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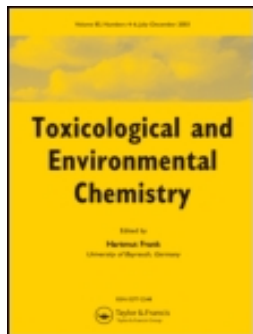


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## Organochlorine levels in human blood from residents in areas of limited pesticide use in Sudan

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Ninety-nine human blood samples were collected from the riverine region of northern Sudan and the traditional and mechanized rain-fed areas of western and eastern Sudan, representing areas of limited pesticide use in Sudan. Blood samples were analyzed for organochlorine pesticides by gas liquid chromatography (GLC) followed by electron-capture detection (ECD). p,p'-DDE (a metabolite of DDT), heptachlor epoxide,  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH), and dieldrin were detected in all locations surveyed. The level of total organochlorine burden was highest in the traditional rain-fed area, followed by the mechanized rain-fed area, and the riverine area. A highly significant correlation was observed between total organochlorine blood burden and the age of the donors ( $r = 0.608^{**}$ ).

**Keywords:** organochlorine insecticides; residues; human blood; Sudan

### Introduction

Organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) (Wania and Mackay 1996; Mackay, Shiu, and Ma 1997) with the potential for bioaccumulation in food crops and animal tissues (Nakata et al. 2002); by atmospheric transport (Park et al. 2011), they may even spread as far as the primeval ecosystems of the polar ice caps (Zhang et al. 2008). Due to their highly lipophilic nature and their persistence, POPs can accumulate in animal and human tissues, resulting in a number of health problems (Mishra, Sharma, and Kumar 2012). A variety of carcinogenic, reproductive, neurological, immunological, and other adverse effects have been reported to be linked with the exposure of humans to these chemicals (Sharma et al. 2009).

In 2001, the Stockholm Convention was adopted in response to the global concerns about POPs; production and usage of POPs-containing commercial products was banned (Ahad et al. 2010; Alamdar et al. 2014).

OCPs, especially dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), have been detected in the environment (Siddiqui et al. 1981; Jensen 1983; Bhatnagar et al. 1992; Banerjee et al. 1997) and in the adipose tissue (Ceron et al. 1995; Jensen 1983), blood (Bhatnagar et al. 1992; Siddiqui et al. 1981), and milk (Banerjee et al. 1997; Kaphalia and Seth 1983) in humans. Blood has been used in many studies as the most easily accessible matrix for assessing pesticide residues in humans.

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OCPs may come into contact with humans in geographical areas away from the point of their application by wind dissipation, water transport, and large-distance trade of contaminated food (Matsumura 1985).

OCPs were first introduced into Sudan in 1945, with DDT first being used in the Gezira Irrigation Scheme to control cotton pests. This was later followed by dieldrin,  $\gamma$ -HCH (lindane), and other members of this group. In 1982, usage of DDT for agricultural purposes was banned. During the following two decades, all members of this group have been banned except endosulfan and DDT, the latter being restricted to use for indoor control of mosquitoes. Dieldrin and heptachlor epoxide were detected in soil of the Gezira Irrigation Scheme (Abdelbagi, Elmahi, and Osman 2000, 2003). Nile fishes, sesame, and cottonseed oils from Sudan have been reported to contain measurable amounts of OCPs ranging from 0.04 to 16.0 mg kg<sup>-1</sup> (Elzorgani 1981, 1976; Elzorgani and Ahmed 1981; Elzorgani, Abdullah, and Ali 1979).

This study was initiated to determine OCP levels in blood of residents in areas of limited pesticide use. There are no studies published so far on this topic for non-agricultural areas of Sudan.

