

## **Organochlorine Residues in Baluchistan/Pakistan: Blood and Fat Concentrations in Humans**

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Organochlorine (OC)-residues are detected in measurable concentrations in various tissues of human beings because of the worldwide pollution of air, water, soil, and foods. The concentrations vary from region to region according to chemical, climatic, socio-economic, and geographic factors. Polluted air is carried over huge distances (Cohen and Pinkerton 1966), and some chemicals are found almost worldwide, e.g. hexachlorobenzene (HCB), which has been used as fungicide and is mainly emitted by chemical industries now (Zell and Ballschmiter 1980).

Persisting pesticides used in agriculture are found in relevant concentrations in agricultural products, meat, and fish: they enter the nutritional chain (Selenka and Eckrich 1983).

As developing countries face economic pressure to increase their agricultural exports cheap pesticides are sometimes used without the precautions necessary to prevent contaminations of water and food. We conducted a small survey monitoring the OC-concentrations in human blood and fat tissue under the aim to detect more recent as well as elder expositions.

### **MATERIALS AND METHODS.**

All 25 probands were inpatients of the Sandeman Provincial Hospital in Quetta, the capital of Baluchistan Province in Pakistan. All underwent surgical operations for different conditions, mostly laparotomies. The age ranges from 10 to 80 years with a median of 25 years. sixteen probands are males and 9 females. All live in Quetta and an area of about 150 kms around.

Available for the analysis were blood from 21 patients

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and fat tissue from 20 patients; i.e. blood and fat from 16, blood only from 5, and fat only from 4. Half a ml heparinized blood were brought on an Extrelut-1-column<sup>R</sup> covered with glass-wool and filled with about 0.8 g decontaminated Extrelut<sup>R</sup> (5 hrs, 600°C) (Eckrich W, personal communication, 1984). After elution with 10 ml n-hexane the eluate was carefully condensed to about 1 ml at a rotating evaporator. Remaining solvent was evaporated with nitrogen, the residue diluted with 10 ml ethylacetate/cyclohexane (1:1) (V/V), and an aliquot of 5 ml taken for gel-chromatography. After careful condensation of the eluate with the pesticides to 5 ml at the rotating evaporator 10 ml iso-octane were added, the mixture being again condensed to 1-2 ml. Then the eluate was brought on a mini-silica-gel column, washed before with 5 ml n-hexane. After rinsing with 1 ml n-hexane and 2 ml of a mixture of toluene/hexane (35/65) further 6 ml toluene/hexane were added for elution. After careful condensation in the rotating evaporator the residue was filled-up to 1 ml with n-hexane. Blanks and positive controls for all chemicals on investigation were measured.

Of the fat-tissue samples 200 mg were homogenized with 50 ml of an hexane/acetone-mixture (2:1) (V/V) using an Ultra-Turrax for 3 minutes. The homogenate was filtered over a 2 cm thick layer of decontaminated sodium sulfate (5 hrs, 550°C) in a weighed round flask of 250 ml volume. The eluate was condensed to few ml using a vacuum evaporator. Remaining solvent was blown off with nitrogen. The residue was weighed and then dissolved in 25 ml ethylacetate/cyclohexane (1:1) (V/V). Again an aliquot of 5 ml was taken for gel chromatography. Further cleaning and preparation followed the treatment of the samples from blood. The eluates were finally analysed through capillary gas-chromatography (Specht and Tillkes 1985). Identification of peaks was performed by using reference materials and mass spectrometry.

## RESULTS AND DISCUSSION.

Results of the analysis for hexachlorcyclohexan (HCH)-isomeres and for 4,4'-dichlordiphenyl-trichlorethane (DDT) as well as 4,4'-dichlordiphenyl-dichlorethane (DDE) are presented in tables 1 and 2. There is a great range of interindividual differences whereas the HCH- and DDT/DDE-values each are concordant in the individual samples: probands with high concentrations of DDT/DDE in blood also have higher levels of DDT/DDE in fat tissue, high levels of DDT are associated with high levels of DDE. The concentration of pesticide-residues was not correlated with age or sex of the patients. The small representation of different age-groups did not allow statistical calculations.

**Table 1 Organochlorine-residues in blood of probands from Quetta/Pakistan (in µg/l)**

Sample	α-HCH	β-HCH	γ-HCH	4,4'-DDE	4,4'-DDT
1	0.47	0.80	0.27	4.66	0.65
2	0.02	3.02	0.18	8.58	0.57
3	0.08	2.22	0.34	9.27	2.09
6	0.21	n.d.	0.19	1.42	0.17
7	1.88	1.92	0.36	0.94	0.85
8	1.78	6.05	0.53	11.37	0.80
9	0.92	1.23	0.31	0.53	0.21
11	n.d.	1.46	0.34	1.58	n.d.
12	0.04	3.02	0.15	32.61	1.90
13	0.77	1.44	0.05	8.44	n.d.
14	0.14	7.16	0.48	30.60	3.23
15	0.30	1.39	0.19	7.57	1.35
16	0.31	1.84	0.73	6.17	n.d.
17	0.07	0.47	0.41	3.32	n.d.
18	n.d.	0.75	n.d.	3.13	n.d.
20	n.d.	0.94	0.31	16.34	0.09
21	n.d.	0.45	n.d.	9.25	4.83
22	n.d.	n.d.	n.d.	8.99	0.17
23	n.d.	0.95	0.29	1.01	n.d.
24	n.d.	1.16	n.d.	18.98	1.61
25	1.33	5.60	0.56	9.64	1.20

