Research Article

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Organochlorine Pesticides in Feathers of Penguins of the Family Pygoscelidae from Livingston and Peterman Islands, Western Antarctica

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Abstract: The aim of the present work is to determine the presence of isomers like αHCH, βHCH, γHCH, 4.4'DDE, 4.4'DDD and 4.4'DDT that compound both the commercial forms of insecticides HCH (Lindane) and DDT in moulting feathers of two Pygoscelidae penguin species, *Pygoscelis antarctica* and *Pygoscelis papua*, from Livingston and Peterman Islands, Western Antarctica. The analyses of the total quantity of the chosen chlorinated pesticides (ΣHCH and ΣDDT) shows significant differences between DDT and HCH concentrations, with quantities of DDT about six times more than this for HCH. In contrast, there were not recorded any differences, either between investigated localities or between penguin species, despite the certain differences in the food spectrum between them. The concentrations of ΣHCH were as follows: for *P. antarctica* from Livingston Island, 0.419 ± 0.178 μg/g; for *P. papua* from Livingston Island, 0.356 ± 0.179 μg/g; and for *P. papua* from Peterman Island, 0.437 ± 0.012 μg/g. The corresponding values for ΣDDT were: for *P. antarctica* from Livingston Island, 2.596 ± 0.312 μg/g; for *P. papua* from Livingston Island, 2.507 ± 0.254 μg/g; and for *P. papua* from Peterman Island – 2.628 ± 0.013 μg/g. Percentage composition of the HCHs compounds showed that β-HCH dominated all investigated samples, being between 55 and 70% of the total quantity of the analyzed HCHs. The major component DDT isomer composes about 2/3 from the total sum or between 72% in feathers of *P. papua* from Livingston Island and 87% for *P. papua* from Peterman Island. There were not obtained any statistically significant differences between investigated species or between two localities.

Key words: penguin feathers, pesticides, Antarctica

Introduction

Persistent organic pollutants (POPs) are considered a substantial risk to environmental and human health and, since 2004, they have been subject to the Stockholm Convention, a legally binding treaty that aims to reduce and ultimately eliminate sources of these chemicals to the environment due to their bioaccumulative and toxic properties (Hoffman *et al*. 2000, Kunisue *et al*. 2003). Many countries have banned the production and use of POPs but concentrations in nature remain high (Fängström *et al*. 2005). They are still frequently found in tissues or fluid samples from many species (Espin *et al*. 2010, Van Drooge *et al*. 2008).

Birds have been used successfully as biomonitors for contamination with POPs in the studies by Furness *et al*. (1993), Jaspers *et al*. (2006a), Papp *et al*. (2005) and others. Since birds are widespread, sensitive to environmental changes and occupy a high position in the food chain, they are very suitable to study bioaccumulation of toxic compounds

(Furness *et al*. 1993). Due to practical and ethical reasons impeding the sacrifice of free-living birds, methods for non-destructive biomonitoring have become necessary to look for alternative sampling methods (Jaspers et al. 2006a; Van Den Steen *et al*. 2006, Bustnes *et al*. 2005, Covaci *et al*. 2002; Dauberschmidt & Wennig 1998). Therefore, bird feathers may be useful as a non-destructive biomonitoring tool for exposure with POPs. The long half-lives of the organochlorine pesticides facilitate repeated cycles of volatilization and deposition resulting in progressive movement away from temperate and tropical source regions towards colder climates. Ultimately, these compounds may experience ''cold-trapping'' at the poles of the earth or at altitude where the colder temperatures further prolong persistence (Nash 2011).

The contamination of the Antarctica reflects the chemical usage in a hemisphere with comparatively little land mass, small human population and historical industry. This, combined with the relatively shorter food chains with few trophic components, low prey diversity and the absence of subsisting human populations, has served to lower the theoretical chemical risk category attributed to Antarctic biota and systems, and therefore also the associated investment into Antarctic POPs research (Laws 1985).

Several attempts have been made to identify and quantify the residues of highly persistent organochlorine compounds in the Antarctic atmosphere and hydrosphere. Sladen et al. (1966) and George & Frear (1966) were the first to report the presence of chlorinated hydrocarbons in Antarctica wildlife. Subsequently, Peterle (1969), Risebrough & Carmignani (1972), Risebrough (1977), Lukowski (1978), Hidaka & Tatsukawa (1981) and Norheim *et al*. (1982) detected these substances in varying quantities in birds and seals from Antarctica. Moreover, Tanabe *et al*. (1982, 1983) carried out extensive studies on the presence of these organochlorines in the Antarctic atmosphere and biosphere, eventually bringing a clear understanding of the transport phenomenon of these compounds into the Antarctic. The concentration levels of various congeners of polychlorinated biphenyls (PCBs) and organochlorine pesticides were determined in krill, bird (emperor penguin) feathers and water were investigated by Sen Gupta *et al.* (1996). Espin *et al*. (2012) used feathers as a biomonitoring tool for organochlorine pesticides (OCP) in a razorbill (*Alca torda*) population.

