Identification and concentration of organochlorine residues in blood of Sudanese workers at Gezira Agricultural scheme

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Abstract

DDT and other pesticides were extensively used in Sudan to control agriculture pests and vectors of malaria, typhus, yellow fever and sleeping sickness. Since 1981, however, DDT use in Sudan is restricted to public health purposes. In 1997, sixty Sudanese males, who were occupational exposed to pesticides in Gezira agricultural scheme were chosen in order to investigate whether there is a correlation between the concentration of these pesticides in the blood and health symptoms such as chronic headache and tremor. The study revealed that there is no correlation between duration of exposure time to pesticides, or concentration of these pesticides in the blood, and these symptoms. It appears, however, that workers suffering from headache or tremor tend to have relatively high organophosphorous (chlorpyriphos) levels in the blood. Extensive epidemic and clinical investigations are recommended to statistically confirm these observations.

1. Introduction

The Gezira scheme was founded in 1913 and is with an area of 153415 ha the most important agricultural scheme in Sudan. It is also the largest Sudanese governmental farm under one management. The Gezira scheme comprises 12 % of the total cultivated area in the country. Main products are cotton, sorghum (dura), wheat, groundnuts and vegetables. With regard to pest management, the total amount of pesticide used in 1995/96 was 65 tons organochlorines (OC) and 68 tons organophosphorous (OP) pesticides [5].

In recent years, man has become increasingly conscious and critical of the environmental pollution by chemicals. OC have been a major cause of concern to ecologists because they are relatively resistant to bio-degradation. OC deposition in biolipids is due to their low solubility in water because of their strongly lipophilic character. These properties explain why OC tend to accumulate in the adipose tissue of the human body to levels that are considered as significant OC residue burdens [8]. Although OC are very persistent, some biodegradation can occur yielding the metabolites shown in Fig 1.

It has been observed that the use of OC in agriculture can lead to chronic intoxications that are characterised by deterioration of the nervous, digestive or cardiovascular systems, or of the blood formation process and the hormone system. Limb tremor and alternations in the electromyograms have been observed in workers exposed to OC and other pesticides during application in the field. The most other reported impairment is the vegetative-vascular type: diencephalic manifestations like headache, dizziness, and paraesthesia of the limbs. Moreover OC pesticides can cause liver and kidney damage [9].

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Positive correlations have been reported between the concentrations of DDT and its derivatives in blood and the human adipose tissue. Blood has therefore been used as an epidemiological tool in the assessment of the total body burden of DDT in various populations, Brown (1970) [1]. With regard to DDT, Hayes and Dale (1969) reported that the severity of clinical signs of poisoning was directly proportional to the concentration of the non-metabolised compounds in the brain.

2. Objective

The study presented in this paper was conducted to determine (i) concentrations of DDT (metabolites included) in the blood of different occupational groups and (ii) to examine whether there is a correlation between co concentrations and the occurrence of clinical symptoms.

3. Subjects and Methods

3.1 Subjects

Blood was collected from 60 Sudanese males working at the Gezira scheme (Central Sudan) in August 1997. Each worker completed a questionnaire regarding age, occupation, body weight, duration of exposure to pesticides, clinical symptoms of chronic intoxication like chronic headache, facial palsy, tremor and abnormal blood pressure. Mean age was 41 years (min/max. 17/70 years), mean body weight 62 kg (min/max. 41/120 kg) and mean duration of exposure 16 years (min/max. 0,11/41 years). The treatment of the results was done on the basis of four different groups of 15 males each. These groups were (A) Maranjan (mainly seed dressers), (B) Agricultural Research laboratory employees (‘white collar’, mainly development of application protocols), (C) Hasahiesa (an area where pesticides were dumped 20 years ago, mainly pesticide applicators) and (D) private sector (mainly fruit garden spraymen). Each group included spraymen, pesticide mixers, supervisors, mechanics, laboratory technicians, store keepers and a few other occupations.

