Legacy and emerging Persistent Organic Pollutants in Antarctic benthic invertebrates near Rothera Point, Western Antarctic Peninsula.

Artem Krasnobaev¹, Guillaume ten Dam^{2,3}, Rita Boerrigter-Eenling², Fang Peng⁴, Stefan P.J. van Leeuwen², Simon A. Morley⁵, Lloyd S. Peck⁵, Nico W. van den Brink^{1*}.

¹ Wageningen University, Dep. Toxicology, PO Box 8000, NL 6700 EA Wageningen, the Netherlands. ² Wageningen Food Safety Research (WFSR), Wageningen Research, PO Box 230, NL 6700 AE Wageningen, the Netherlands.

³ DSP-systems, Food Valley BTA12, Darwinstraat 7a, 6718 XR Ede, The Netherlands.

⁴ Luxembourg Institute of Health, rue Thomas Edison 1A-B, 1445 Strassen, Luxembourg.

⁵ British Antarctic Survey, Natural Environment Research Council (NERC), Cambridge, the UK.

* corresponding author. Address: sub-division of Toxicology. Postbus 8000, 6700 EA Wageningen, The Netherlands. E-mail: <u>nico.vandenbrink@wur.nl</u>

Appendix 3. Statistics.

Appendix 3A. Explanation of Bonferonni Correction

Bonferonni correction is a method for accounting for statistical inferences in dependent variables¹. The question whether to employ a Bonferonni correction is not trivial and must be case-specific^{2,3}. Recent research has formulated 3 major rationales for its usage⁴, 2 of which are clearly not applicable to the current study. First, here it is not imperative to avoid the type I error (as it may have been in medical research) and, second, we have a distinctly defined null-hypothesis. However, the choice of rejecting or accepting the last rationale – presence of an *universal* null hypothesis is less straightforward.

The research question "Are there differences in concentrations of POPs between different sampling locations?" presents a broad nature and a high level of aggregation of data, which may be also intercorrelated. Therefore, a Bonferonni correction is required here to avoid analytical overexploitation on the dependent variable. On the other hand, the question "Are there differences in concentration of PCBs, PBDEs or OCPs" has a much lower level of generalization: the relative concentrations of PCBs are almost exactly the same across the species; the PBDEs are mainly represented by one congener (BDE-209); and the differences in OCPs were calculated on the basis of an individual compounds. As such, the choice was not to use Bonferonni (or other) correction for the latter research question, but instead to consider the statistical significance on 3 different levels, according to the actual p-value.

- 1. Dunn, O. J. Multiple Comparisons among Means. J. Am. Stat. Assoc. 56, 52–64 (1961).
- Streiner, D. L. & Norman, G. R. Correction for Multiple Testing: Is There a Resolution? *Chest* 140, 16–18 (2011).
- 3. Cabin, R. J. & Mitchell, R. J. To Bonferroni or Not to Bonferroni: When and How Are the Questions. *Bulletin of the Ecological Society of America* **81**, 246–248
- 4. Armstrong, R. A. When to use the Bonferroni correction. Ophthalmic Physiol. Opt. 34, 502–

508 (2014).



Appendix 3B. Significance of differences of interspecies concentrations of PCBs and PBDEs sums.

NS Not Significant

S2

Appendix 3C. Significance of differences of interspecies concentrations of (individual) OCPs.

SCF = Sea cucumber A = Ascidian SST = Sea star SU = Sea urchin SL = limpet

Statistics values = The Mann-Whitney U statistic, equal to min(U for x, U for y) if alternative is equal to None (deprecated; exists for backward compatibility), and U for y otherwise

SCF A

HCB no difference found HCH alphastatistics 148.0 p-value 5.5810663821847943e-05 HCH beta- no difference found HCH gammastatistics 207.0 p-value 0.0020490070085374974 HCH deltastatistics 254.0 p-value 0.018798077703420662 HCH epsilonstatistics 214.0 p-value 0.002955611333006968 Heptachlor statistics 67.0 p-value 8.589039170678466e-08 Oxychlordan statistics 183.0 p-value 0.0005290999203994083 Hept. ep. (B) statistics 271.0 p-value 0.03648480179427856 DDE o,p'statistics 243.0 p-value 0.011773985067118608 Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'-226.0 p-value 0.005376340858529473 statistics End. alphastatistics 190.0 p-value 0.0007977540471249249 Mirex no difference found

SCF SST

HCB no difference found HCH alpha- no difference found HCH beta- no difference found HCH gammastatistics 53.0 p-value 0.006491206628688183 HCH deltastatistics 72.0 p-value 0.03671712578800128 HCH epsilon- no difference found Heptachlor statistics 75.0 p-value 0.04642780339796862 Oxychlordan statistics 37.0 p-value 0.0010746700946077331 Hept. ep. (B) no difference found DDE o,p'- no difference found Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'statistics 46.0 p-value 0.0030721169771417084 End. alpha- no difference found Mirex no difference found SCF SL HCB no difference found HCH alpha- no difference found HCH betastatistics 53.0 p-value 0.0403826648016666 HCH gammastatistics 45.0 p-value 0.01844421285352491 HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Oxychlordan no difference found Hept. ep. (B) no difference found DDE o,p'statistics 52.0 p-value 0.03681913506015133 Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'statistics 23.0 p-value 0.001247217297723964 End. alpha- no difference found Mirex statistics 24.0 p-value 0.0014345563960383074 SCF SU HCB statistics 28.0 p-value 0.0214800730142125 HCH alpha- no difference found HCH beta- no difference found HCH gammastatistics 32.0 p-value 0.034647042755779005 HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Oxychlordan statistics 14.0 p-value 0.0029734377243145716 Hept. ep. (B) no difference found DDE o,p'statistics 10.0 p-value 0.0015467246613805905 Ch. trans (g) no difference found Ch. cis- (a) statistics 35.0 p-value 0.04837703685437085 DDE p,p'statistics 23.0 p-value 0.011197615987668465 End. alpha- no difference found Mirex statistics 27.0 p-value 0.018947105700081363