**Table 2 Organochlorine-residues in fat tissue of probands from Quetta/Pakistan (in mg/kg)**

Sample	α-HCH	β-HCH	γ-HCH	4,4'-DDE	4,4'-DDT
1	0.04	1.62	0.03	5.16	0.71
2	0.01	4.36	n.d.	15.35	2.05
3	0.06	2.35	0.42	7.11	1.88
5	0.05	0.57	0.02	2.41	0.82
6	0.02	0.38	n.d.	n.d.	1.44
8	4.08	21.05	n.d.	81.83	10.10
9	n.d.	0.86	n.d.	1.14	0.07
11	0.02	0.14	n.d.	0.64	0.16
12	0.37	1.38	n.d.	12.54	1.17
13	0.47	0.95	0.04	4.36	0.67
15	0.21	0.72	0.03	2.93	0.62
16	0.12	0.88	0.03	3.38	0.62
18	0.06	1.68	n.d.	4.32	1.65
19	0.30	5.89	n.d.	5.66	1.52
20	0.09	0.53	n.d.	12.04	0.84
21	0.01	0.18	n.d.	2.74	1.42
22	n.d.	0.32	n.d.	31.24	2.07
24	0.06	0.70	n.d.	6.88	0.91
26	0.07	0.90	0.01	3.50	0.54
27	0.12	1.78	n.d.	17.92	0.54

n.d.: not detectable

We could not allocate higher and lower levels of pesticide-residues to different places of residence or different environments of the patients: we found for each village or living area low and high concentrations.

Not included into the tables is one eminent finding: there was no measurable concentration of hexachlorbenzene (HCB) in any sample from Quetta.

The role of organochlorines as pathogenic agents is questioned as no obvious symptoms of chronic intoxication of man have been reported excluding all other pathogenetic impacts. On the other hand we have strong indicators of an impact of DDT on the activity of microsomal enzymes in the liver (Zielhuis 1978, Kolmodin-Hedman 1974). Exposure to  $\gamma$ -HCH is found associated with an hyper- $\alpha$ -lipoproteinaemia (Carlson and Kolmodin-Hedman 1977). Chronic exposure to HCH is associated with neuropathies, alterations of EEG and EMG, tinnitus and psychotic episodes (Schüttman 1972), and immunosuppression (Hosler et al. 1980).

Pesticide-residues in man extremely vary from country to country, even from region to region (Astolfi et al. 1974, Siddiqui et al 1981, Albert et al. 1980, Abbott et al. 1981, Niessen et al. 1984). Doing the analyses in 1987 in Germany we compared the levels from Quetta with levels from Northwestern Germany (Löffelmann G, unpublished data, Bochum, FRG, 1987) (Table 3 and 4).

Table 3: Median- and max.-levels of chlorinated hydrocarbons, blood, Quetta and W-Germany ( $\mu\text{g/l}$ )

		Quetta	F R G
4,4'-DDT	median	0.61	0.13
	max	4.83	0.38
4,4'-DDE	median	8.58	2.4
	max	32.61	6.5
$\alpha$ -HCH	median	0.08	0.004
	max	1.88	0.018
$\beta$ -HCH	median	1.39	0.33
	max	6.05	1.70
$\gamma$ -HCH	median	0.29	0.035
	max	0.56	0.31

Table 4: Median- and max.-concentrations of chlorinated hydrocarbons, fat, Quetta and W-Germany (mg/kg)

		Quetta	F R G
4,4'-DDT	median	0.87	0.09
	max	10.10	0.42
4,4'-DDE	median	4.76	0.90
	max	81.83	4.30
$\alpha$ -HCH	median	0.06	0.002
	max	4.08	0.035
$\beta$ -HCH	median	0.89	0.13
	max	21.05	0.40
$\gamma$ -HCH	median	--	0.005
	max	0.42	0.27

Except the median for  $\gamma$ -HCH in fat tissue the median-concentrations of all pesticides are much higher in Quetta than in Germany. For  $\gamma$ -HCH-levels in fat from Quetta no median has been calculated as in 13 out of 20 samples the concentration of  $\gamma$ -HCH was below the limit of detection.

The fact that no HCB was detected in any sample from Quetta is interesting. We suggest that HCB is not applied as fungicide and not used in chemical plants in the area. The global spread of HCB in the air alone does not cause a contamination of human blood and fat tissue as it could be suspected from the findings of other investigators (Schauerte et al. 1982).

Recent studies (Drijver et al. 1988) and preliminary results of our own work in Northern Germany indicate that residues of OC are mobilised during lactation and secreted with breastmilk. The OC then accumulate in the infant's fat-tissue again. As long as we do not know about pathogenic or copathogenic effects of organochlorine-derivates and as prolonged breastfeeding is essential for the survival of children in developing countries, efforts should be made to decrease environmental contaminations and human exposure.

#### Acknowledgments.

We appreciate the extremely kind cooperation of the staff of the Departments of Surgery and Anaesthesia of the Sandeman Provincial Hospital, Quetta/Pakistan.

We also thank Mrs.Dr.A.Lommel, Kiel, FRG, who revised a huge number of publications on organochlorine-residues in humans and animals.

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Received March 6, 1989; accepted June 9, 1989.