Blood (5 ml) was taken from each worker and placed in a 10 ml glass tube containing 0.1 ml of 20 % aqueous potassium oxalate as an anticoagulant. The mixture was gently agitated by inversion and stored for one day at 5°C prior to analysis.

3.2 Sample preparation

Extraction and clean-up was done at the Agricultural Research Corporation Laboratory in Sudan. The sample was given on a silica gel column (20 g) equilibrated with acetone. The pesticides were eluted with 17 ml of acetone and collected in a 50 ml mixing cylinder. Anhydrous sodium sulphate was added and the mixture was shaken gently for three minutes. Redistilled hexane (10 ml) was added and the mixture was agitated by inversion as before. Phase separation occurred in a separator funnel. The upper layer (hexane) was filtered through glass wool and some anhydrous sodium sulphate was added. The filtrate was dried by evaporation at room temperature over night. The dry samples were transported to the Federal Centre for Nutrition and Foods, Detmold site, Germany, for pesticide identification and quantification.

OC- and OP-pesticide standards containing 80 µg/ml 2’,2,6,6’-Tetrachlorbiphenyl and 135 µg/ml Methacrifos used as internal standard were purchased from Ehrenstorfer (Augsburg, Germany). Samples were dissolved in 1 ml of isooctane (Merck, Darmstadt, Germany).

A gas chromatograph (Varian GC 3600 and GC 3700) equipped with ECD and nitrogen-phosphorous detectors were used. Separation was done on quartz capillary columns of different polarity (50-m * 0.32 mm i.d., film thickness 0.25 µm, CP-Sil-8CB/C18 column) and (50-m * 0.32 mm i.d., film thickness 0.4 µm, CPSIL-19CB column). An aliquot (1 µl) was automatically

injected in the split-less mode and oven temperature was stepwise increased as follows. For OC-pesticide-analyses, GC 3600: 75°C for 1,2 min, then increase to 169 °C (30°C/min), to 250 °C (1.8°C/min), and to 265 °C (2.5 °C/min). For OP-pesticide-analyses GC 3700: 70°C for 1 min, then increase to 265 °C (5°C/min). This temperature was held for 14 min. The injector temperature ranged from 50 to 260 °C and the ECD was operated at 310 °C. Calibration curves for pesticides were recorded with the i.s. method by measuring peak heights vs concentrations. Linear relationships were obtained between 0 and 5 mg/kg with a correlation coefficient of 0.999. Identity of pesticides was confirmed by GC-coupled mass spectrometry (Shimadzu GC/MS QP 2000 equipped with 30-m * 0.25 mm i.d., film thickness 0.25 µm, DB5 column). For ionisation 70 eV electron impact energy was used.

4. Results and Discussion

Because pesticide concentration was high in all samples for analysis, a dilution step (1 to 15 fold) was introduced. Mean concentrations of these pesticides were calculated for all workers and separately for each group (see Tab. 1). DDE accounted for 64 % of total blood DDT. This result nicely compares with those reported by other authors [8,6] who found that DDE accounted for 88 % and 78 %, respectively. No close correlation was found between pesticide concentration and age, body weight, duration of exposure, or occupation. In other studies also no correlation between age, body weight, etc. [2,7] was detected, although there are a few reports suggesting that positive correlations between these health parameters and pesticide concentrations in blood [3,4,11] may exist. Comparisons between our study and previous Sudanese studies [3,4,11], which report blood DDE concentrations in the range of 0,01-012 mg/l; 0,003-0,051 mg/ml and a mean 2,85 ± 0,5 mg/l respectively (for our results see table 1) are hampered by the fact that different analytical methods were used. In our study capillary column separation and GC/MS were used whereas other authors used packed columns [3,4,11]. Having the above mentioned restrictions in mind one can nevertheless conclude those blood concentrations of Gezira scheme workers were very high at that time (1997) by comparison with values determined for workers in other tropical countries [2,6,7,8,10,13]. It is proposed that this difference is due to different methods of pesticides application in the fields. In the Gezira scheme, especially on private sector land, pesticides are often applied manually without any protective devices. There is information that this situation has changed for the better in the recent years. It is therefore possible that pesticide concentration in the blood of workers are now lower than observed in 1997. It is nevertheless interesting that our results are similar to those of one other study conducted in the tropics [12] which also report DDT blood levels for spraymen in the range of 0,009 to 0,548 mg/l (for a detailed account of our results see table 2). It is probable that these workers also did not use protective devices.