Moreover, the penguins are restricted to the Antarctic environment and therefore not exposed to more contaminated environments as other migrated representatives of the Antarctic fauna (Caio *et al.* 2013). As an additional factor, penguins due to their trophic position close to the food web base, since krill represents more than 70% of their diet (Croxall & Lishman 1987) give a clear picture of available constant amount of pesticides in the endemic Antarctic biota.

Penguin feathers are very suitable for such type if investigations due to two general reasons: 1. Sample collecting method is completely non-invasive and does not affects the status and integrity of the penguins' colony. 2. Penguin's feathers are superimposed to organochlorines both from the adipose gland and from the water.

The aim of the present work is to determine the presence of organochlorines like α-hexachlorocyclohexane (α-HCH), β-isomer of hexachlorocyclohexane (β-HCH), γ-hexachlorocyclohexane (γ-HCH), dichlorodiphenyldichloroethylene (4.4'DDE), dichlorodiphenyldichloroethane (4.4'DDD) and dichlorodiphenyltrichloroethane (4.4'DDT) in penguins feathers of two species, *Pygoscelis antarctica* and *Pygoscelis papua*, from two Western Antarctic islands (Livingston and Peterman), for which data on the occurrence of pesticides are scarce or nonexistent.

Materials and Methods

Only moulting feathers of Gentoo penguins (*P. papua*) and Chinstrap penguin (*P. antarctica*) inhabiting South Shetland Islands, Livingston (62º38´ S, 60º24´ W) and Petermann (65°10′ S, 64º10´ W), were collected in Antarctic summer season of 2010. Feathers were extracted with a mixture of 1 : 1 = diethyl ether : n-hexane. The extract was filtered and evaporated to a dry residue. The dry residue was dissolved in n-hexane in a concentration of 0.1 g/ml and purification by the presence of fat, according to the standard BDS EN 1528-3:1996. 8.3.3. The purified extract was analyzed for organochlorine pesticides by gas chromatography procedure set forth in BDS EN 1528-4: 1996, Annex B, item. B.1.1.

Variation and factor variances analysis were applied using Leveni test for the equality of dispersions inside groups and nonparametric Kruskal-Wallis test to confirm the hypotheses. Empirical data were analyzed using the statistical package SPSS 20.

Results and Discussion

As it is well known, both commercial forms of insecticides, HCH (Lindane) and DDT, are a mixture of several closely–related isomers (Metcalf 2002). The investigation presents the quantities of each one

of them separately as well as their sum. The obtained results of the organochlorines' content in averaged samples of penguins' feathers from Livingston and Peterman Islands, Western Antarctica, are presented in Table 1.

The percentage composition of every one of the studied isomers that compound each one of the studied insecticides is shown on Fig. 1 and 2.

The percent value of β-HCH dominated all the investigated samples (Fig. 1). It varied between 55 and 70% of the total quantity of the analyzed HCHs. Data analysis did not display any statistically significant differences ($p \geq 0.05$) in the isomers presence either between species or between different locations.

The results of organochlorine compounds in penguin's feathers obtained by Sen Gupta *et al*. (1996) in East Antarctica presented a slightly higher to those amounts of HCHs but authors did not investigate the presence β-HCH. A similar proportion of HCH isomer residues were detected by Fasola *et al*. (1997) for birds in Northern Italy, although this most toxic isomer of insecticide 'Lindane' and its by-products have been banned for use for more than 30 years. In penguin feathers, it has been registered in concentrations more than 10 times higher than the limit values obtained by Lakaschus *et al*. (2002) and Nash (2011).

DDE and DDD are the major metabolites and environmental breakdown products (Environmental Health Criteria Monograph 1979) but they did not consist more than about 30% of the total compound of DDT's. The major component DDT isomer composes about 2/3 from the total sum or between 72% for penguin feathers for *P. papua* from Livingston Island to 87% for *P. papua* from Peterman Island. No statistically significant differences were found.

