Effects of Food Deprivation in Rats Previously Exposed to Mirex

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Food deprivation has been shown to alter the toxicity and tissue distribution of organohalogens in animals previously exposed to these compounds (DAHLGREN et al., 1972; DALE et al., 1963; ECOBIOCHON and SASCHENBRECKER, 1969; VILLENEUVE, 1975; ZABIK and SCHEMMEL, 1973). Such effects are attributed to the mobilization of fat stores and subsequent release of the organohalogen. The present study was carried out as part of a general program designed to investigate the effects of food deprivation on the toxicity of pesticides. Mirex (perchlorocyclopentanodecane) was chosen for this study because of lipophilicity, low biodegradability and demonstrated presence in the environment. Mirex is currently under close scrutiny by regulatory and environmental agencies in Canada and the United States because of its appearance in Lake Ontario fish.

MATERIALS AND METHODS

Seventy-two male Sprague-Dawley rats (Bio-Breeding Laboratories Inc., Ottawa, Ontario) weighing 375-400 g. were divided randomly into four groups. Mirex (98% purity, Hooker Chemicals and Plastics Corp., Niagara Falls, N.Y.) was administered by gavage in corn oil (0.5 mg/100 g. body weight) for 14 days at levels of 0, 0.1, 1.0 and 10 mg/kg. During this period the animals were fed ad libitum with standard laboratory feed (Master Feed). Food consumption was monitored and feces collected from each animal daily. Six animals from each group were killed twenty-four hours after the last dose was administered (sub-group 1) and tissues removed from biochemical and tissue residue analysis. Six animals from each group continued to feed ad libitum (sub-group 2) while the remaining 6 animals were placed on 25% of their normal food intake for 8 days (sub-group 3). All remaining animals were then killed. The blood was examined for hemoglobin concentration, hematocrit value, erythrocyte count, total and differential counts of leucocytes, mean corpuscular volume and mean hemoglobin content. Serum was collected and assayed for sorbitol dehydrogenase activity (Calbiochem, La Jolla, Cal.) lactic dehydrogenase activity (Autoanalyzer II method No. SE-4-0021FJ4) and mirex content. Tissue residues of mirex were determined using a glc-ec method reported previously for the determination of hexachloro-

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benzene (Villeneuve et al, 1974). Tissues analyzed included brain, heart, spleen, kidney, liver, and adipose tissue. Microsomal enzyme (aniline hydroxylase) activity of fresh liver homogenates was determined as outlined by Becking (1973) using a 15 minute incubation time. Protein content of liver was determined by the biuret method (Gornall et al, 1948). Another portion of the liver was examined histologically. Statistical analysis where applicable was carried out using the Students t test.

RESULTS AND DISCUSSION

Body weight changes and food intake during the 14 days of mirex administration are shown in Table 1.

TABLE 1

Initial body weight, body weight gain and food intake of rats dosed with mirex for 14 days[a].

Level of mirex[b]	Initial body weight(g)	Body weight gain(g)	Food intake (g/day/animal)
0	356 ± 14	51 ± 15	21.5 ± 1.8
0.1	357 ± 15	44 ± 13	20.3 ± 2.4
1.0	356 ± 18	46 ± 18	20.3 ± 1.4
10	357 ± 13	28*± 18	18.6*± 2.1

[a] Values represent the mean ± S.D. of 18 rats.

[b] Level of mirex in mg/kg body weight.

* Denotes significant difference from 0 group at $P \leq 0.05$.

A significant reduction in body weight and food intake was observed at 10 mg/kg mirex. During the 8 day period following mirex administration the ad libitum groups gained from 20-30 g, while the animals on 25% normal food intake lost from 62-75 g.

The results for organ weights expressed as a percent of body weight are shown in Table 2.

TABLE 2

Organ weights (% body weight) of rats administered mirex and subjected to food deprivation[a].

Sub-Group[b]	Brain	Heart	Liver	Spleen	Kidney
		0 mg/kg mirex			
1	0.53	0.33	2.96	0.17	0.73
2	0.56	0.37	2.49	0.19	0.74
3	0.59	0.34	2.13	0.15	0.69
		0.1 mg/kg mirex			
1	0.50	0.33	2.68	0.16	0.68
2	0.49	0.33	2.52	0.18	0.74
3	0.60	0.36	2.17	0.15	0.67
		1.0 mg/kg mirex			
1	0.52	0.33	3.44*	0.17	0.70
2	0.53	0.34	3.08*	0.17	0.71
3	0.60	0.36	2.86*	0.15	0.72
		10 mg/kg mirex			
1	0.53	0.34	5.98*	0.17	0.67
2	0.54	0.33	6.04*	0.15	0.72
3	0.64	0.33	5.23*	0.15	0.71

[a] Values represent the mean of 6 rats.

[b] See text for details of treatment.

* Denotes significant difference from corresponding sub-group receiving 0 mirex.

TABLE 3

Effect of mirex and food restriction on several biochemical parameters in the rat[a].

