

Residue Levels of Polychlorinated Terphenyls, Polychlorinated Biphenyls and DDT in Human Blood

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Since 1966 when the environmental pollution by PCBs was detected by Jensen, many investigators identified PCB residues not only in the samples from environment such as bird, fish and animal but also in human milk, blood and adipose tissue. These results indicate that PCBs are widely dispersed through the biota.

PCTs which are used in similar industrial application to PCBs may be expected to accumulate in biological systems because of the similar chemical properties which these two substances share.

While their presence in environment has been reported by several investigators(1-6), PCTs have not recieved so far as wide attention as PCBs.

Zitko identified the PCT residues in the eggs and the fatty tissue of herring gull(1), and Freudenthal found its presence in the river water, oyster and human fat(2). It was shown by Thomas that paperboard and food packing material contained PCTs(3). Nishimoto identified PCT residues in human fat and milk, but not in such food materials as meat, dairy products, vegetable and fish, and in the samples of water and sludge(4). Minagawa reported the residues of PCTs in human fat, some food and food packing materials and wild birds(5). We reported the levels of PCT in human fat collected from the Tokyo area(6).

This paper describes the detection of PCTs as well as PCBs and DDT compounds in human blood.

Materials and Methods

The analyses were made with 27 samples of human blood offered by the volunteers, 19 males and 8 females from 21 to 57 years old, in Tokyo Metropolitan Research Laboratory of Public Health in November 1973. No occupational contacts with PCBs and PCTs were recognizable with these subjects.

above.

We confirmed PCT residues in human blood using gas chromatograph-mass spectrometer, Simazu-LKB 9000, coupled to Simazu GC-MS PAC 300 computer. As the quantity of the sample blood collected from the subjects was not sufficient to allow confirmation using the mass spectrometer, the 600 ml of whole blood was purchased from Japan Red Cross Central Blood Center. This blood sample was shown to contain 3 ppb of PCT residues, when calculated from the peak heights in its chromatogram.

Results and Discussion

The gas chromatograms of PCT and PCB residues in the blood are shown in Fig.1 and Fig.2, respectively.

The peak profiles in the chromatograms of human blood and fat which we reported in the previous paper are quite similar to that of KC-C. No contamination of PCBs and PCIs during the analytical procedure was ascertained.

All the samples examined were found to contain substantial quantities of PCTs, PCBs and DDE (Table 1).

Table 1, Residue levels of PCBs, PCTs and DDE in Human Blood (ppb on whole blood basis)

Age	Sex	PCB	PCT	DDE	Age	Sex	PCB	PCT	DDE
24	M	3.1	7.0	7.9	38	M	5.8	1.3	13.8
"	"	3.4	5.7	12.2	40	"	3.0	4.0	12.2
25	"	2.8	4.5	18.2	42	"	2.5	7.2	6.7
26	"	3.0	11.8	7.9	"	"	2.5	4.2	8.5
"	"	5.1	0.7	17.2	54	"	5.7	2.0	19.6
27	"	3.0	4.9	10.1	21	F	2.3	7.8	9.3
"	"	2.2	1.1	6.4	22	"	4.0	19.6	12.8
29	"	5.1	1.8	12.1	"	"	2.7	4.2	6.0
"	"	2.7	4.0	3.8	23	"	3.2	2.6	9.6
30	"	3.5	1.9	14.0	25	"	2.5	5.3	8.5
31	"	2.3	2.9	12.4	35	"	2.9	10.5	3.2
35	"	1.9	3.3	9.8	36	"	2.7	3.2	10.3
"	"	2.8	4.9	18.2	46	"	3.1	4.0	18.9
36	"	2.4	5.4	13.7					
		PCB	PCT	DDE					
Mean value		3.2	5.0	11.2					
Max.		5.8	19.6	19.6					
Min.		1.9	0.7	3.2					
σ		1.05	3.95	4.5					

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E in (s)	
CT	DDE
3.3	13.8
4.0	12.2
4.2	6.7
4.2	8.5
4.0	19.6
4.8	9.3
4.6	12.8
4.2	6.0
4.6	9.6
4.3	8.5
4.5	3.2
4.2	10.3
4.0	18.9