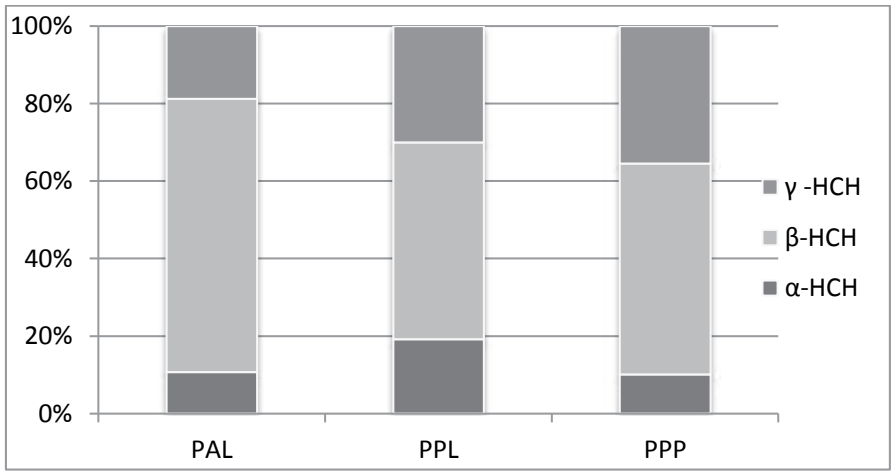
The present results for HCHs are two to three times higher than those obtained by Sen Gupta *et al.*

Table 1. Mean concentrations (± SD) of selected pesticides in the feathers of *Pygoscelis antarctica* and *Pygoscelis papua* from Livingston and Peterman Islands (μg/g). PAL – *Pygoscelis antarctica* from Livingston Island; PPL – *Pygoscelis papua* from Livingston Island; PPP – *Pygoscelis papua* from Peterman Island

		N	Mean	SD	Std. Error	95% Confidence Interval for Mean		Min.	Max.
						Lower Bound	Upper Bound		
α-HCH	PAL	24	0.043	0.013	0.003	0.037	0.049	0.020	0.062
	PPL	15	0.058	0.006	0.002	0.055	0.061	0.050	0.068
	PPP	3	0.044	0.004	0.002	0.034	0.054	0.040	0.048
β-HCH	PAL	24	0.285	0.145	0.030	0.224	0.347	0.000	0.430
	PPL	15	0.154	0.054	0.014	0.124	0.185	0.000	0.204
	PPP	3	0.238	0.004	0.002	0.229	0.247	0.234	0.241
γ -HCH	PAL	24	0.076	0.039	0.008	0.060	0.093	0.015	0.123
	PPL	15	0.091	0.041	0.011	0.069	0.114	0.000	0.129
	PPP	3	0.155	0.005	0.003	0.144	0.167	0.151	0.160
DDE	PAL	24	0.326	0.057	0.012	0.302	0.350	0.252	0.390
	PPL	15	0.313	0.071	0.018	0.274	0.353	0.237	0.383
	PPP	3	0.227	0.004	0.002	0.217	0.237	0.223	0.231
DDD	PAL	24	0.400	0.041	0.008	0.383	0.418	0.335	0.481
	PPL	15	0.411	0.011	0.003	0.406	0.417	0.397	0.424
	PPP	3	0.276	0.004	0.002	0.267	0.284	0.272	0.279
DDT	PAL	24	1.856	0.263	0.053	1.745	1.967	1.427	2,140
	PPL	15	1.783	0.172	0.045	1.688	1.878	1.600	1.943
	PPP	3	2.125	0.006	0.003	2.112	2.139	2.120	2.131

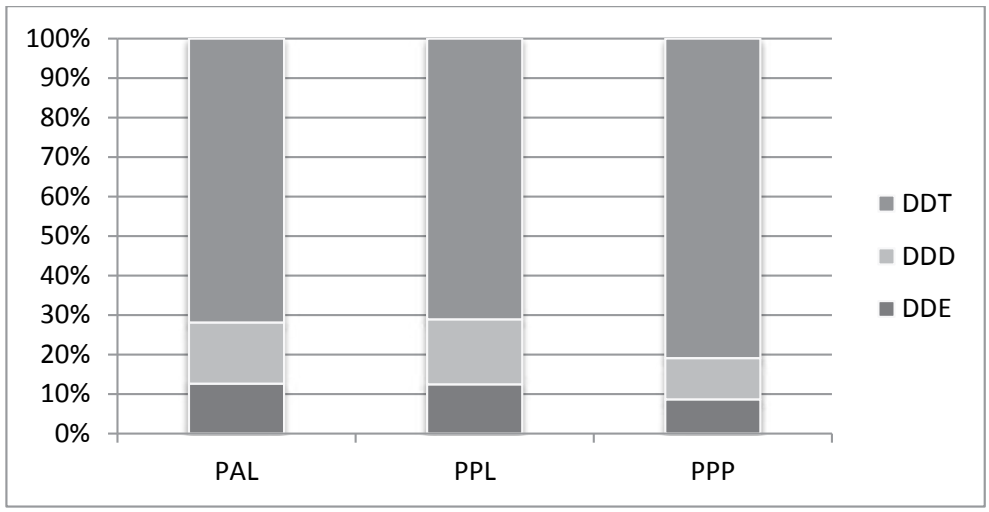
Table 2. Total quantity (mean ± SD) of chlorinated pesticides (ΣHCH and ΣDDT) in μg/g in penguin feathers. PAL – *Pygoscelis antarctica* from Livingston Island; PPL – *Pygoscelis papua* from Livingston Island; PPP – *Pygoscelis papua* from Peterman Island

