DDTs AND OTHER PERSISTENT ORGANIC POLLUTANTS IN PLASMA OF DELIVERING WOMEN FROM SELECTED AREAS OF SOUTH AFRICA – RESULTS OF A PILOT STUDY

Röllin HB [a,] Sandanger TM [b], Odland J Ø [c],

[a] South African Medical Research Council (SA MRC), PO Box 87373, Houghton 2041, Johannesburg, South Africa; hrollin@mrc.ac.za
[b] Norwegian Institute for Air Research (NILU), Tromsø, Norway; tsa@nilu.no
[c] Institute of Community Medicine, University of Tromsø, Norway; jon.oyvind.odland@ism.no

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Introduction:
Worldwide there is a need for comprehensive research to identify sources and distribution of persistent toxic substances in various environmental compartments and in humans. Persistent organic pollutants (POPs) have a high affinity for bioaccumulation (due to their hydrophobic and /or lipophilic nature) and biomagnifications in the environment and living organisms, including humans. These substances have an ability to exert negative health effects on humans that are often subtle, long-term, sometimes trans-generational and difficult to measure even in long term epidemiological studies in large populations. Furthermore, the most vulnerable periods for toxic impact of POPs and other pollutants on human development are at the embryonic and foetal stages, as these substances are known to influence reproductive health, pregnancy outcomes, reduce defence against diseases, affect children's physical and mental development, and/or increase the risk of cancer [1,2,3].

Several multidisciplinary international projects are currently in progress. These are firstly studying the sources and levels of persistent toxic substances (PTS) in people residing in different geographical regions and secondly ascertaining the relationship between levels of these chemicals and health [4,5,6,7,8].

At present, no comprehensive data on the levels of these contaminants in ecosystems and humans in the Southern Hemisphere exist and research linking environmental exposures with human health outcomes in general population are scarce. South Africa, situated in the Southern Hemisphere and having dimensions both of developed and developing country is of particular importance to the global research in the science of environmental pollutants and human health outcomes. The subject of this paper is to report on a pilot study performed in South Africa under the auspices of AMAP that measured levels of PTS including POPs in maternal plasma at seven geographical regions that included two endemic malaria areas (one coastal and one inland where DDT is sprayed indoors for malaria vector control).

Materials and Methods

Seven sites that differed in the type of environmental pollution were purposely selected for the pilot study. These were rural, urban, industrial, fishing site situated at the Atlantic Ocean, coastal DDT spraying malaria site situated at the Indian Ocean, an inland DDT spraying malaria site and a mining site (Figure 1). Study subjects were women who were admitted for delivery at the local provincial hospitals and volunteered to participate in the study. They signed an informed consent form and agreed to donate blood and urine before delivery and cord blood samples immediately after delivery. They also agreed to answer socio-economic questionnaire by interview and allow access to their post delivery records.

A sterile, vacutainer disposable system for blood collection was used. Tubes that collected maternal blood for plasma fraction were spun by clinical laboratory on site, transferred into acid pre-washed tubes, immediately frozen at -20°C and shipped in the frozen state to the University of Tromsø, Tromsø, Norway.

All samples were analyzed for selected POPs at NILUs laboratory in Tromsø. Plasma samples were extracted on an Oasis HLB (540 mg; Waters Corp.) solid phase extraction (SPE) column according to the method by Sandanger et al[9]. In short, internal standards were added to 2 ml of plasma that was mixed with equal volumes of formic acid and water and left overnight in the refrigerator. The compounds were eluted from the HLB column using 15 ml 10 % methanol in dichloromethane. The sample was evaporated to dryness, before it was dissolved in 0.5 ml of n-hexane and eluted through a column containing 1 g activated Florisil (60-100 mesh; Fisher, Pittsburgh, PA, USA). The fraction containing the POPs was eluted using 7 ml hexane/dichloromethane (9/1). The extraction and clean up procedures were automated using a Rapidtrace Automated SPE workstation (Zymark Corp.) and evaporation was performed using a heated vacuum evaporator; the Rapidvap (Labconco Corp., Kansas City, MO). A large number of C13 labelled compounds were used as internal standards (indicated in Table 1). The extracts were analysed on a MD-800 HRGC-MS.