Ten grams of whole blood in a 50 ml centrifuge tube was shaken vigorously with 20 ml of ethanol. Five grams of potassium hydroxide was added to this and heated at 90°C for 1 h. After cooling, the content was extracted with 2 additional 20 ml portions of n-hexane. The hexane extract was separated by centrifugation and transferred to a 50 ml separate funnel and washed twice with 20 ml of sulphuric acid(96-98%), followed by passing through the column consisted of each 1 g of silicic acid, Florisil and anhydrous sodium sulfate piled on this sequence. PCTs, PCBs and DDE are eluted from the column with hexane and initial 100 ml of fraction was collected. The eluate was concentrated to 1 ml prior to gas chromatography.

The checking of possible contamination during the analytical procedure was made on the type of syringes which were used for blood collection by washing them with hexane which subsequently followed the same analytical procedure as the blood samples.

The gas chromatograph used was a Simazu GC-5AP3 instrument fitted with ⁶³Ni electron capture detector. The gas chromatographic operating conditions for PCB and DDE analyses were as follows :

Column dimensions : 2 m' x 3 mm (i.d.) glass,
Column packing : 2% OV-1 on Gas-Chrom Q 80/100 mesh,
Column temp. : 200°C, Detector temp. : 250°C
The operating conditions for PCT analysis were as follows :
Column dimensions : 1.5 x 3 mm (i.d.) glass,
Column packing : 2% OV-1 on Gas-Chrom Q 80/100 mesh,
Column temp. : 280°C, Detector temp. : 320°C.

Quantitation of PCB residues was made by comparing respective height of several peaks in the sample with the height of corresponding peak in the chromatogram of a mixture of KC-500 and KC-600 in the proportion of 1:2 which showed a similar peak profile to that of sample blood. The calculation of PCB quantity in each peaks of the standard mixture followed the method presented by Ugawa et al(7) KC-500 and KC-600 are technical PCBs produced by Kanegafuchi Chemical Industry Co. in Japan, corresponding to Aroclor 1254 and 1260 respectively.

The PCT residues in the blood samples were quantified by comparison of total peak height of the 12 major peaks given by KC-C and those given by the samples. KC-C is a technical PCTs produced by the same company as described

The mass spectrum shown in Fig.3 identifies some PCTs present in human blood.

The average concentrations of PCTs, PCBs and DDE were 5.0 ppb, 3.2 ppb and 11.2 ppb, respectively.

So far as we are aware, no one has reported presence of PCT residues in human blood. Statistical analyses showed significant difference at the 5% level between residue levels of PCTs and PCBs, and no correlation between them.

It is of interest that the level of PCT residues in human blood is higher than that of PCBs in spite of lesser industrial output of the former ; the amount of PCBs produced and imported in Japan up to 1971 was 58,000 t (8) and that of PCTs is estimated as 2,000 - 3,000 t.

The levels of DDE obtained in this study consist of the sum of DDE originally present in the sample blood and those transformed from p,p'-DDT by alcoholic alkaline hydrolysis which converts p,p'-DDT to p,p'-DDE and p,p'-DDD to p,p'-MDE (10).

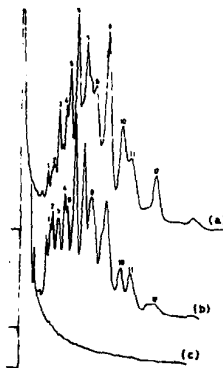


Figure 1. Gas chromatogram of PCT residues in human blood.

(a) : KC-C. (b) : Human blood. (c) : Reagent blank.

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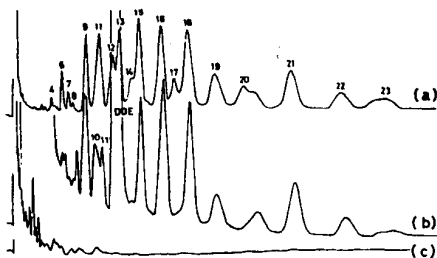


Figure 2. Gas chromatogram of PCB residues in human blood.

(a) : PCB standard (KC500 : KC600 = 2 : 1).
(b) : Human blood. (c) : Reagent blank.