Figure 3 shows the frequency distribution of DDT and metabolite concentration in the blood samples. Figure 4 illustrates the concentration of DDT and metabolites *versus* occupation. It turned out that there was no clear-cut relationship between occupation and DDT and metabolite levels. Nevertheless, some individuals (especially from the group „others“, n=15, see table 2) with higher DDT levels were exposed for a relatively short time, while most with lower DDT levels (spraymen) were exposed for longer periods of time (data not shown). Because neurological symptoms such as chronic headache and tremor were general complaints, attempts were made to correlate symptoms with blood pesticide levels. Our results did however not reveal any significant correlation between symptoms and DDT or metabolite levels. The data pool was too small to conclude on whether there was a relationship with the time of exposure [see ref. 9,12]. It appears, however, that workers having in their blood high levels of OC such as lindane, α-HCH, γ-HCH, heptachlor and/or of OP such as chlorpyriphos were prone to produce neurological symptoms (table 2).

In conclusion, our study suggests that it was not only DDT (the use of which is restricted in Sudan to malaria control) but also other pesticides of the OC- and OP-type that constituted health risks for workers at the Gezira scheme. The use of these pesticides must be re-evaluated having in mind the effects on the ecology. Spraymen are at high risk and the use of protective devices is strongly recommended. Firms are encouraged to develop devices suitable for tropical conditions in order to increase the acceptance of protective devices. There is information that mechanization of pesticide application has been furthered at Gezira scheme. This means that pesticide contamination of the workers may have been reduced in the recent years.
It should be emphasized that an extensive study of pesticide concentrations in Sudanese agricultural workers is required before generalising the results obtained at the Gezira scheme.

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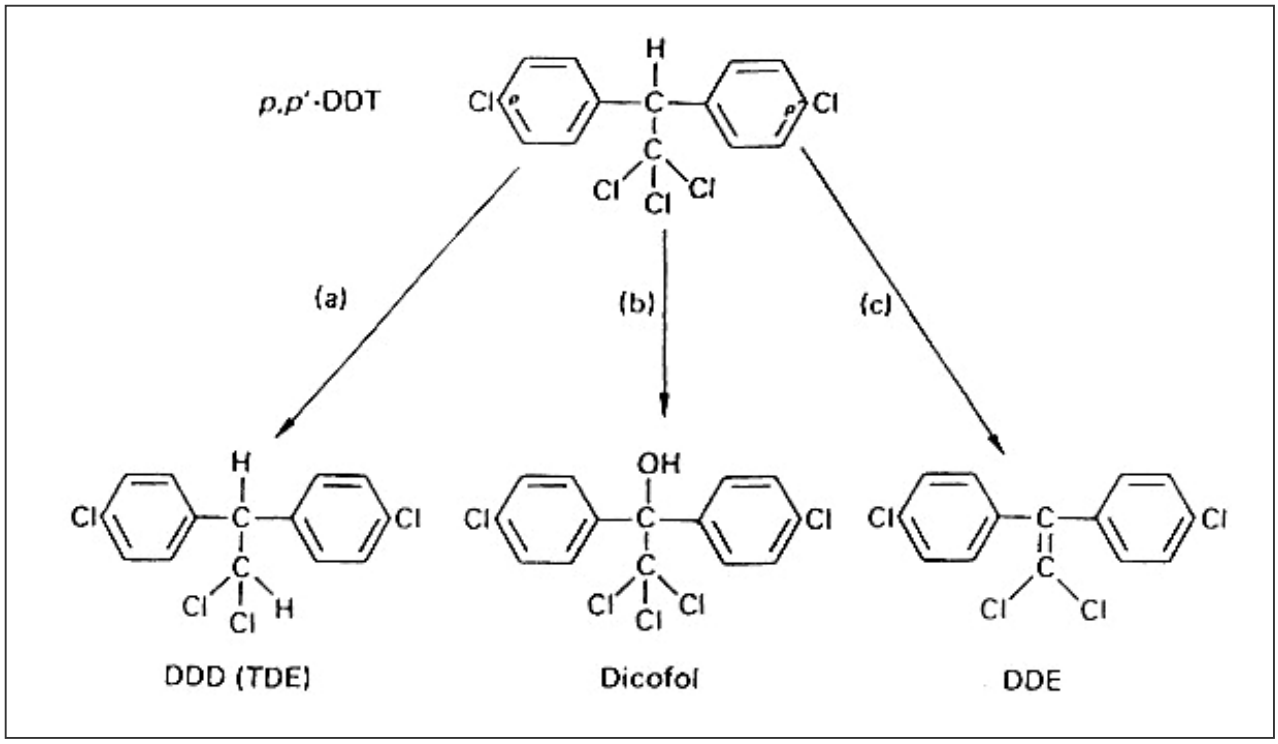