Statistical analyses: Statistical analyses were conducted using the Stata release 10 (StataCorp, 2007).

Ethical considerations: The study protocol was submitted to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, South Africa and unconditional approval was obtained (Protocol M040314).

Figure 1. Study sites of pilot

Results and Discussion

Levels of POPs measured in maternal plasma in different study sites are presented in Table 1. The following PCB congeners were quantified; PCB 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187. Levels were low in all samples and even close to LODs for most congeners and they are therefore not discussed further. Similarly, the levels of PCB's, DDTs and other pesticides quantified in the rural site were found to be extremely low and close to LODs with only pp-DDE, β-HCH, HCB being detectable. For this reason these results are not included in the Table 1.

High levels of p,p'DDE and γ -HCH were found in the majority of samples, with p,p'-DDE being the dominant compound, even though γ -HCH concentrations were higher in 30% of the samples. The concentrations of DDTs were elevated in most participants of this study with large regional differences. Significant differences between sites were found for *p,p'*-DDE and *p,p'*-DDT (p=0.0001), with the highest levels being in the coastal malaria/DDT site with geometric mean levels of 5177 and 1797 ng/g lipids for *p,p'*-DDE and *p,p'*-DDT respectively and lowest in the

Atlantic site. The levels of p,p'DDE in the coastal malaria site were however not significantly higher than in the inland malaria site where some individuals showed low levels. In decreasing order of concentrations of DDTs were coastal malaria site > inland malaria site > mining > urban >industrial >Atlantic>rural. In the two most contaminated communities all DDTs except the p,p'-DDD were detected in all samples. Across all communities the p,p'-DDE and p,p'-DDT levels were highly correlated with a r^2 value of 0.7. Within the communities with lower levels of DDTs, the urban community had a few individuals with elevated DDE and DDT levels indicating the presence of fresh sources. There were no individuals with elevated levels in the Atlantic coast community.

Table 1: Levels of POPs in maternal plasma from selected communities in South Africa. Geometric mean levels and ranges are presented in ng/g lipids for all compounds.