Species and location	ΣHCH	ΣDDT
PAL	0.419 ± 0.178	2.596 ± 0.312
PPL	0.356 ± 0.179	2.507 ± 0.254
PPP	0.437 ± 0.012	2.628 ± 0.013



PAL – Pygoscelis antarctica from Livingston Island; PPL – Pygoscelis papua from Livingston Island; PPP – Pygoscelis papua from Peterman Island

Fig. 1. Percentage composition of the HCHs compounds in penguin's feathers from Livingston and Peterman islands



PAL – Pygoscelis antarctica from Livingston Island; PPL – Pygoscelis papua from Livingston Island; PPP – Pygoscelis papua from Peterman Island

Fig. 2. Percentage of the isomers of DDT in penguin's feathers from Livingston and Peterman islands

(1995) from the surroundings of the Dakshin Gangotri (Antarctica) but authors analyzed feathers of Emperor penguins (*Aptenodytes forsteri*) that, especially during brooding, do not have direct contacts with the water and are not fed. Lukowski (1983a) found a similar profile of DDTs in adipose tissue of three species of penguins collected in the proximity of the Arctowski Station in Admiralty Bay but at significantly lower concentrations for *P. adeliae*, *P. antarctica* and *P. papua*. In all the cases, the content of DDT residues was lower than 1 mg/kg (ppm) but the authors have no data for its concentration in moulting feathers.

Comparable concentrations of HCH and DDT were found in the studies of Taniguchi *et al*. (2009) and Caio *et al*. (2013) in the fat tissue of three species of penguins, *P. adeliae, P. antarctica* and *P. papua,* captured in the vicinity of the Brazilian and the Polish Antarctic Station on King George Island.

The analyses of the total quantity of the chosen chlorinated pesticides (ΣHCH and ΣDDT) showed significant differences between DDT and HCH concentrations, with DDT quantities being about six times higher than this for HCH. In contrast, there were not recorded any differences either between investigated localities or among penguin species, despite the certain differences in the food spectrum of these species. The concentrations of the chlorinated pesticides are presented in Table 2.

The obtained data most likely reflect the circumpolar movement of waters surrounding Antarctica. These movements of the same water masses transport contaminations via distant flows.

There are two hypotheses about the relationships between organochlorines, penguin species and their geographical location. The null hypothesis H_0 can be formulated as "there is no relationship between penguin species and its geographic location, and the type of pesticide"; the alternative H_1 hypothesis is "there is a correlation between penguin species and its geographical location, and the type of pesticide." Both hypotheses can be represented by the average scores of the samples as follows:

$$H0: \bar{X}_{PAL} = \bar{X}_{PPL} = \bar{X}_{PPP}$$
$$H1: \bar{X}_{PAL} \neq \bar{X}_{PPL} \neq \bar{X}_{PPP}.$$

The Levene test shows that for all the discussed parameters the condition of equality of variances in groups PAL, PPL and PPP makes the results questionable. Therefore, for final confirmation of the hypotheses additional non-parametric test of Kruskal-Wallis was applied. It showed that except DDT for α-HCH, β-HCH, γ-HCH, DDE and DDD is considered the alternative hypothesis H1 because Chi Square was statistically significant indicator for such variables.

Asymp. Sig. α-HCH = 0.002 < α = 0.05; Asymp. Sig. β-HCH = 0.020 < α = 0.05; Asymp. Sig. γ-HCH = 0.003 < α = 0.05; Asymp. Sig. DDE= 0.006 < α = 0.05; Asymp. Sig. DDD = 0.006 < α = 0.05; Asymp. Sig. DDT = 0.105 > α = 0.05.

As regards to ΣHCH and ΣDDT, the results were obtained at a guaranteed probability of P = 95% (with significance level α = 5% - P + α = 100%). According ANOVA test for ΣHCH and ΣDDT, the null hypothesis H_0 is accepted as the following conditions are fulfilled:

Sig. F □HCH = 0.530 > α = 0.05 and Sig. F □DDT = 0.595 > α = 0.05.

Conclusions

The concentration levels of various organochlorine compounds in penguin feathers clearly indicate the wide variation in the occurrence and distribution of organochlorine contaminants in the Antarctic environment. Further studies in the region on the level of contamination of these compounds will be of utmost importance from the point of view of environmental pollution in this pristine environment. The above research activities will ultimately be most effectively addressed as part of comprehensive longitudinal studies focusing on complementary research questions across a broad spatial range and according to best practice protocols.

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