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Chlordane Residues in Rat and Monkey Tissues Following Subchronic Inhalation Exposure to Technical Chlordane

Abdallah M. Khasawinah

Velsicol Chemical Corporation, 5600 N. River Road,
Rosemont, Illinois 60018, USA

Chlordane is a cyclodiene insecticide that was introduced in 1945. It has been used for control of insects on vegetables, small grains, oil seeds, potatoes, sugarcane, sugar beets, fruits, nuts, cotton and jute. In recent years its use in the U.S.A. has been limited to termite control.

The metabolism of chlordane and its residues in animals following oral intake has been documented (Balba and Saha 1978, Barnett and Dorough 1974, Dorough and Hemken 1973, Polen, et al. 1970, Street and Blau 1972, Tashiro and Matsumura 1977). However, there are no published studies on chlordane residues in animals following inhalation exposure. This study sought to investigate the residues in the blood and tissues of rats and monkeys (both sexes) during 90-day subchronic inhalation exposure to technical chlordane. The effect of discontinued exposure on the depuration of residues in rats was also investigated.

MATERIALS AND METHODS

In a subchronic 90-day inhalation toxicity study, four groups each of albino rats and cynomolgus monkeys were exposed to technical chlordane vapors and aerosol at target concentrations of 0, 0.1, 1.0 and 10 ug/l. (Khasawinah et al. 1988). The exposure regiment consisted of 8 hours per day, 5 days a week for a 90-day duration. At days 57, 90 and 180 (90-days after last exposure) five rats from each group and each sex were sacrificed. Blood, liver and adipose tissues samples were removed for analysis. Additionally blood samples were taken pre- and post exposure from 2 female and 2 male rats of the 0.1 ug/l group during the first five days of exposure and from one male and one female rat on each of the following 2 days. Pre exposure samples were collected by orbital sinus puncture and post exposure and on non-exposure days samples were collected by cardiac puncture. The

Table 1. Average total chlordane residues in monkey blood & tissues

Exposure (ug/l)	Interval (days)	Tissue	Av. Total Residue (ppm) Male	Female
0.1	90	A	3.4 ± 1.1	2.6 ± 0.4
1.0	90	A	23.7 ± 0.8	21.3 ± 0.5
10.0	90	A	173.5 ±20.1	122.4 ±36.1
0.1	90	L	0.75 ± 0.31	0.80± 0.27
1.0	90	L	3.43 ± 0.94	2.70± 0.69
10.0	90	L	10.80 ± 2.04	9.56± 1.96
0.1	31	R	0.001± .001	ND
1.0	31	R	0.009± .002	0.009±0.003
10.0	31	R	0.083± .018	0.095±0.055
0.1	90	R	0.003± .001	0.003±0.001
1.0	90	R	0.024± .006	0.032± .011
10.0	90	R	0.173± .038	0.200± .159
0.1	31	P	0.002± .002	0.001± .001
1.0	31	P	0.016± .005	0.015± .005
10.0	31	P	0.174± .040	0.191± .086
0.1	90	P	0.004± .001	0.006± .001
1.0	90	P	0.045± .015	0.061± .028
10.0	90	P	0.390± .050	0.358± .177

A=Adipose, L=Liver, R=Red Blood Cells, P=Plasma

latter procedure was terminal. For the monkeys, blood samples were taken from six monkeys from each sex and exposure group at day 31 of exposure and at terminal sacrifice time of 90 days exposure. Liver and adipose tissue samples were also taken from the monkeys at the 90-day sacrifice time. Blood was collected into tubes containing heparin, centrifuged to separate plasma and red blood cells. All samples were stored frozen until analysis.

Extraction procedures varied for the blood and tissues. Plasma and RBC samples (1g) were extracted in conc. formic acid (1 ml) for 0.5 min., followed by 3 x 10 ml portions of pentane. The extract was passed over anhy. Na_2SO_4, followed by the addition of 1 ml of n-hexane. The extract was then reduced to 1 ml on a 65°C water bath, followed by treatment with 0.5 ml conc. H_2SO_4 for 0.5 min. The hexane layer was transferred quantitatively to a tube containing 100 mg solid Na_2CO_3 and diluted to 5.0 ml for analysis.