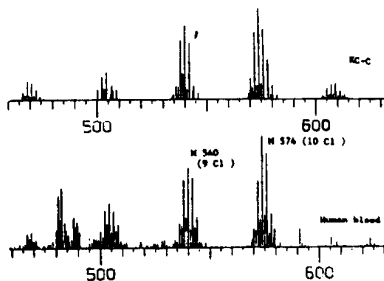


Figure 3. Mass spectrum of PCT residues in human blood at ionization energy of 70 eV.

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As far as the levels of these substances are concerned, DDT compounds were most abundant in the blood.

Available data (9) indicate that about 70% of the DDT in human fat and milk is present in the form of DDE and no p,p'-DDD is detectable. As DDT and its metabolites are not individually analysable, we are unable to demonstrate the absence of DDD in human blood. However, if we assume that the constitution of DDT compounds in human blood is not significantly different from those in human fat and milk, we may consider that the levels obtained in this study represent approximate levels of DDT in the sample blood.

The Ministry of Health and Welfare reported that the average level of PCB residues was 7.8 ppb in human blood collected from 128 subjects in the 7 prefectures where PCB pollution is regarded high, and the average level of PCBs was about 3 ppb (n=16) in Simane prefecture where the pollution is supposedly low (11). A study on the 37 Yusho patients and equal number of control subjects showed that the average PCB concentration in the blood of the former was twice as high as of the latter (6 ppb compared with 3 ppb) (12). The level of PCBs in Osaka prefecture was 3 ppb, much the same as obtained in this study (13).

Acknowledgement

The authors wish to thank Dr. G. Ohi for the preparation of the sample and helpful discussion.

We are also indebted to Dr. H. Yagiu for his valuable advices and encouragement.

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**Lack of Cytogenetic Effects in Bone Marrow
and Spermatogonial Cells in Rats
Treated with Polychlorinated Biphenyls
(Aroclors 1242 and 1254)***

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The polychlorinated biphenyls (PCBs) are used primarily as coolants and insulators in the electrical industry (ANONYMOUS 1972). The stability of these compounds made them useful in the past as industrial stabilizers for paints, rubber, asphalt, printers' ink and pesticides. However, in recent years, the use of PCBs has been restricted to enclosed application, for example, in electrical capacitors and transformers.

PCB's have gained worldwide recognition as pollutants of ecosystems. Traces of these compounds have been found in a range of species from fish to man (HANSEN et al. 1971; KOLBYE 1972; ZITKO 1972). There is increasing concern over the threat these environmental contaminants pose to human health; this concern has its origins in the adverse reactions, observed after treatment of experimental animals with PCBs and after accidental exposure of humans to high levels of PCBs. Humans are exposed to the PCB's through contaminated air, water, and food (STANOVICK et al. 1973; TARRANT and TATTON 1968; VEITH and LEE 1970). In the "Yusho" incident in Japan, approximately 1,000 humans consumed rice oil contaminated with PCBs (KURATSUNE et al. 1972). PCBs have been found in human adipose tissue and in human milk (BIROS et al. 1970).

The PCBs exert a variety of biological effects. The most common subjective symptoms described by male and female "Yusho" patients were dark brown pigmentation of nails, acne-like skin eruptions, pigmentation of the skin, increased eye discharge, hyperemia of the conjunctiva, and swelling of the upper eyelids (KURATSUNE et al. 1969). Studies in rats have shown that the PCBs induce several liver microsomal enzymes (ALVARES et al. 1973; BRUCKNER et al. 1973; LITTERST et al. 1972), reduce the urinary levels of estrogen and dehydroepiandrosterone in the boar (PLATONOW et al. 1972), produce hyperplasia and dysplasia of the gastric mucosa in male Rhesus monkeys (ALLEN and NORBACK 1973), increase host susceptibility to viral infection (FRIEND and TRAINER 1970),

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synergize the toxicity of pesticide residues (LICHTENSTEIN et al. 1969), and inhibit the growth of human cells in culture (LITTERST and LICHTENSTEIN 1971).