Fig. 1: Metabolites formed from DDT, (a) reductive dechlorination, (b) oxidation, (c) dehydrochlorination.

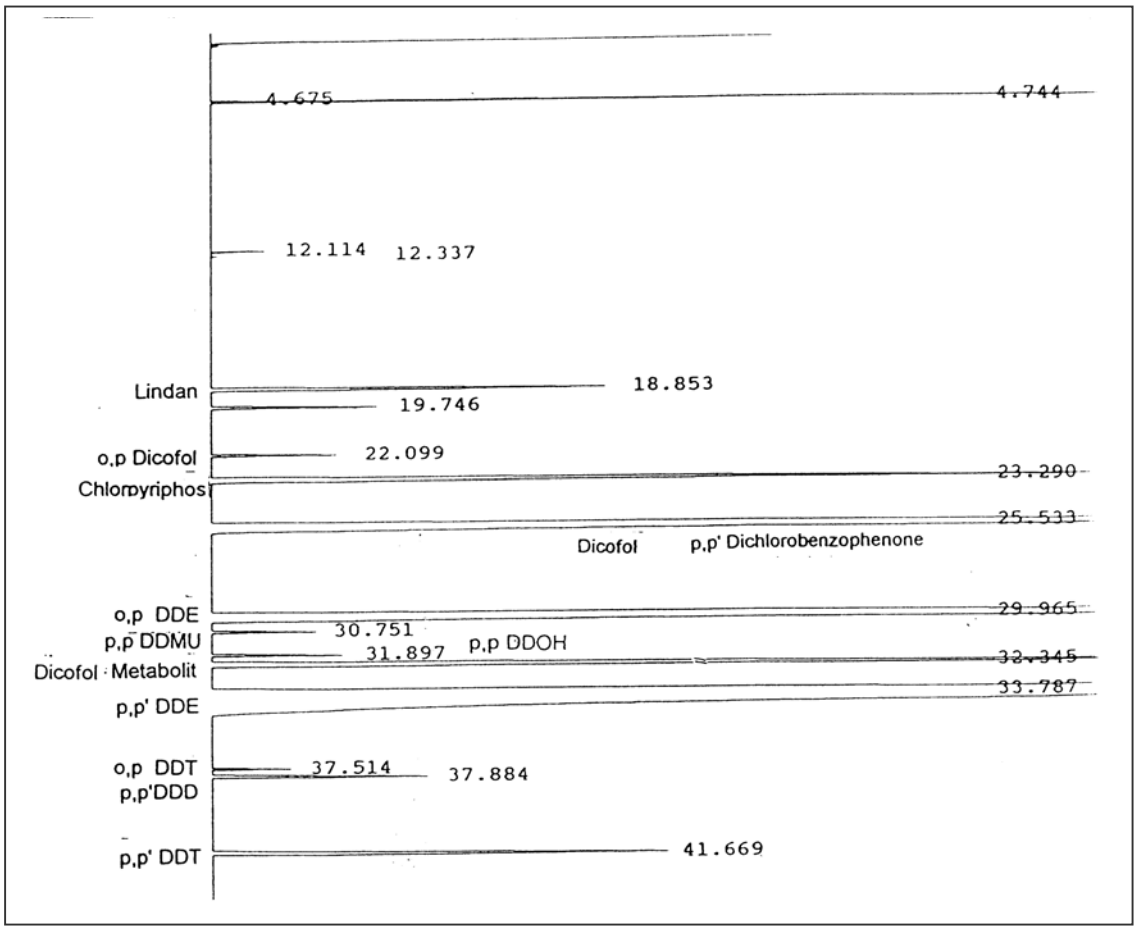

Fig. 2: Gas chromatographic separation of DDT-isomers and metabolites in extracts from blood. ECD was used for detection.

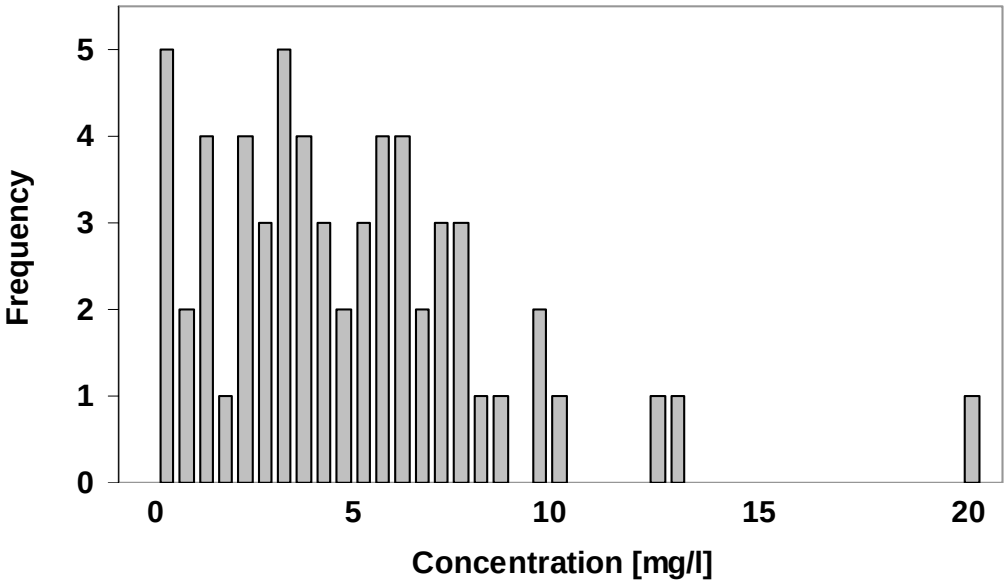

Fig. 3: Number of individuals with different DDT concentrations (metabolites included) in the blood