Table 2. Average total chlordane residues in rat blood

Exposure (ug/l)	Interval (days)	Tissue	Av. Total Residue (ppm) Male	Female
0.1	5	R	0.007	0.010
0.1	57	R	0.007± .001	0.010± .003
1.0	57	R	0.052± .008	0.059± .004
10.0	57	R	0.178± .033	0.355± .057
0.1	90	R	0.011± .002	0.015± .006
1.0	90	R	0.038± .003	0.075± .014
10.0	90	R	0.204± .046	0.335± .103
0.1	D	R	0.001± .0001	0.006± .001
1.0	D	R	0.009± .002	0.015± .004
10.0	D	R	0.028± .004	0.074± .023
0.1	57	P	0.007± .002	0.006± .004
1.0	57	P	0.056± .010	0.048± .018
10.0	57	P	0.230± .127	0.287± .033
0.1	90	P	0.008± .003	0.012± .003
1.0	90	P	0.045± .011	0.059± .024
10.0	90	P	0.212± .071	0.330± .118
0.1	D	P	0.001± .001	0.004± .001
1.0	D	P	0.014± .006	0.012± .004
10.0	D	P	0.038± .010	0.091± .031

D=Depuration, R=Red Blood Cells, P=Plasma

The rat adipose (1 g) was extracted in a tissue grinder with five 2.0 ml portions of n-hexane. A 2.0 ml portion of the extract was removed to a tube and diluted to 5.0 ml with n-hexane followed by treatment with 1.0 ml conc. H_2SO_4 and mixed gently by inverting (to avoid emulsion formation) until the hexane layer was clear. The mixture was centrifuged and the hexane layer removed quantitatively. The acid phase was washed with an additional 2.0 ml n-hexane. The hexane phase was treated a second time with conc. H_2SO_4. The hexane phase was then reduced to 2.0 ml and mixed with 2 mg of 9:1 Na_2SO_4: Na_2CO_3. The monkey adipose (1g) was homogenized for 3 min. in 25 ml n-hexane followed by rinsing in hexane to a 50.0 ml volume. A 20.0 ml portion of the extract was removed and reduced on a water bath to 5.0 ml and treated twice with conc. H_2SO_4 as above.

Liver samples (2.0g) were extracted by homogenizing (2 min) in 25.0 ml acetonitrile and filtering through

glass wool and rinsed to a final volume of 50.0 ml. A 12.5 ml aliquot was removed and concentrated on a water bath to 0.1 ml. This was diluted to 5.0 ml with n-hexane and treated once with con. H_2SO_4 as above.

Table 3. Average total chlordane residues in rat tissues

Exposure (ug/l)	Interval (days)	Tissue	Av. Total Residue (ppm) Male	Female
0.1	57	A	3.7 ± .2	6.2 ± 1.1
1.0	57	A	14.3 ± .8	26.2 ± 2.9
10.0	57	A	47.2 ± 9.6	156.6 ±17.9
0.1	90	A	3.7 ± .3	6.9 ± 1.7
1.0	90	A	16.0 ± 3.5	37.4 ± 6.3
10.0	90	A	69.3 ±14.8	198.2 ±72.1
0.1	D	A	1.3 ± .3	3.3 ± .4
1.0	D	A	5.6 ± .6	11.4 ± 1.0
10.0	D	A	14.3 ± 1.0	39.7 ± 2.8
0.1	57	L	0.23± .07	0.25 ± .10
1.0	57	L	0.90± 0.20	1.15 ± .15
10.0	57	L	3.46± .70	4.74 ± 1.53
0.1	90	L	0.16± .02	0.37 ± .11
1.0	90	L	1.12± .24	1.90 ± .30
10.0	90	L	4.22± 1.22	6.84 ± 1.23
0.1	D	L	0.06± .03	0.09 ± .02
1.0	D	L	0.20± .06	0.53 ± .15
10.0	D	L	0.49± .08	1.13 ± .18

A=Adipose, L=Liver, D=Depuration

Extracts were analyzed on GC/ECD (Fig. 1) for six chlordane components and two chlordane metabolites.