In studies of the reproductive effects of PCBs, KEPLINGER et al. (1971) fed chickens 10 or 100 ppm of Aroclor 1242 or 100 ppm of Aroclor 1254 and reported loss of body weight, decreased thickness of egg shells, and poor hatchability of eggs. KIMBROUGH (1971) reported that fewer offspring were born after Aroclor 1254 was fed to rats at 100 ppm, and that the pups born were smaller and had decreased survival time as compared with the control group. Fetal loss, as exemplified by fewer offspring, is consistent with potential dominant lethal effects. The latter have as a basis cytogenetic damage (EPSTEIN et al. 1972). This report presents the results of the cytogenetic investigation of bone marrow and spermatogonial cells of rats after their treatment with Aroclors 1242 and 1254.

Materials and Methods

Aroclor 1242 was obtained from Mr. W.B. Papageorge of Monsanto Industrial Chemicals Co. and Aroclor 1254 was obtained from Mr. Sidney Williams of the Chemical Technology Branch of the Food and Drug Administration. Both samples, according to information supplied by Monsanto, were free of contamination by the dibenzofurans.

Random-bred male Osborne-Mendel rats, weighing 180-250 g were randomly assigned to groups of eight. Aroclor 1242 was given orally at single doses of 1250, 2500, or 5000 mg/kg, or as a multiple dose of 500 mg/kg/day for 4 days. Our usual 5-day regimen was not accomplished because of the debilitated condition of the animals after the compound had been administered for 4 days. Aroclor 1242 was administered as an undiluted solution at 5000 mg/kg and as a solution in corn oil at the other dosages. Aroclor 1254 in corn oil was given in a multiple regimen for 5 days at doses of 75, 150, or 300 mg/kg/day. Controls received corn oil. The rats were killed 24 hours after the single doses or 24 hours after the last dose in the multiple regimen. Three hours before killing, Colecimid was injected intraperitoneally at a dose of 4 mg/kg.

Bone marrow was aspirated from both femurs and processed according to the method of LEGATOR et al. (1969) with a slight modification of the incubation time; in this investigation the cells were allowed to incubate in the hypotonic solution for only 20 minutes. Spermatogonial cells were processed according to the method of HOO and BOWLES (1971). One hundred cells per animal (50 from each of two slides) were examined

for chromosomal abnormalities and one thousand cells per animal (500 per slide) for mitotic inhibition. Results from the treated groups were compared with control values by a χ^2 -test. For bone marrow and spermatogonial aberrations, the individual proportion of affected cells for each animal was transformed to the Freeman-Tukey arc sine to satisfy the assumptions of the χ^2 -test (MOSTELLER and YOUTZ 1961). The transformations were not done for mitotic inhibition because the sample size was adequate to satisfy χ^2 -test assumptions.

The spermatogonial procedure was not performed with rats given Aroclor 1254, since Aroclor 1242, the more toxic of the compounds, did not produce chromosomal aberrations in spermatogonia. In addition, it was felt that a companion dominant lethal investigation of both compounds would yield more definitive information about germ cell effects.

Results

Toxicity

Table 1 shows that the administration of Aroclor 1242 at 1250 mg/kg did not cause the death of any animals but body weight was reduced an average of 5 g. In the group given 2500 mg/kg, 4 of 8 rats expired within 24 hours and in the group given 5000 mg/kg, 3 of 8 expired. Weight losses in these groups were 20 and 26 g, respectively. The multiple doses of 500 mg/kg/day for 4 days caused the death of 5 of 8 rats within 96 hours after the first administration and a mean weight loss of 11 g, showing the cumulative toxic effect of this compound.

TABLE 1

Toxicity of Aroclor 1242 and 1254 after Oral Administration to the Osborne-Mendel Rat

Group	Dose (mg/kg)	No. Dead/ No. Treated	Mean Weight Loss (g)
Control	Corn oil	0/8	-
Aroclor 1242	500 X 4	5/8	11
Aroclor 1242	1250 X 1	0/8	5
Aroclor 1242	2500 X 1	4/8	20
Aroclor 1242	5000 X 1	3/8	26
Control	Corn oil	0/8	-
Aroclor 1254	75 X 5	0/8	-
Aroclor 1254	150 X 5	0/8	18
Aroclor 1254	300 X 5	1/8	34

Aroclor 1254, although producing very few deaths, did cause a reduction in weight which exceeded that of the rats treated with Aroclor 1242. The subacute doses of 150 and 300 mg/kg produced a mean reduction in weight of 18 and 34 g, respectively (Table 1).

