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Lack of Cytogenetic Effects in Bone Marrow and Spermatagonial 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 compunds 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 origin 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 consistant with potential dominant lethal affects. The latter have as a basis cytogenetic damage (EPSTEIN et al. 1972). This raport 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 the method of HOO and BOWLES (1971). One hundred cells per animal (50 from each of two slides) were examined

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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 t-test. For bone marrow and spermatogonial aberrations, the individual proportion of effected cells for each animal was transformed to the Fraeman-Tukey arc sine to satisfy the assumptions of the t-test (MOSTELLER and YOUTZ 1961). The transformations were not done for mitotic inhibition because the sample size was adequate to satisfy t-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-Mendal 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

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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

[a] One hundred cells examined per animal.

[b] One 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.

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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 t-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 t-test (MOSTELLER and YOUTZ 1961). The transformations were not done for mitotic inhibition because the sample size was adequate to satisfy t-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.

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Group	Dose (mg/kg)	No. Dead/ No. Treated	Mean Weight Loss (g)
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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

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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

[a] One hundred cells examined per animal.

[b] One 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.

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TABLE 3

Number of Bone Marrow Cells with Abnormalities and 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]

[a]One hundred cells examined per animal.

[b]One thousand cells counted per animal.

[c]Significantly 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 affect 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.

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TABLE 4

Number of Spermatogonial 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	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	3/300=0.010	20/3000=0.007[c]
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[d]

[a] One hundred cells examined per animal.

[b] One thousand cells counted per animal.

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

[d] 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.

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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 abberations 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 affect 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 visible 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

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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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