## Materials and methods

### Chemicals

Analytical standards (99% pure) of DDT,  $\beta$ -HCH, heptachlor epoxide, aldrin, and dieldrin were obtained from Bayer Middle East Ltd. (Khartoum, Sudan). Standard solutions of 1–10 mg L<sup>-1</sup> each were prepared using analytical grade *n*-hexane (Fisons, Manchester, UK). Analytical grade acetone and *n*-hexane (Fisons) were redistilled prior to usage for extraction and cleanup of samples. Analar grade silica gel (60 G) for column chromatography (Applichem GmbH, Darmstadt, Germany), anhydrous Na<sub>2</sub>SO<sub>4</sub>, potassium oxalate (C<sub>2</sub>K<sub>2</sub>O<sub>4</sub>), and KOH were used (BDH, Poole, England).

### Sampling areas

A total of 99 human blood samples (5 mL each) were collected from male donors from five locations representing the agricultural zones of Sudan with limited pesticide use. The samples within each area were selected randomly from different age groups and body weights. The areas surveyed included the riverine areas of Dongola and Elgolid (Northern Sudan), the mechanized rain-fed area of Eastern Sudan (Elgedarif), and the traditional rain-fed areas of Elobaid and Um Rawaba (Western Sudan).

### Sample collection

The donors were questioned about their age, medical history, and length of stay in the region. Candidates were selected if they were residents of that region for the last 10 years and had never traveled out of that region for a significant period. Donors who had chronic disease histories were rejected. The body weights of the donors were recorded using a portable scale. Blood samples were collected by a medical assistant by venous puncture using disposable syringes and transferred immediately to 10-mL vials containing 0.1 mL of an aqueous solution (20%) of potassium oxalate (BDH) as anticoagulant. Vials were tightly closed, labeled with date and sample code number, placed in an ice chest at

–5 °C, and within 2–3 days were transferred to the Central Pesticide Analytical Laboratory, Wad Medani, Sudan, for analysis.

### **Extraction and clean-up**

Blood samples were shaken and gently poured into a chromatographic column (8 mm id × 50 mm) containing acetone pre-wetted silica gel (20 g) held in place with glass wool plug at the bottom. The analytes were eluted with 17 mL acetone (Fisons). The eluents were collected in 100 mL mixing cylinders, and 33 mL of an aqueous solution (2%) of Na<sub>2</sub>SO<sub>4</sub> and 10 mL of re-distilled *n*-hexane were added. The mixtures were carefully shaken for 2 min with an open top to avoid buildup of pressure, transferred to separation funnels, and left standing for 10 min for separation of layers. The extracts (hexane phase) were filtered through cotton wool and mixed with 20 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> (BDH). The solvent was removed by means of a rotary evaporator (Buechi, Flawil, Switzerland) at 40 °C. The dry residues were dissolved in 10 mL hexane and kept in closed vials at –10 °C until gas chromatographic analysis. Recoveries were controlled with samples spiked with solutions (5 mg L<sup>–1</sup>) of the pesticide standards and found to be greater than 90% for all.

### **GLC analysis**

Duplicate samples of each extract were analyzed on a GLC instrument (Carlo Erba Fracto Vap 2101, Milano, Italy), equipped with a Ni<sup>63</sup> electron-capture detector (ECD) and 2 m length × 2 mm (internal diameter, i.d.) glass column packed with 5% OV-210 chromosorb WHP 80–100 mesh. The flow of the N<sub>2</sub> makeup gas of the ECD was 30 mL min<sup>–1</sup>. The operating temperature conditions of the column, injection port, and detector were 200 °C, 250 °C, and 350 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.5 mL min<sup>–1</sup>. Aliquots of 1 µL of sample extract were injected in the splitless mode, opening of the splitter 0.5 min after injection. Extracts of blood samples and analytical standards were injected in an alternating pattern. The oven temperature program was started at 60 °C isothermal for 1 min, increased to 135 °C at a rate of 15 °C min<sup>–1</sup>, followed by an increase of 5 °C min<sup>–1</sup> to 200 °C as end temperature. Concentrations of the pesticides were estimated from the peak areas. Calibration curves were obtained by plotting the concentrations of the standards versus the corresponding peak areas with regression coefficients (*R*<sup>2</sup>) ranging between 0.9895 and 0.9995. The absolute limits of detection (LOD) were 32 pg for β-HCH, 20 pg for aldrin, 25 pg for heptachlor epoxide, 31 pg for DDE, and 30 pg for dieldrin. Blood extracts that displayed the highest concentrations of the pesticides were re-analyzed on the same gas chromatograph but with a glass column (2 m × 2 mm i.d.) packed with 2.5% SE 30 on chromosorb WHP 80–100 mesh.