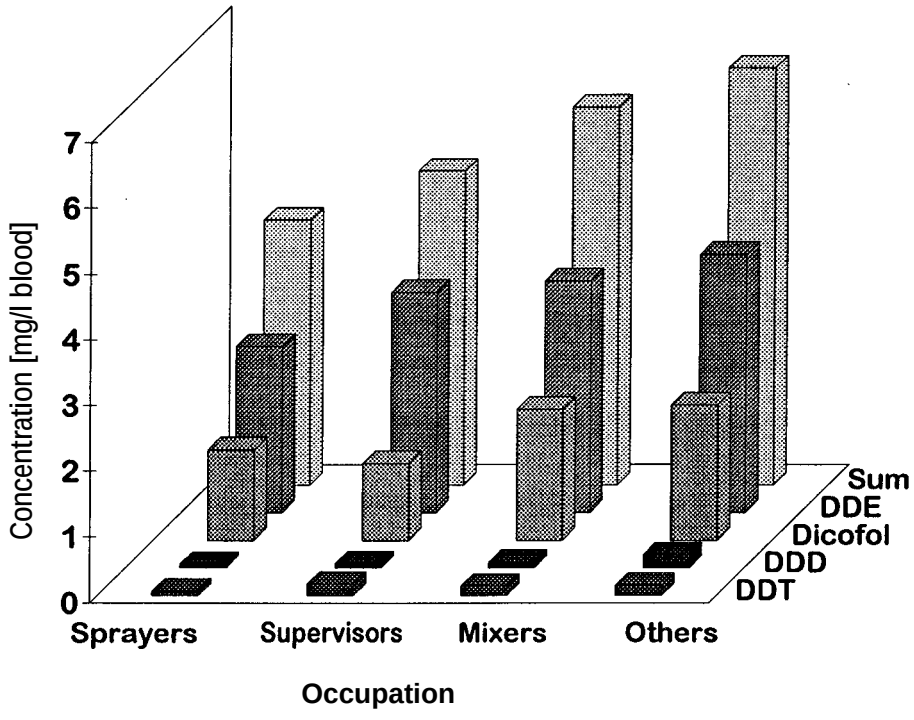

Fig. 4: Mean concentrations of DDT and metabolites in blood samples
(Mean body weight, mean age and mean time of exposure. Sprayers: 57 kg, 40 y, 20 y; supervisor: 68 kg, 46 y, 18 y; mixer: 64 kg, 32 y, 8 y; other: 67 kg, 42 y, 11 y).

Table 1: DDT and metabolites detected in blood samples from Gezira scheme workers

Worker groups	Total (60 workers)			Group A (15 workers) *"Seed dressers"*		Group B (15 workers) *"White collar"*		Group C (15 workers) Hasahiesa (dump)		Group D (15 workers) „Fruit garden spraymen"	
Pesticides	range	Mean	s. d.[1]	mean	s. d.[1]	mean	s. d.[1]	mean	s. d.[1]	mean	s. d.[1]
	[mg/l]			*[mg/l]*		*[mg/l]*		*[mg/l]*		*[mg/l]*	
DDT/metabolites total	**0,01 - 0,58**	**0,12**	**± 0,11**	**0,16**	**± 0,15**	**0,18**	**± 0,11**	**0,11**	**± 0,06**	**0,05**	**± 0,03**
p,p' DDT	0,005 - 0,37	0,08	± 0,07	0,1	± 0,10	0,11	± 0,06	0,08	± 0,04	0,04	± 0,02
o,p DDT	0,002 - 0,21	0,04	± 0,05	0,06	± 0,06	0,07	± 0,06	0,03	± 0,02	0,02	± 0,01
DDE total	**0,08 - 13,64**	**3,15**	**± 2,41**	**3,93**	**± 3,71**	**3,59**	**± 2,22**	**2,89**	**± 1,37**	**2,16**	**± 1,41**
p,p' DDE	0,06 - 12,37	2,79	± 2,16	3,51	± 3,34	3,18	± 1,97	2,56	± 1,21	1,89	± 1,24
o,p DDE	0,01 - 1,27	0,36	± 0,27	0,42	± 0,37	0,42	± 0,29	0,33	± 0,17	0,27	± 0,18
DDD total	**0,002 - 1,03**	**0,10**	**± 0,16**	**0,19**	**± 0,29**	**0,07**	**± 0,04**	**0,08**	**± 0,06**	**0,05**	**± 0,04**