A SST

HCB no difference found HCH alphastatistics 43.0 p-value 0.001747528523787258 HCH beta- no difference found HCH gammastatistics 55.0 p-value 0.006285922691210745 HCH deltastatistics 66.0 p-value 0.017590141696240954 HCH epsilon- no difference found Heptachlor statistics 49.0 p-value 0.003383780759939502 Oxychlordan statistics 75.0 p-value 0.03691377495557096 Hept. ep. (B) statistics 21.0 p-value 0.00010811454406201593 DDE o,p'- no difference found Ch. trans (g) statistics 65.0 p-value 0.016109750067359065 Ch. cis- (a) no difference found DDE p,p'- no difference found End. alpha- no difference found Mirex no difference found

A SL

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HCB no difference found
HCH alpha- no difference found
HCH beta- no difference found
HCH gamma-
statistics 51.0 p-value 0.027579118888853754
HCH delta- no difference found
HCH epsilon- no difference found
Heptachlor no difference found
Oxychlordan no difference found
Hept. ep. (B) no difference found
DDE o,p'- no difference found
Ch. trans (g) no difference found
Ch. cis- (a) no difference found
DDE p,p'- no difference found
End. alpha- no difference found
Mirex
 statistics 33.0 p-value 0.003907784690770291
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A SU

HCB

statistics 32.0 p-value 0.029859558464299187 HCH alpha- no difference found HCH beta- no difference found HCH gamma- no difference found HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Oxychlordan no difference found Hept. ep. (B)

statistics 23.0 p-value 0.009778282270008598 DDE o,p'- no difference found Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'- no difference found End. alphastatistics 24.0 p-value 0.01116994174316034 Mirex statistics 35.0 p-value 0.04161606632084231 SST SL HCB no difference found HCH alpha- no difference found HCH betastatistics 11.0 p-value 0.01712888443293505 HCH gamma- no difference found HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Oxychlordan no difference found Hept. ep. (B) no difference found DDE o,p'statistics 15.0 p-value 0.045168795869826826 Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'statistics 9.0 p-value 0.009936767845410874 End. alpha- no difference found Mirex statistics 4.0 p-value 0.0021317156915536613 SST SU HCB statistics 7.0 p-value 0.022750131948179195 HCH alpha- no difference found HCH betastatistics 9.0 p-value 0.041518219688779105 HCH gamma- no difference found HCH deltastatistics 8.0 p-value 0.030974075706740573 HCH epsilon- no difference found Heptachlor statistics 9.0 p-value 0.041518219688779105 Oxychlordan statistics 7.0 p-value 0.022750131948179195 Hept. ep. (B) statistics 7.0 p-value 0.022750131948179195 DDE o,p'statistics 5.0 p-value 0.011705298080558346 Ch. trans (g) statistics 8.0 p-value 0.030974075706740573 Ch. cis-(a)statistics 5.0 p-value 0.011705298080558346 DDE p,p'statistics 7.0 p-value 0.022750131948179195 End. alpha- no difference found Mirex no difference found

SL SU

HCB no difference found HCH alpha- no difference found HCH beta- no difference found HCH gamma- no difference found HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Oxychlordan no difference found Hept. ep. (B) no difference found DDE o,p'statistics 5.0 p-value 0.02565995179403558 Ch. trans (g) no difference found Ch. cis- (a) no difference found DDE p,p'statistics 6.0 p-value 0.03701762392744832 End. alpha- no difference found Mirex no difference found

Appendix 3D. Significance of differences of location concentrations of (individual) OCPs.

Note: For PCBs and PBDEs no differences were found at all.

Cucumbers

HCB no difference found HCH alpha- no difference found HCH beta- no difference found HCH gammastatistics 30.0 p-value 0.0018462476541649497 HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Aldrin no difference found Isodrin no difference found Oxychlordan no difference found Heptachlor epoxide (iso B) statistics 48.0 p-value 0.02143405940857073 DDE o,p'- no difference found Chlordane trans- (gamma) no difference found Chlordane cis- (alpha) no difference found DDE p,p'- no difference found Endosulfan alphastatistics 54.0 p-value 0.04161696569853907 DDD o,p'- (TDE) no difference found Dieldrin no difference found Endrin no difference found DDD p,p'- (TDE) no difference found Endosulfan beta- no difference found heptachlor endo epoxide (iso A) no difference found Methoxychlor no difference found Mirex no difference found

Stars

HCB statistics 4.0 p-value 0.03630046972497867 HCH alpha- no difference found HCH beta- no difference found HCH gamma- no difference found HCH delta- no difference found HCH epsilon- no difference found Heptachlor no difference found Aldrin no difference found Isodrin no difference found Oxychlordan no difference found Heptachlor epoxide (iso B) no difference found DDE o,p'- no difference found Chlordane trans- (gamma) no difference found Chlordane cis- (alpha) no difference found DDE p,p'- no difference found Endosulfan alpha- no difference found

DDD o,p'- (TDE) no difference found Dieldrin no difference found Endrin no difference found DDD p,p'- (TDE) no difference found Endosulfan beta- no difference found heptachlor endo epoxide (iso A) no difference found Methoxychlor no difference found Mirex no difference found