The limit of detection for each of the six chlordane components (Compound C, Compound E, heptachlor, *cis*- and *trans*- chlordane, and *trans*- nonachlor) and oxychlordane and heptachlor epoxide was 0.5 ppb in the blood and 2.0 ppb in the tissues. Recoveries from the fortified control samples processed in same manner as the test samples ranged from 92.3 - 101%.

The conc. H_2SO_4 treatment was used as a clean up procedure to improve the GC resolution of the chlordane residues. This treatment is known to remove

interferences with no adverse effect on the residues (Hernandez et al. 1987).

Table 4. Percent distribution of chlordane residues in 10 ug/l 90-day exposure groups (males)*

Tissue	Compound C	Oxychlordane	Heptachlor Epoxide	trans-Nonachlor
		Monkeys		
R	23	18	17	38
P	14	14	12	55
A	11	14	7	62
L	16	19	18	44
		Rats		
R	41 (14)	36 (85)	14 (1)	2 (0)
P	29 (7)	43 (92)	23 (1)	5 (0)
A	19 (5)	51 (90)	13 (11)	13 (4)
L	29 (8)	43 (89)	24 (2)	4 (1)

A=Adipose, L=Liver, R=Red Blood Cells, P=Plasma
*Figures in brackets represent 90-day depuration. Male and female data in monkeys were comparable. Data from the 0.1 and 1.0 ug/l groups were also comparable. Female rats had higher oxychlordane and lower compound C percents compared to male rats.

RESULTS AND DISCUSSION

This study was part of a subchronic inhalation toxicity study. It was not intended to be a metabolism, excretion and pharmacokinetics study. Therefore the emphasis in this study was to determine the chlordane residues and its metabolites and to determine any species and sex differences, as well as to determine the effects of discontinued exposure on depuration of residues. Important findings are reported here.

Residues in blood and tissues were directly proportional in a logarithmic fashion to the inhalation exposure level (Tables 1, 2, 3). The adipose contained the highest residues. The ratio of the total adipose residues to the liver residues was 5-20, while that of the adipose to the total blood (serum and RBC) residues was 200-300 after 90 days of exposure to technical chlordane.

In monkeys plasma residue concentrations were nearly

twice of the RBC's, while in rats they were comparable (Tables 1 and 2). The rat data (Table 2), suggests that residues in the blood reach equilibrium after very short period of exposure (4-5 days) and remain constant in spite of the continued exposure. Rat blood samples taken from the 0.1 ug/l exposure group during the first week showed level of 0.007-0.01 ppm by the fifth day of exposure and remained relatively constant in the 57 and 90 day samples. This observation is consistent with the known short half life of chlordane in the blood (Ewig et al. 1985). The monkey data also (Table 1) shows that blood residues did not show significant increase after the first sampling period. Tissue residues also seem to plateau after certain period of exposure (Table 3). The rat data (Table 3) suggest that at the high exposure level of 10 ug/l residues continued to increase (though slightly) through the exposure period. This may be an indication that metabolism and elimination of chlordane is more efficient at the lower exposure levels.

The effect of discontinued exposure on the depuration of residues was examined in rats (Tables 2 & 3). There was 75-90% reduction in the residues over a 90-day period. The sharpest decline occurred in the liver and blood. The source of the residues in the blood must be the result of mobilization and metabolism of Chlordane in the tissues mainly adipose.

An important observation was the sex difference in rats regarding residue concentrations of chlordane and its metabolites (Tables 2 & 3). Females had consistently higher residues (2-3 times) more than the males. This was also observed in rats by other researchers following oral feeding of cis- and trans-chlordane isomers (Street and Blau 1972). Such a sex difference was not observed in monkeys.