Mitotic Inhibition

The administration of Aroclor 1242 had no demonstrable effect on mitotic division of rat bone marrow cells (Table 2).

TABLE 2

Number of Bone Marrow Cells with Abnormalities and in Mitosis after Treatment of Individual Male Osborne-Mendel Rats with Aroclor 1242

Aroclor 1242 (mg/kg)	Number of Cells with Chromosomal Abnormalities ^a	Number of Cells in Mitosis ^b
0	1, 1, 0, 1, 0, 0, 1, 0	42, 38, 32, 22, 44, 64, 40, 40
Total proportion	4/800=0.005	322/8000=0.040
500 X 4	0, 0, 0	38, 32, 27
Total proportion		97/3000=0.032
1250 X 1	0, 0, 0, 0, 1, 1, 0, 0	44, 57, 62, 54, 32, 28, 24, 24
Total proportion	2/800=0.003	325/8000=0.041
2500 X 1	0, 0, 0, 0	54, 60, 26, 26
Total proportion		166/4000=0.042
5000 X 1	0, 1, 5, 0, 0	28, 45, 38, 50, 45
Total proportion	6/500=0.012	206/5000=0.041

^aOne hundred calls examined per animal.

^bOne thousand cells counted per animal.

Although the number of mitotic cells in rats given the multiple doses of 500 mg/kg was reduced, the decrease was not statistically significant. As seen in Table 3, Aroclor 1254 at 75 mg/kg did not inhibit mitosis in bone marrow but doses of 150 and 300 mg/kg produced indexes of 0.029 and 0.031, respectively, as compared with a control index of 0.041.

for chromosomal abnormalities and one thousand cells per animal (500 per slide) for mitotic inhibition. Results from the treated groups were compared with control values by a χ^2 -test. For bone marrow and spermatogonial aberrations, the individual proportion of affected cells for each animal was transformed to the Freeman-Tukey arc sine to satisfy the assumptions of the χ^2 -test (MOSTELLER and YOUTZ 1961). The transformations were not done for mitotic inhibition because the sample size was adequate to satisfy χ^2 -test assumptions.

The spermatogonial procedure was not performed with rats given Aroclor 1254, since Aroclor 1242, the more toxic of the compounds, did not produce chromosomal aberrations in spermatogonia. In addition, it was felt that a companion dominant lethal investigation of both compounds would yield more definitive information about germ cell effects.

Results

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Table 1 shows that the administration of Aroclor 1242 at 1250 mg/kg did not cause the death of any animals but body weight was reduced an average of 5 g. In the group given 2500 mg/kg, 4 of 8 rats expired within 24 hours and in the group given 5000 mg/kg, 3 of 8 expired. Weight losses in these groups were 20 and 26 g, respectively. The multiple doses of 500 mg/kg/day for 4 days caused the death of 5 of 8 rats within 96 hours after the first administration and a mean weight loss of 11 g, showing the cumulative toxic effect of this compound.

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Aroclor 1242	1250 X 1	0/8	5
Aroclor 1242	2500 X 1	4/8	20
Aroclor 1242	5000 X 1	3/8	26
Control	Corn oil	0/8	-
Aroclor 1254	75 X 5	0/8	-
Aroclor 1254	150 X 5	0/8	18
Aroclor 1254	300 X 5	1/8	34

Aroclor 1254, although producing very few deaths, did cause a reduction in weight which exceeded that of the rats treated with Aroclor 1242. The subacute doses of 150 and 300 mg/kg produced a mean reduction in weight of 18 and 34 g, respectively (Table 1).

