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Toxicity of Photomirex with Special Reference to Porphyria, Hepatic P-450 and Glutathione Levels, Serum Enzymes, Histology and Residues in the Quail and Rat

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Photomirex (8-monohydromirex) has been identified as an environmental contaminant in both the southern United States and in the Lake Ontario region of North America (HALLETT et al. 1976). Little information is available on the potential toxicity of this compound. HALLET et al. (1978) reported the oral LD_{50} of this compound to be between 200-300 mg/kg. This same study revealed the compound to be strongly lipophilic and non-mutagenic in an Ames bacterial test. Photomirex has also been shown to cause histological changes in the liver and thyroid and to cause increases in mixed function oxidase and sorbitol dehydrogenase activities (RITTER et al. 1978, VILLENEUVE et al. 1979). The present study was carried out to determine the hepatotoxic and porphyrogenic potential of this compound in the rat and quail.

MATERIALS AND METHODS

Photomirex was prepared using a procedure as described by HALLET et al. (1978). Identity of the product was confirmed by gas chromatography, gas chromatography-mass spectrometry, and nuclear magnetic resonance techniques. Purity was determined to be 96%. The remaining 4% was identified as 2,8-dihydromirex (dihydromirex).

Toxicity of four oral dosages. Eight male adult Japanese quails weighing approximately 120 g were divided in four groups of two animals each. The groups were dosed orally using gelatin capsules containing 0, 100, 500 or 1000 mg photomirex/kg body weight on day 1, 2, 7 and 14. The animals had free access to Trouw's turkey feed and water. Excreta were collected on day 8, 15, 22, 29 and 36 for the analysis of the porphyrin pattern (STRIK 1973). After being on test for 37 days the animals were lightly anesthesized and exsanguinated via the abdominal aorta. Serum enzymes and constituents were determined in a multi-channel autoanalyzer (Technicon SMA 12/60 micro). The liver and kidneys were excised and weighed. A sample of the fresh liver and a kidney was fixed in a phosphate-buffered formalin for routine histology examination (VILLENEUVE et al. 1979). The remaining liver and kidney were used for the following analysis:

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Liver: Total porphyrins, porphyrin pattern (HARMSEN & STRIK, in press; STRIK & HARMSEN, in press). Macroscopic and microscopic porphyrin fluorescence (STRIK 1973). glutathione (HISSEN & HILF 1976) P-450 Content (OMURA & SATO 1964) and residue of Photomirex (HALLET et al. 1978).

Kidney: Porphyrin pattern and microscopic porphyrin fluorescence

Twenty-seven day Subacute Toxicity

A. Quail Experiment: Ten male adult Japanese quails (120 g) were equally divided into two groups. The animals were housed individually and fed diets containing 0 or 100 ppm photomirex for 27 consecutive days. Excreta were collected at weekly intervals throughout the experiment.

B. Rat Experiment: Similarly, ten female U-strain rats weighing about 180 g were assigned to two groups of five animals each. The groups were kept on diets containing 0 or 100 ppm photomirex for 27 days. Urine and feces were collected twice weekly for the analysis of the porphyrin pattern.

After twenty-seven days both Japanese quails and rats were killed by exsanguination. Histological examination, porphyrins, glutathione, P-450 content, residue levels and serum biochemical analysis similar to that described for the four oral dosages experiment were performed.

RESULTS AND DISCUSSION

Toxicity of four oral dosages. The body weights of the control and photomirex treated quails did not show any significant difference. Liver weight was significantly ($P < 0.05$) increased in the quails dosed with 500 and 1000 mg/kg photomirex (Table 1). Glutathione levels (only performed in highest dosed groups and controls) did not differ. P-450 content was significantly increased ($P \leq 0.05$) (Table 1).

Cryostat sections of the liver and kidney examined by fluorescence microscopy did not reveal any fluorescence due to porphyrins. Porphyrins with one, two (protoporphyrin), three, four (coproporphyrin) and five carboxylic groups were detected in the excreta of quail from all groups. The percentages of distribution for the five porphyrins in the control animals were found to be 5-26 (one), 0-43 (two), 22-43 (three), 0-22 (four) and 9-26 (five carboxylic acid groups) on 15th, 22nd, 29th and 34th day of the test. Except for the 1000 mg/kg treated group which was found to have percentages of 0-18, 0-14, 0-33, 12-62 and 18-64, respectively; the remaining groups were within ranges of control values. The copro- and pentacarboxylic fractions were increased about three fold above the control values on the 15th, 22nd and 29th day of the test. This indicates enzyme induction, as a result of increased heme synthesis. No porphyrins were detectable in the kidney or liver by the thin layer chromatography method described by STRIK (1973). Residue levels in the liver are shown in Table 2.

TABLE 1

Effect of four oral doses of photomirex on the liver weight, hepatic glutathione level and P-450 content of Japanese quail.[a]

Dose (mg/kg)	Liver weight (g)	Glutathione (μ/250 g)	P-450[b]
0	25. ± 0.2	310 ± 36	0.35
100	2.1 ± 0.1		0.25
500	3.3 ± 0.4		0.60[c]
1000	3.6 ± 0.7	334 ± 15	0.45[c]

[a]Where appropriate values represent mean ± S.D.

