophenol from Tick *Amblyomma cajennense* by GC/MS-SIM

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**Table S1.** Cochran's test results of 2,6-DCP quantification by internal standard method

| 2,6-DCP / (ng mL$^{-1}$) | Measurements | | | | | | Average | sd | Variance |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | | | |
| 5 | 0.098 | 0.098 | 0.098 | 0.097 | 0.097 | 0.098 | 0.098 | 0.001 | $2.67 \times 10^{-7}$ |
| 8 | 0.113 | 0.112 | 0.113 | 0.112 | 0.113 | 0.113 | 0.113 | 0.001 | $2.67 \times 10^{-7}$ |
| 10 | 0.148 | 0.152 | 0.150 | 0.150 | 0.147 | 0.151 | 0.150 | 0.002 | $3.47 \times 10^{-6}$ |
| 30 | 0.301 | 0.301 | 0.297 | 0.297 | 0.298 | 0.298 | 0.299 | 0.002 | $3.47 \times 10^{-6}$ |
| 50 | 0.456 | 0.456 | 0.456 | 0.455 | 0.454 | 0.456 | 0.456 | 0.001 | $7.00 \times 10^{-7}$ |
| 100 | 0.846 | 0.845 | 0.846 | 0.846 | 0.845 | 0.846 | 0.846 | 0.001 | $2.67 \times 10^{-7}$ |
| $\sum$ variance | | | | | | | | | $8.43 \times 10^{-6}$ |
| Cochran (experimental) | | | | | | | | | 0.412 |
| Cochran (critical; k = 6; n = 6; $\alpha$ = 0.05) | | | | | | | | | 0.445 |

**Table S2.** Dixon's test results of 2,6-DCP quantification by internal standard method

| 2,6-DCP/(ng mL$^{-1}$) | Measurements | | | | | | $Q_{95\%}$ (experimental) | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Up | Down |
| 5 | 0.097 | 0.097 | 0.098 | 0.098 | 0.098 | 0.098 | 0 | 0 |
| 8 | 0.112 | 0.112 | 0.113 | 0.113 | 0.113 | 0.113 | 0 | 0 |
| 10 | 0.147 | 0.148 | 0.150 | 0.150 | 0.151 | 0.152 | 0.2 | 0.2 |
| 30 | 0.297 | 0.297 | 0.298 | 0.298 | 0.301 | 0.301 | 0 | 0 |
| 50 | 0.454 | 0.455 | 0.456 | 0.456 | 0.456 | 0.456 | 0 | 0.5 |
| 100 | 0.845 | 0.845 | 0.846 | 0.846 | 0.846 | 0.846 | 0 | 0 |
| $Q_{95\%}$ (critical, H = 6; $\alpha$ = 0.05) = 0.63 | | | | | | | | |

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**Table S3.** Grubb's test results of 2,6-DCP quantification by internal standard method

| 2,6-DCP/(ng mL$^{-1}$) | Average | sd | $G_{95\%}$ (experimental) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| 5 | 0.098 | 0.001 | −1.29 | −1.29 | 0.65 | 0.65 | 0.65 | 0.65 |
| 8 | 0.113 | 0.001 | −1.29 | −1.29 | 0.65 | 0.65 | 0.65 | 0.65 |
| 10 | 0.150 | 0.002 | −1.43 | −0.90 | 0.18 | 0.18 | 0.72 | 1.25 |
| 30 | 0.299 | 0.002 | −0.90 | −0.90 | −0.36 | −0.36 | 1.25 | 1.25 |
| 50 | 0.456 | 0.001 | −1.79 | −0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| 100 | 0.846 | 0.001 | −1.29 | −1.29 | 0.65 | 0.65 | 0.65 | 0.65 |

$G_{95\%}$ experimental = (Xi-average)/sd; $G_{95\%}$ (critical, n = 6, $\alpha$ = 0.05) = 1.89

**Table S4.** Cochran's test results of 2,6-DCP quantification by standard addition method

| 2,6-DCP/(ng mL$^{-1}$) | Measurements | | | | | Average | sd | Variance |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | |
| 0 | 2565 | 2893 | 2148 | 2572 | 2887 | 2613.0 | 305.649 | 9.34×10$^4$ |
| 25 | 7747 | 7745 | 7752 | 7749 | 7747 | 7748.0 | 2.646 | 7.00 |
| 50 | 12078 | 13348 | 13782 | 12085 | 13372 | 12933.0 | 796.222 | 6.34×10$^5$ |
| 100 | 23250 | 25060 | 24226 | 23150 | 25090 | 24155.2 | 939.061 | 8.82×10$^5$ |
| 150 | 33620 | 35635 | 34422 | 33710 | 35535 | 34584.4 | 965.455 | 9.32×10$^5$ |
| $\Sigma$ variance | | | | | | | | 2541337 |
| Cochran (experimental) | | | | | | | | 0.367 |
| Cochran (critical: k = 5; n = 5; $\alpha$ = 0.05) | | | | | | | | 0.544 |

**Table S5.** Dixon's test results of 2,6-DCP quantification by standard addition method

| 2,6-DCP/(ng mL$^{-1}$) | Measurements | | | | | $Q_{95\%}$ (experimental) | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Up | Down |
| 5 | 2148 | 2565 | 2572 | 2887 | 2893 | 0.01 | 0.56 |
| 8 | 7745 | 7747 | 7747 | 7749 | 7752 | 0.43 | 0.29 |
| 10 | 12078 | 12085 | 13348 | 13372 | 13782 | 0.24 | 0.00 |
| 30 | 23150 | 23250 | 24226 | 25060 | 25090 | 0.02 | 0.05 |
| 50 | 33620 | 33710 | 34422 | 35535 | 35635 | 0.05 | 0.04 |

$Q_{95\%}$ (critical, H = 5; $\alpha$ = 0.05) = 0.71

**Table S6.** Grubb's test results of 2,6-DCP quantification by standard addition method

| 2,6-DCP/(ng mL$^{-1}$) | Average | sd | $G_{95\%}$ (experimental) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 |
| 5 | 2613.000 | 305.649 | −1.52 | −0.16 | −0.13 | 0.90 | 0.92 |
| 8 | 7748.000 | 2.646 | −1.13 | −0.38 | −0.38 | 0.38 | 1.51 |
| 10 | 12933.000 | 796.222 | −1.07 | −1.07 | 0.52 | 0.55 | 1.07 |
| 30 | 24155.200 | 939.061 | −1.07 | −0.96 | 0.08 | 0.96 | 1.00 |
| 50 | 34584.400 | 965.455 | −1.00 | −0.91 | −0.17 | 0.98 | 1.09 |

$G_{95\%}$ experimental = (Xi-average)/sd; $G_{95\%}$ (critical, n = 5, $\alpha$ = 0.05) = 1.71

**Table S7.** 2,6-DCP contents[a] (ng mL$^{-1}$ *per* female) in hexane extracts determined by internal standard and standard addition methods from two *A. cajennense* populations

| Population (extract) | Quantification method | | Average |
|---|---|---|---|
| | Internal standard (n = 6) | Standard addition (n = 10) | |
| I | 2.17 ± 0.13 | 2.19 ± 0.14 | 2.18 ± 0.14 A |
| II | 2.13 ± 0.04 | 2.12 ± 0.07 | 2.12 ± 0.06 A |
| Average | 2.15 ± 0.09 a | 2.15 ± 0.12 a | |

[a]Averages followed by the same capital letter in the columns and by the same small letter in the rows did not share significant differences at 5% probability by Tukey test.