### **Results**

Gas chromatographic analysis of blood samples revealed the presence of p,p'-DDE, heptachlor epoxide, β-HCH, and dieldrin in blood samples from all locations at frequencies of 100%, 100%, 92%, and 98%, respectively (Table 1). Aldrin was not detected. The level of total organochlorine burden was relatively higher in the traditional rain-fed area (mean 69 µg L<sup>–1</sup>) followed by the mechanized rain-fed area (54 µg L<sup>–1</sup>) and the riverine area (56 µg L<sup>–1</sup>) (Table 1).

In all 20 blood samples collected from the Dongola area, p,p'-DDE, heptachlor epoxide, and dieldrin were detected at levels of 46.75, 18, and 6.5 µg L<sup>–1</sup>, respectively.

Table 1. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in agricultural sectors surveyed.

Insecticides/sector	<i>N</i>	Riverine	Traditional rain-fed	Mechanized rain-fed	Grand mean and range	Samples tested +ve (%)
		40	39	20		
$\beta$ -HCH	Mean	7	15	5	10	92
	Range	ND–44	ND–36	ND–29	ND–44	
Heptachlor epoxide	Mean	12	13	14	13	100
	Range	1–36	2–31	2–82	1–82	
p,p'-DDE	Mean	32	35	31	33	100
	Range	9–77	13–87	4–174	9–174	
Dieldrin	Mean	5	6	4	5	98
	Range	1–19	Nd–18	Nd–11	Nd–19	
Total OC burden		56	69	54		

OC, organochlorines; ND, not detected; *N*, samples analyzed.

Coefficient of correlation (*r*) of total OC burden with age = 0.608\*\* {(i.e. highly significant ( $p \leq 0.01$ ))}.

Coefficient of correlation (*r*) of total OC burden with body weight = -0.204.

$\beta$ -HCH was found in 70% of the samples at a mean level of  $4.75 \mu\text{g L}^{-1}$  (Table 2). There was no correlation to age or body weight. From the Elgolid area, p,p' DDE, heptachlor epoxide,  $\beta$ -HCH, and dieldrin were detected in all 20 blood samples at levels of 17, 5.5, 8.5, and  $2.75 \mu\text{g L}^{-1}$ , respectively (Table 3). There was a weak positive correlation between p,p'-DDE and age of the donor, and between heptachlor epoxide and p,p'-DDE and body weight of the donor.

All 20 blood samples collected in the Elgedarif area, the centre of the mechanized rain-fed sector, contained p,p'-DDE and heptachlor epoxide at levels of 42.25 and  $14.25 \mu\text{g L}^{-1}$ , respectively.  $\beta$ -HCH and dieldrin were detected in 19 samples at concentrations of 5 and  $5 \mu\text{g L}^{-1}$ , respectively (Table 4). A positive correlation was noted between p,p'-DDE and age of the donor, but correlation was weak between levels of p,p'-DDE, dieldrin, and heptachlor epoxide, and donor body weight.

Table 2. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in the blood of residents of the Dongola region and correlation to the age of the donors.

Age group (years)	Bd	$\beta$ -HCH		Heptachlor epoxide		p,p'-DDE		Dieldrin	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
20–25	4	5	ND–11	22	13–36	59	46–77	6	3–10
26–35	8	6	ND–21	17	13–24	38	16–66	6	2–19
36–45	3	4	ND–7	14	10–20	41	28–65	7	4–11
46–70	5	4	ND–10	19	14–28	49	35–64	7	2–9
OC level means		4.75		18		46.75		6.5	
<i>r</i>		-0.162		-0.102		-0.015		0.145	
<i>N</i> + ve (%)		70		100		100		100	

OC, organochlorines; Bd, number of blood donors; ND, not detected; *r*; coefficient of correlation; *N*, samples tested.