	Industrial (N 9)		*Mining (N 11)*		*Inland DDT (N 12)*		*Coastal DDT (N 11)*		*Urban (N 16)*		*Atlantic (N 12)*	
	GM	***Range***	**GM**	***Range***	**GM**	***Range***	**GM**	***Range***	**GM**	***Range***	**GM**	***Range***
o,p'-*dde*	2.0	0.6-7.0	4.1	0.5-53	7.5	3.2-18.4	5.1	1.9-13.3	3.8	0.6-8.7	2.8	0.4-11
p,p'-*Dde**	64	21-448	488	42-3407	1966	114-11602	5178	1243-14482	109	15-1440	41.1	21-125
o,p'-DDD	2.3	0.6-9.7	3.6	0.5-33.1	8.1	3.4-16.3	8.6	2.7-24.8	3.4	0.4-26.8	4.4	0.5-13.7
p,p'-DDD	0.6	0.3-3.3	7.1	0.3-259	0.7	0.2-20.8	0.8	0.2-17.7	0.7	0.2-7.8	1.0	0.2-4.8
o,p'-DDT	0.8	0.4-2.4	9.8	0.3-259	49.8	3.9-319	100	33-192	0.8	0.2-5.2	1.0	0.3-4.2
p,p'-DDT*	2.3	1.4-17.8	63.4	1.2-2095	726	43-5278	1797	534-3588	2.4	0.9-122	1.9	1.1-5.7
dde/ddt ratio	4.9	1.4-45	1.3	0.1-16.0	0.5	0.1-1.7	0.5	0.2-1.4	7.1	0.7-33.2	3.4	0.8-12.4
α-HCH*	1.1	0.7-2.3	0.8	0.5-1.1	1.7	0.5-5.3	1.4	0.5-4.4	0.8	0.4-11.1	0.7	0.5-0.8
HCB*	5.4	2.5-9.0	3.1	1.8-8.4	2.6	1.6-6.0	3.4	2.0-7.9	4.5	1.9-44.3	5.5	2.4-9.7
β-HCH*	8.4	1.4-19.6	7.0	1.4-36.4	5.7	1.8-20.4	2.4	1.3-8.6	10.6	1.2-44.3	7.8	1.7-21.7
γ-HCH*	150	19-1066	150	51-1248	1081	181-3137	896	401-2839	1.4	0.5-10.3	336	118-1035
Heptachlor	2.6	2.2-3.0	2.5	1.7-3.4	2.3	0.2-3.5	2.7	1.7-5.2	2.4	1.3-3.3	2.2	1.7-2.6
oxy-CD	1.3	0.3-3.1	2.2	0.4-6.0	0.5	0.1-1.7	0.5	0.3-0.8	1.0	0.3-2.1	0.6	0.2-1.3
t-CD*	0.2	0.1-0.2	0.2	0.1-0.2	0.2	0.0-0.2	0.2	0.1-0.3	0.2	0.0-0.2	0.1	0.1-0.1
c-CD	0.1	0.0-2.7	0.1	0.0-0.4	0.1	0.0-0.1	0.1	0.0-0.1	0.1	0.0-4.1	0.1	0.0-0.0
t-NC*	2.7	1.0-6.8	5.0	1.6-12.6	0.4	0.0-0.9	0.8	0.3-2.6	1.3	0.3-4.1	1.0	0.4-2.8
c-NC	0.4	0.0-1.1	0.7	0.2-2.1	0.1	0.0-0.1	0.2	0.0-1.1	0.2	0.0-0.6	0.1	0.0-0.3
Mirex	0.4	0.3-0.4	0.3	0.2-0.4	0.3	0.2-0.4	0.4	0.2-0.7	0.3	0.2-0.6	0.4	0.2-1.5

*These compounds were also used as labelled internal standards.

Among other pesticides measured, the differences were significant for all sites except heptachlor, t-CD and c-CD. γ-HCH was the other dominant pesticide that was found to be elevated overall with the exception of the urban community. The geometric mean values are displayed in Figure 2. Similarly to DDTs, the highest levels of γ-HCH were found in the inland and coastal malaria sites. The levels of DDT/DDE were only weakly correlated to the levels of γ -HCH. The concentrations of the other pesticides were significantly lower than the DDTs and γ -HCH, but

nevertheless present. β-HCH displayed a pattern very different from the α-HCH, with the lowest level found in the coastal malaria village and the highest level (10.6 ng/g lipids) found in the urban community.

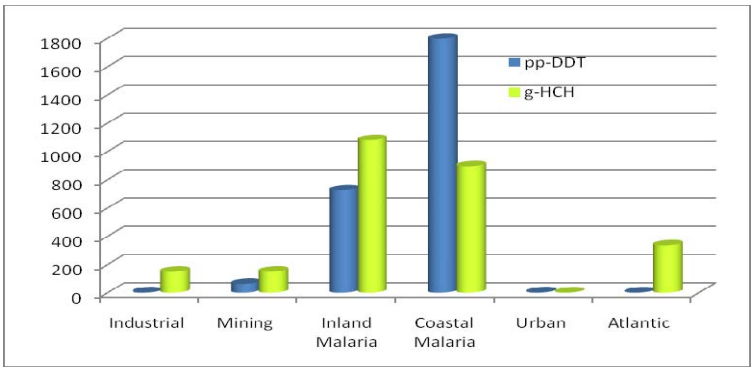

Figure 2: Comparison of γ -HCH and *p,p'*-DDT geometric mean levels (ng/g lipids) in the different communities.

Conclusions:

- The levels of DDTs are elevated in areas where indoor spraying against malaria takes place.
- Further studies on the potential health effects of DDT in the region are required.
- γ -HCH is also elevated in areas where DDT is sprayed, but limited correlation between these compounds was found.

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