p,p' DDD	0,002 - 0,21	0,02	± 0,036	0,05	± 0,05	0,04	± 0,03	0,04	± 0,03	0,02	± 0,02
p,p' DDMU	0,004 - 0,27	0,03	± 0,052	0,07	± 0,08	0,03	± 0,03	0,05	± 0,04	0,03	±0,03
p,p' DDOH	0,22 - 0,85	0,53		0,53	± 0,44						
Dicofol total	**0,05 - 5,87**	**1,57**	**± 1,18**	**2,09**	**± 1,84**	**1,32**	**± 0,84**	**1,58**	**± 0,79**	**1,28**	**± 0,82**
Dicofol +(*)	0,03 - 3,98	1,09	± 0,84	1,49	± 1,30	0,86	± 0,59	1,11	± 0,58	0,91	± 0,58
o,p Dicofol	0,005 - 0,29	0,07	± 0,07	0,09	± 0,08	0,04	± 0,04	0,09	± 0,08	0,06	± 0,04
Other metabolites	0,02 - 1,63	0,41	± 0,30	0,51	± 0,46	0,43	± 0,28	0,38	± 0,19	0,32	± 0,19
(* p,p' Dichlorbenzophenon)											
HCH/isomers[2] total	**0,001 - 0,13**	**0,023**	**± 0,024**	**0,014 (n=7)**	**± 0,014**	**0,058 (n=6)**	**± 0,053**	**0,015 (n=6)**	**± 0,012**	**0,012 (n=7)**	**± 0,011**
Lindan	0,001 - 0,109	0,02	± 0,027	0,008	± 0,005	0,051	± 0,043	0,014	± 0,012	0,012	± 0,012
delta HCH	0,001 - 0,025	0,01	± 0,009	0,012 (n=3)	± 0,012	0,017 (n=2)	± 0,0007	0,002 (n=1)		0,002 (n=1)	
alpha HCH	0,004 - 0,005	0,005									
Heptachlor	**0,005 - 0,134**	**0,036**	**± 0,046**	**0,008 (n=2)**	**± 0,003**	**0,066 (n=5)**	**± 0,057**	**0,015 (n=2)**	**± 0,007**	**0,01 (n=2)**	**± 0,008**
Chlorpyrifos[3]	**0,01 - 0,28**	**0,13**	**± 0,084**	**(n=0)**	**-**	**(n=0)**	**-**	**0,12 (n=4)**	**± 0,07**	**0,13 (n=15)**	**± 0,088**