Table 3. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in the blood of residents of the Elgolid region and correlation to the age of the donors.

Age group (years)	Bd	$\beta$ -HCH		Heptachlor epoxide		p,p'-DDE		Dieldrin	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
20–25	3	3	2–3	4	4–4	13	9–18	2	1–2
26–35	5	14	4–44	5	2–9	16	9–24	3	1–9
36–45	6	6	2–8	8	7–10	18	12–28	3	1–10
46–70	6	11	3–35	5	1–9	21	10–30	3	1–8
OC level means		8.5		5.5		17		2.75	
<i>r</i>		–0.044		0.147		0.389		0.059	
<i>N</i> + ve (%)		100		100		100		100	

OC, organochlorines; Bd, number of blood donors; *r*, coefficient of correlation; *N*, samples tested.

All 19 blood samples from the Elobaid area contained p,p'-DDE, heptachlor epoxide,  $\beta$ -HCH, and dieldrin at levels of 20.25, 8.5, 10.75, and 4.25  $\mu\text{g L}^{-1}$ , respectively (Table 5). A weak correlation was determined between p,p'-DDE and heptachlor epoxide and age, whereas there was no clear correlation between levels of any of the detected pesticides and body weights of the donors. All 20 samples collected in the Um Rawaba area contained p,p'-DDE and heptachlor epoxide at levels of 54.75 and 17.5  $\mu\text{g L}^{-1}$ , respectively.  $\beta$ -HCH and dieldrin were detected in 19 samples at concentrations of 18.5 and 7  $\mu\text{g L}^{-1}$ , respectively (Table 6). A weak correlation was determined between the blood levels of p,p'-DDE and heptachlor epoxide, and age. There was no clear correlation between levels of the detected pesticides and body weights of the donors.

### Discussion

Residues of one or more OCPs were found in the blood of all of the blood donors in the areas of limited pesticide use. This confirms their potential to be transported from other areas and their environmental persistence (Matsumura 1985; POPs 2004; PIC 2004). Aldrin was not detected in any of the analyzed samples, but this can be explained by its conversion to dieldrin (Matsumura 1985; Gannon and Bigger 1958).

Table 4. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in the blood of residents of the Elgadarif region and correlation to the age of the donors.

Age group (years)	Bd	$\beta$ -HCH		Heptachlor epoxide		p,p'-DDE		Dieldrin	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
20–25	9	7	2–29	12	6–17	16	11–22	4	1–11
26–35	5	2	ND–4	23	2–82	12	4–20	5	ND–11
36–45	3	6	1–9	10	6–16	39	12–92	2	1–4
46–70	3	5	3–8	12	10–13	102	17–174	9	8–9
OC levels mean		5		14.25		42.25		5	
<i>r</i>		–0.061		–0.088		0.656		0.295	
<i>N</i> + ve (%)		95		100		100		95	

OC, organochlorines; Bd, number of blood donors; ND, not detected; *r*, coefficient of correlation; *N*, samples.

Table 5. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in the blood of residents of the Elobaid region and correlation to the age of the donors.

Age group (years)	Bd	$\beta$ -HCH		Heptachlor epoxide		p,p'-DDE		Dieldrin	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
20–25	8	12	3–24	7	2–19	19	13–30	5	1–18
26–35	5	7	1–26	6	2–13	16	10–22	5	ND–18
36–45	3	11	8–17	13	6–17	24	17–30	4	1–7
46–70	3	13	7–17	8	6–12	22	15–30	3	2–5
OC level means		10.75		8.5		20.25		4.25	
<i>r</i>		0.101		0.268		0.296		–0.153	
<i>N</i> + ve (%)		100		100		100		100	

OC, organochlorines; Bd, number of blood donors; *r*, coefficient of correlation; ND, not detected; *N*, samples tested.