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0	1, 1, 0, 1, 0, 0, 1, 0	42, 38, 32, 22, 44, 64, 40, 40
Total proportion	4/800=0.005	322/8000=0.040
500 X 4	0, 0, 0	38, 32, 47
Total proportion		97/3000=0.032
1250 X 1	0, 0, 0, 0, 1, 1, 0, 0	44, 57, 62, 54, 32, 28, 24, 24
Total proportion	2/800=0.003	325/8000=0.041
2500 X 1	0, 0, 0, 0	54, 60, 26, 26
Total proportion		166/4000=0.042
5000 X 1	0, 1, 5, 0, 0	28, 45, 38, 50, 45
Total proportion	6/500=0.012	206/5000=0.041

^aOne hundred cells examined per animal.

^bOne thousand cells counted per animal.

Although the number of mitotic cells in rats given the multiple doses of 500 mg/kg was reduced, the decrease was not statistically significant. As seen in Table 3, Aroclor 1254 at 75 mg/kg did not inhibit mitosis in bone marrow but doses of 150 and 300 mg/kg produced indexes of 0.029 and 0.031, respectively, as compared with a control index of 0.041.

TABLE 3

Number of Bone Marrow Cells with Abnormalities end in Mitosis after Treatment of Individual Male Osborne-Mendel Rats with Aroclor 1254

Aroclor 1254 (mg/kg)	Number of Cells with Chromosomal Abnormalities ^a	Number of Cells in Mitosis ^b
0	1, 0, 0, 1, 0, 1, 0, 1	35, 40, 38, 45, 60, 38, 40, 35
Total proportion	4/800=0.005	331/8000=0.041
75 X 5	1, 0, 1, 0, 0, 1, 1, 0	61, 46, 40, 24, 38, 42, 36, 29
Total proportion	4/800=0.005	316/8000=0.040
150 X 5	0, 0, 0, 1, 0, 1, 0, 0	12, 38, 22, 40, 40, 30, 18, 35
Total proportion	2/800=0.003	235/8000=0.029 ^c
300 X 5	0, 1, 1, 1, 0, 1, 0	48, 28, 40, 16, 32, 26, 29
Total proportion	4/700=0.006	219/7000=0.031 ^c

^aOne hundred cells examined per animal.

^bOne thousand cells counted per animal.

^cSignificantly different from control value at $p < 0.05$, one-tail t -test.

Table 4 shows that at acute dosages of 1250 and 2500 mg/kg, Aroclor 1242 had no effect on mitosis of spermatogonial cells but 5000 and 500 mg/kg x 4 significantly decreased the rate of division.

Chromosomal Abnormalities

There was no evidence that Aroclor 1242 administered to rats at 1250 or 2500 mg/kg produced a significant number of chromosomal aberrations in bone marrow cells (Table 2). In the group given 5000 mg/kg, the number of abnormalities appeared to be increased, but the result was considered insignificant inasmuch as the response was due mainly to one animal. The subacute dosage of 500 mg/kg also failed to produce chromosomal damage. All lesions seen were of the single chromatid type.

TABLE 4

Number of Spermatogonial Cells with Abnormalities and in Mitosis after Treatment of Individual Male Osborne-Mendel Rats with Aroclor 1242

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98, 45,
40, 35
=0.041

40, 24,
36, 29
=0.040

22, 40,
18, 35
=0.029[§]

40, 16,
39
=0.031[§]

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Aroclor 1242 (mg/kg)	Number of Cells with Chromosomal Abnormalities [§]	Number of Cells in Mitosis [§]
0	0, 0, 0, 2/68, 1, 0, 0, 0	11, 18, 14, 2, 13, 15, 28, 18
Total proportion	3/768=0.004	119/8000=0.015
500 X 4	0, 2, 1	4, 8, 8
Total proportion	3/3000=0.010	20/3000=0.007 [§]
1250 X 1	0, 0, 1, 1, 0, 0, 0, 0	8, 5, 12, 22, 18, 20, 6, 11
Total proportion	2/800=0.003	102/8000=0.013
2500 X 1	0, 1, 1, 0	20, 20, 12, 18
Total proportion	2/400=0.005	70/4000=0.018
5000 X 1	0, 1, 3, 0, 0	10, 12, 6, 8, 9
Total proportion	4/500=0.008	45/5000=0.009 [§]

[§]One hundred cells examined per animal.