The values in brackets give the numbers of blood samples with a metabolited detected. 1) s. d., standard deviation 2) analysed in blood extracts between 2 and 26 workers
3) analysed in blood extracts from 19 workers

Table 2: Occupation and levels of DDT and metabolites in blood samples from Gezira scheme workers

Occupation	Sprayers (n=27)			Supervisors (n=12)			Mixers (n=6)			Others (n=15)		
	mean	s. d.[1]	n	mean	s. d.[1]	n	mean	s. d.[1]	n	mean	s. d.[1]	n
DDT Isomers / metabolites	*[mg/l]*			*[mg/l]*			*[mg/l]*			*[mg/l]*		
DDT total	0,07	± 0,06	27	0,18	± 0,11	12	0,15	± 0,09	6	0,16	± 0,15	15
DDE total	2,53	± 1,71	27	3,36	± 2,1	12	3,53	± 2,39	6	3,93	± 3,48	15
DDD total	0,06	± 0,04	27	0,07	± 0,04	12	0,09	± 0,10	6	0,2	± 0,28	15
Dicofol total	1,38	± 0,87	27	1,17	± 0,72	12	1,99	± 1,51	6	2,06	± 1,64	15
Total in mg/kg body weight	0,073	± 0,05	27	0,076	± 0,046	12	0,094	± 0,066	6	0,102	± 0,088	15
Other organochl. insecticides												
HCH and Isomers	0,016	± 0,016	13	0,043	± 0,056	7	0,005	± 0,005	2	0,022	± 0,014	4
Heptachlor	0,011	± 0,006	5	0,081	± 0,053	4	0,01		1	0,006		1
Organophosphorous insecticides												
Chlorpyrifos *(only group C and D)*	0,13	± 0,084	18			0			0	0,053		1
Persons with clinical symptoms												
Headache			9			*0*			1			7
No HCH detected			5						0			4
No Heptachlor detected			6						0			6
Tremor			13			*0*			1			3
No HCH detected			7						0			1
No Heptachlor detected			10						0			3

1) s. d., standard deviation