Oxychlordane, compound C, heptachlor epoxide and trans-nonachlor accounted for at least 95% of the residues in blood and tissues (Table 4). Occasionally, traces of heptachlor, cis- and trans-chlordane (major components of technical chlordane) were detected in tissues. This confirms the efficient metabolism of these isomers (Barnett and Dorough, 1974). In monkeys, trans- nonachlor (a major component of technical chlordane) was the dominant residue comprising nearly 50% of the total residue. In rats trans- nonachlor was minor one, while the oxychlordane metabolite was a major one. This suggests that rat liver is more efficient in the metabolism of trans-nonachlor than the monkey liver. In Vitro studies have reported that rat liver is more

efficient than human liver in the metabolism of trans-nonachlor. During the depuration period oxychlordane became dominant (90%) in rat and blood tissues.

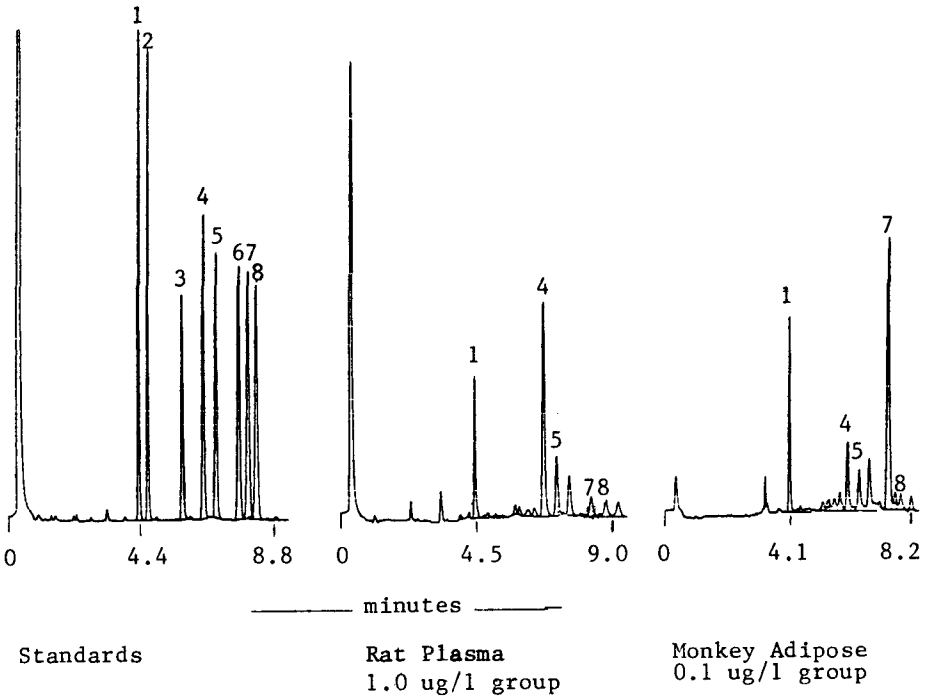

Figure 1. Representative GLC chromatograms.
1: Compound C, 2: Heptachlor, 3: Compound E, 4: oxychlordane, 5: heptachlor epoxide, 6: trans-chlordane, 7: trans-nonachlor, 8: cis-chlordane
GC Column: Fused silica, WCOT, 15m x 0.25mm i.d., 0.2 mm film thickness OV-17(SP-2550). Gas chromatograph: Hewlett Packard, model 5880 with Ni-63 EC detector. Carrier gas: Helium 2ml/min. Purge gas: 5% methane in argon 30ml/min. Temperature 300°C (detector), 250°C (injector). Column temperature 100°C initial for 0.5 min., ramped at 30°C per min. to 180°C and held for 4 min. A Hewlett Packard model 7671A auto sampler and a Varian Vista model 401 were used.

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