Sub-Group[b]	Sorbitol Dehydrogenase (mu/ml)	Lactic Dehydrogenase (mu/ml)	Aniline Hydroxylase (nmoles PAP/60 min/mg protein)	Liver Protein (mg/g liver)
		0 mg/kg mirex		
1		527 ± 313	12.9 ± 3.0	118.3 ± 13.0
2	2.55 ± 1.38	294 ± 104	12.9 ± 5.1	116.0 ± 6.9
3	5.11 ± 1.40	542 ± 212	6.14 ± 2.0	103.3 ± 5.2
		0.1 mg/kg mirex		
1		975* ± 333	18.8* ± 3.9	121.9 ± 12.9
2	2.25 ± 1.20	813 ± 210	11.9 ± 2.1	113.7 ± 8.9
3	5.56 ± 1.88	790 ± 283	9.55* ± 2.1	110.0 ± 3.8
		1.0 mg/kg mirex		
1		1326* ± 699	19.0* ± 2.9	144.0 ± 29
2	3.36 ± 0.44	959* ± 408	22.7 ± 1.93	120.2 ± 6.1
3	6.96 ± 2.47	1378* ± 686	18.2* ± 5.3	115.0* ± 6.3
		10 mg/kg mirex		
1		1050 ± 688	19.0* ± 3.1	148.0* ± 27
2	12.9* ± 9.0	816* ± 311	17.0 ± 4.6	138.0* ± 20
3	22.8* ± 9.2	1426* ± 775	16.7* ± 4.6	129.7* ± 10.7

[a] Values represent the mean of 6 animals ± S.D.

[b] See text for details of treatment.

* Denotes significant difference from corresponding sub-group receiving 0 mirex.

Liver was the only organ to be significantly altered by mirex treatment and increased size was observed in all groups at 1.0 and 10 mg/kg mirex. Mirex's ability to enlarge the liver was not augemented by food restriction.

Neither mirex nor food restriction caused alterations in the hematological parameters monitored in this study.

Effects of mirex on the biochemical parameters monitored in this study are shown in Table 3. Sorbitol dehydrogenase activity (SDH) was significantly elevated at 10 mg/kg mirex. Data was not available on this enzyme in animals killed after 14 days. Lactic dehydrogenase activity (LDH) was increased in animals dosed with 0.1 mg/kg mirex and killed after 14 days. Significant increases in this enzyme were also observed in all groups of animals receiving 1.0 and 10 mg/kg mirex except that group killed after 14 days which received 10 mg/kg mirex. Microsomal enzyme activity was increased at all dose levels of mirex in animals killed after 14 days and also in animals subjected to food deprivation. Liver protein levels were increased in animals receiving 1.0 mg/kg mirex and subjected to food deprivation and in all groups receiving 10 mg/kg mirex.

The residue profile of mirex in selected tissues is shown in Table 4. The levels for the 0 mirex group are not shown but all values were less than 0.2 ppm. The values quoted in the table are not corrected for recovery which ranged, depending upon the tissue, from 80-100%. Highest levels of mirex were found in fat followed by liver, serum, kidney, heart and spleen. Although the differences were not always statistically significant, tissues (except fat) from animals fed ad libitum for 22 days showed a trend toward lower mirex levels when compared to the corresponding sub-group killed after 14 days. By the same token food deprivation resulted in a trend toward elevated levels of mirex in all tissues examined when compared to rats fed ad libitum.

The analysis of feces samples for mirex during the study showed that there was no difference in the rate of excretion of mirex in animals subjected to food deprivation.

Histopathological examination of the liver revealed that mirex administration resulted in an increased fatty infiltration in the centrilobular region. This effect was dose-dependent and was not affected by food deprivation.

The results presented above indicate that food deprivation causes a relocation of mirex residues due to mobilization of fat depots. However no overt toxic symptoms were observed in any of the rats even at the highest dose level. The hematology was not disturbed either by mirex alone or in combination with the stress of food.

TABLE 4

Tissue residue profile of rats fed mirex and subjected to food deprivation[a].

Sub-Group[b]	Serum	Liver	Heart	Brain	Kidney	Spleen	Fat
			0.1 mg/kg mirex				
1	N.D.	3.24	0.99	1.36	1.02	0.65	3.93
2	N.D.	1.67	0.39*	0.24	0.53*	0.24*	7.05
3	N.D.	4.30	0.94**	0.38	1.53**	0.70**	10.1
			1.0 mg/kg mirex				
1	18.9	79.3	19.1	4.96	16.4	4.60	29.7
2	4.50*	35.2	4.27	2.42	7.36	3.15	65.4*
3	6.34	52.0	5.60	3.13	11.2**	3.17	125**
			10 mg/kg mirex				
1	269	317	102	50	122	71	437
2	114*	243	52*	25*	105	38*	714
3	188	318	131**	58**	136	73**	370

[a] Values represent average ppm of 6 rats.

[b] See text for details of treatment.

N.D. Not detectable (0.01 ppm).

* Denotes significant difference from Sub-Group 1 at $P \leq 0.05$.

** Denotes significant difference from Sub-Group 2 at $P \leq 0.05$.

deprivation. The pathological lesions observed were not exacerbated by food deprivation. The only indication that food deprivation might increase the biological activity of mirex was the fact that animals at all dose levels of mirex and subjected to food deprivation showed increased aniline hydroxylase activity whereas the ad libitum groups did not. Tissue residue analysis revealed that brain, heart and liver showed increased mirex residues due to food deprivation while fecal residue analysis indicated that food restriction had no effect on the excretion of mirex. One can only conclude from these results that food deprivation does not significantly alter the toxicity of mirex, at least at the levels reported here.

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