In the riverine area of Northern Sudan, p,p'-DDE,  $\beta$ -HCH, and dieldrin were detected in most blood samples. Their presence in human blood samples from this region may be associated with the consumption of contaminated food such as cottonseed oil, sesame oil, and contaminated cotton seed cake for animals. These contaminated products are usually produced in areas where OC insecticides were heavily applied (Elzorgani 1981; Elzorgani and Ahmed 1981). Furthermore, residents of this region are fish eaters, both fresh and salted, and Nile fishes have been reported to contain measurable levels of OCPs (Elzorgani 1976; Elzorgani and Ahmed 1981; Elzorgani, Abdullah, and Ali 1979). In addition, in this region, DDT had been used extensively in malaria control campaigns, and  $\gamma$ -HCH (lindane) for the control of storage pests in their main cash crop faba beans (*Vicia faba* L.) (Sudan Pesticide Registrar, pers. comm.). These findings are similar to those of Subramaniam and Solomon (2006) who discovered high concentrations of  $\beta$ -HCH and DDE in the serum of agricultural and non-agricultural people in India.

Blood samples from both the mechanized and traditional rain-fed areas were found to contain measurable levels of DDE, heptachlor epoxide,  $\beta$ -HCH, and dieldrin. The possible sources of exposure again are consumption of contaminated food, especially dried

Table 6. Organochlorine insecticide residues ( $\mu\text{g L}^{-1}$ ) in the blood of residents of the Um Rawaba region and correlation to the age of the donors.

Age group (years)	Bd	$\beta$ -HCH		Heptachlor epoxide		p,p'-DDE		Dieldrin	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
20–25	4	18	15–20	18	7–25	54	37–64	6	2–11
26–35	3	19	12–26	13	11–15	47	33–55	8	3–15
36–45	9	19	ND–36	15	9–29	38	19–62	4	ND–8
46–70	4	18	15–25	24	15–31	80	63–87	10	8–15
OC level means		18.5		17.5		54.75		7	
<i>r</i>		0.174		0.289		0.234		0.085	
<i>N</i> + ve (%)		95		100		100		95	

OC, organochlorines; Bd, number of blood donors; ND, not detected; *r*, coefficient of correlation; *N*, samples tested.



fish, cotton, and sesame seed oils. The majority of the residents were seasonal workers and small farmers who also consumed dried fishes as a protein staple. Direct spray drift and dust storms may bring measurable levels of OCP residues in direct contact with the human body. These airborne contaminants may be deposited, contaminate the soil, and subsequently the crops grown there. Note that the sampling areas in these two regions were along the axis of seasonal winds that blow from the areas of irrigated cotton and sugarcane production where these pesticides have been heavily used. The use of OCPs in the control of desert locust (*Schistocerca gregaria* Forsk.), sesame seed bug (*Elasmolomus sordidus* F.), and dura andat (*Agonoscelis pubescens* thunb.) cannot be excluded as another source of soil and plant contamination. Measurable soil levels of  $\gamma$ -HCH, heptachlor epoxide, and dieldrin have been reported in Sudanese soils of both limited and intensive pesticide use (Abdelbagi, Elmahi, and Osman 2000, 2003). A highly significant correlation was found between total OC blood burden and age of the donors ( $r = 0.608^*$ ), the highest between DDE levels and age of the donors of the Elgadarif region ( $r = 0.656^*$ ). Correlations with age are expected since these compounds are lipophilic in nature, and therefore they tend to accumulate in the body fat over prolonged exposure (Matsumura 1985). Correlations with body weight were weak and this may be due to the limited range of donors.

The level of total organochlorine burden detected was highest in the traditional rain-fed area, followed by the mechanized rain-fed area and the riverine area. Significant correlation was observed between total organochlorine blood burden and the age of the donors. This indicates risks and concerns that people in the studied areas are exposed to OCPs through different ways and that these pesticides have a potential for long-range transport.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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