[§]One thousand cells counted per animal.

[§]Significantly different from control value at $P < 0.01$, two-tailed t -test.

[§]Significantly different from control value at $P < 0.05$, two-tailed t -test.

Aroclor 1254 failed to produce a significant number of chromosomal abnormalities in bone marrow at the dosages tested. The proportions of abnormal cells were 0.005, 0.003, and 0.006 for the groups given 75, 150, and 300 mg/kg, respectively (Table 3).

No cytogenetic abnormalities were produced in spermatogonial cells after the administration of Aroclor 1242 at 1250 and 2500 mg/kg; the proportions of cells with abnormalities were 0.003 and 0.005, respectively, as compared to a mean of 0.004 in the control group (Table 4). Greater numbers of abnormalities were produced in the groups treated with 5000 and 500 mg/kg but the differences were not large enough to be statistically significant. The lesions produced were all of the single chromatid type.

Discussion

The results presented here clearly demonstrate the cumulative toxicity of Aroclor 1242 when administered in a multiple regimen. The 500 mg/kg dosage given acutely would have caused few if any deaths, as indicated by the results obtained with the 1250 mg/kg dosage (Table 1); however, when 500 mg/kg was given each day for 4 days, more than 50% of the animals died. Although the Aroclors are known to be relatively non-toxic in acute administration, we have found that when testing for possible mutagenic effects the usual assumptions do not always apply. Therefore both acute and multiple regimens were instituted in this investigation.

When negative data with respect to chromosomal aberrations are presented utilizing compounds of this type, questions are raised concerning the accessibility of the tissues to the compounds. The mitotic index serves as an indicator, although not definitively, of the bioavailability of the compound under investigation. Generally, if inhibition of cell division is observed it is felt that the compound or a breakdown product does penetrate the tissue in question. This precludes a generalized toxic condition in the animal as a factor. Therefore, although chromosomal aberrations were not seen, the lack of such an effect was not considered to be a result of the inaccessibility of the tissue to the compounds. In addition, since these compounds are highly lipid-soluble, the cell membrane would be expected to offer little resistance to their passage.

Mitotic inhibition of bone marrow cells was completely absent with exception of the subacute dosage in the study of Aroclor 1242, and was barely evident in the study of Aroclor 1254. The inhibition produced by Aroclor 1254, although not very marked, may be due to a generalized toxic condition at the highest dose levels; both levels produced comparable inhibition. The mean weight loss of these groups appeared to be dose-related and it had been expected that the inhibition would parallel this dose-response effect.

With respect to spermatogonial cells, the inhibition produced by Aroclor 1242 appears to be genuine in that the data are not consistent with the view that a generalized toxic condition of the animals caused mitotic inhibition. If this were the etiology of the inhibition, then the single dose of 2500 mg/kg should have produced inhibition as well as the multiple doses of 500 mg/kg, since both produced approximately the same degree of toxicity. The significance of the mitotic inhibition of spermatogonial cells is open to speculation. It was originally thought that mitotic inhibition could lead to a reduction in the number of viable sperm in approximately 10 weeks, the amount of time required for spermatogonia to become mature sperm in the rat, and at that time the reduction could influence the reproductive performance of male animals. This

possibility has been investigated and it appears not to be the case (GREEN, SAURO, and FRIEDMAN, manuscript in preparation). Two factors which may have prevented the manifestation of a reduction in reproductive performance are that ample numbers of spermatogonia were able to divide and become mature sperm at week 10, or that the inhibition of mitosis may have been concomitant with a slowing of the entire spermatogenic cycle, causing post-gonial cells to require 10 weeks or more for maturation.

The investigation of the mutagenic potential of these compounds through the cytogenetic examination of rat bone marrow and spermatogonia cells does not preclude the possibility that point mutations occurred, since they are not measurable in the procedure. There are, however, very few instances in which point mutations are known to occur without chromosomal aberrations occurring at a higher concentration in the same or in another system. In any case, on the basis of this study, the Aroclors 1242 and 1254 possess no mutagenic potential as assessed by cytogenetic analysis of rat bone marrow and spermatogonia.

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