[b]nmol/mg protein (for extinction units quail; rat = 91/mM,cm). Results represent mean values obtained with 2 quails.

[c]Significantly different from control ($P \leq 0.05$).

Photomirex produced lesions which appeared to be dose dependent in the livers of quails. The cells in the livers of the control quails were of uniform size with the cytoplasm being uniformly eosinophilic. Livers from the animals treated with 100 mg/kg had widespread midzonal cytoplasmic inclusions and occasional necrotic hepatocytes. The livers of the two quails of the 500 mg/kg dose group were similar to those of the 100 mg/kg dose group but were more severely affected and had markedly lobular patterns due to cytoplasmic vacuolation which involved the pericentral and midzonal areas. The hepatic lesions in the 1000 mg/kg group were less severe.

Renal changes in this experiment were minimal and the organs were for the most part cytologically and architecturally normal.

Serum biochemical parameters (Na^+, K^+, PO_4^{-3}, total bilirubin, alkaline phosphatase, total protein, Ca^{++}, cholesterol, glucose, uric acid, lactic dehydrogenase, SGOT) were not altered by the photomirex treatments. This is consistent with the absence of severe necrotic lesions in the liver and kidneys.

TABLE 2

Photomirex residues in liver of quail dosed four oral dosages[a]

Dose (mg/kg)	Photomirex residue (ppm, wet tissue)
0	0.59
100	66
500	492
1000	1056

[a]Each value denotes the mean obtained with two animals.

Twenty-seven day Subacute Toxicity.

A. Quail Experiment. The body and liver weights of the control and photomirex fed quails did not show any significant difference. The glutathione levels of two randomly selected livers from controls and two from the photomirex dosed group did not show any difference (control: 304 $\pm$ 10; treated: 342 $\pm$ 43). The control cytochrome P-450 had a value of 17.3 whereas the treated animal had 38.5 (extinction unit/g liver). Cyrostat sections of liver and kidney examined under the fluorescence microscope did not show any fluorescence characteristic of porphyrins. In the excreta of the quail (treated and controls) porphyrins with one, two, three, four and five carboxylic groups were detected. In both groups the percentage values ranged respectively: 12-41, 5-19, 14-37, 9-30, 10-33%. Residue levels are shown in Table 3.

TABLE 3

Photomirex residues in liver of quail fed 0 or 100 mg/kg photomirex for 27 days

Dose (mg/kg)	Photomirex residue[a] (ppm, wet tissue)
0	0.46
100	188

[a]Mean values obtained with five animals.

There were mild cytyoplasmic vacuolar changes in the two of five livers in the control group. These were judged to be normal. Histological lesions in the treatment group appeared to be above the level of detection in the livers of two animals (residues of photomirex 95 and 107 ppm). The changes consisted of fine panlobular cytoplasmic vacuolation and cytoplasmic enlargement. The more affected livers had increased vacuolation and increased vacuolar size (residue levels of photomirex 250 and 187 ppm). The most severely injured liver had a residue level of 305 ppm and a marked panlobular cytoplasmic vacuolation. No changes were found in the kidneys in this experiment.

B. Rat Experiment. The body weights of the control and photomirex fed rats did not change significantly. A significant increase in liver weight was observed in the photomirex treated group (control: 6.24 $\pm$ 0.68, treated: 8.26 $\pm$ 0.80 at $P \leq 0.05$). Glutathione levels (44.8 $\pm$ 38 for the controls and 47.5 $\pm$ 41 for photomirex), P-450 levels, urinary total porphyrins and porphyrin patterns were not different. The percentages of distribution for proto-, copro-, penta-, heptacarboxylic and urophyrin were found to be 19-44; 35-61; 0-8; 0-9; and 6-27 respectively.

TABLE 4

Photomirex residues in liver of rats
fed 100 mg/kg photomirex during 27 days.

Dose (mg/kg)	Photomirex residue[a] (ppm, wet tissue)
0	0.13
100	229

[a]Results are expressed as mean values obtained with five animals.

Histologically the rats in the control group as expected had normal livers typical of those of young rats with prominent cytoplasmic basophilia, abundant cytoplasmic and frequent mitosis.

Rat livers of the photomirex fed group possessed a moderate degree of injury. The common observation was a moderate lobular pattern in the liver cells due to pericentral cytoplasmic enlargement with peripheralized cytoplasmic basophilia and an overall reduction in cytoplasmic density. In addition, cells had the characteristic nuclear vesiculation and anisokaryosis. The affected area generally involved about one third of the lobule and was well above that of a minimal detectable lesion. No changes were found in the kidneys of these animals.

CONCLUSIONS

Photomirex, given either as single oral doses up to 1000 mg/kg to quails or as 100 ppm diet to quails and female rats for 27 days, caused moderate hepatic changes consisting of an increase in liver weight and cytoplasmic enlargement of hepatocytes. Hepatic cytochrome P-450 contents of quails but not rats were also increased. There were no indications of severe damage to the livers of these animals (alterations in glutathione level and serum profile, porphyria and necrosis).

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