column (yield of adsorbate-aa fraction, 5.0% of total extractive).

Paper chromatography proved to be the effective final step in locating the odor precursor substance. The bulk of the material was recovered from zones 4 and 5 (representing respectively regions R_f 0.3–0.4 and R_f 0.4–0.5) in BAW and zones 6 and 8 in H_2O. In terms of sensory significance, the area of most interest was found to be zone 5 from BAW and zone 8 from H_2O. Further study revealed that the zones of interest coincided closely with two UV-absorbing components, identified from their spectral properties (UV, IR, NMR) to be the purine derivatives inosine and hypoxanthin. Relevant R_f values for these two compounds are given below:

	BAW	H_2O
inosine	0.33	0.72
hypoxanthin	0.46	0.55
tyrosine	0.45	0.76

The two-dimensional chromatographic data revealed that the precursor substance is not inosine or hypoxanthin, although in one-dimensional chromatography in each of the two solvent systems used it coincided closely with one or other of the two purines. The two-dimensional chromatographic location of the precursor substance appeared to coincide closely with that of tyrosine, found also as a component of fraction 3. Heating of authentic tyrosine, inosine, and hypoxanthin under the standard conditions, or of samples of the two purines recovered from two-dimensional chromatography, did not reproduce the unpleasant odors previously recognized (inosine gave a characteristic burnt caramel odor and deposited copious black precipitates, while hypoxanthin was not affected by heating). Due to lack of material it was not possible to establish any chemical characteristics for this precursor substance to aid in its physical detection. Sensory assessment remained the only means of monitoring its existence.

This work establishes that precursor substances contributing to the characteristic odor of mutton are present in the lean meat, and these can be isolated and fractionated by techniques applicable to the fractionation of aromatic amino acids and N-heterocyclics. The chemical nature of the precursor(s) remains to be elucidated.

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Induction of Hepatic Mixed Function Oxidases by Photomirex

Various parameters associated with the hepatic mixed function oxidase system were studied in hepatic microsomal preparations of male rats exposed to a dietary level of 50 ppm photomirex for 15 days. Hydroxylation of pentobarbital and hexobarbital was increased in excess of twofold while hydroxylation of aniline was increased approximately 1.5-fold. N-Demethylation of aminopyrine was elevated approximately threefold while that of ethylmorphine was only slightly increased. Components of the electron transport system and substrate binding to microsomal protein were also found to be significantly increased by exposure to photomirex. These results suggest that photomirex is similar to mirex and Kepone in its potential for altering hepatic function.

Components of the hepatic drug metabolizing enzyme system are readily induced by numerous environmental contaminants. Examples of this phenomenon are provided by the potent induction of the hepatic mixed function oxidases by mirex (Baker et al., 1972; Mehendale et al., 1973) and its structural analogue Kepone (Mehendale et al., 1977; 1978) in both male and female rats. Mirex has been widely employed in the southeastern United States as the insecticide of choice for control of the fire ant (Shapley, 1971). Ivie et al. (1974) demonstrated that several photodegradation products appear following exposure of this fully chlorinated, ten-carbon compound to sunlight.

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Table I. Effect of Photomirex Preexposure on Aromatic and Aliphatic Hydroxylation and *N*-Demethylase Activities in Rat Liver Microsomes[a]

substrate	control diet		50 ppm photomirex diet	
	nmol (g of liver)$^{-1}$ (30 min)$^{-1}$	nmol (mg of protein)$^{-1}$ (30 min)$^{-1}$	nmol (g of liver)$^{-1}$ (30 min)$^{-1}$	nmol (mg of protein)$^{-1}$ (30 min)$^{-1}$
aniline[b]	282 ± 34	7.4 ± 0.9	506 ± 41*[f]	10.6 ± 0.6*
pentobarbital[c]	171 ± 38	4.5 ± 1.0	510 ± 41*	10.7 ± 0.8*
hexobarbital[c]	1036 ± 198	27.1 ± 5.0	2420 ± 130*	50.5 ± 2.1*
aminopyrine[d]	286 ± 43	7.5 ± 1.0	997 ± 138*	20.7 ± 2.4*
ethylmorphine[e]	2572 ± 225	67.6 ± 5.1	3955 ± 515*	82.3 ± 9.3

[a] Male rats were maintained on either a control rat chow diet or one containing 50 ppm photomirex for a 15-day period. On day 16 the rats were decapitated and their livers were promptly excised and microsomes were prepared using standard differential centrifugation procedures. Results are means ± SE for four run in duplicate. [b] According to Kato and Gillette (1965). [c] According to Brodie et al. (1953). [d] According to LaDu et al. (1955). [e] According to Nash (1953) and Cochin and Axelrod (1959). [f] (*) Significantly different from control at the $p < 0.05$ level.

Table II. Effect of Photomirex Preexposure on Substrate Interactions with Cytochrome P-450 in Hepatic Microsomes[a]

substrate	control		50 ppm photomirex	
	ΔOD × 10^3 (mg of protein)$^{-1}$ mL^{-1}	ΔOD × 10^3 (g of liver)$^{-1}$ mL^{-1}	ΔOD × 10^3 (mg of protein)$^{-1}$ mL^{-1}	ΔOD × 10^3 (g of liver)$^{-1}$ mL^{-1}
pentobarbital[b]	4.9 ± 0.4	185 ± 23	6.4 ± 0.4*	304 ± 21*
hexobarbital[b]	5.2 ± 0.3	199 ± 18	8.2 ± 0.9*	388 ± 36*
aniline[b]	10.2 ± 0.8	390 ± 46	20.5 ± 1.6*	980 ± 84*

[a] Details of treatment are given in Table I. Results are means ± SE for four animals assayed individually in duplicate. [b] Measured according to Schenkman et al. (1967). [c] (*) Significantly different from control at the $p < 0.05$ level.

Table III. Effect of Photomirex Preexposure on NADPH-Cytochrome *c* Reductase and NADPH-Dehydrogenase in Hepatic Microsomes[a]

	control		50 ppm photomirex	
	nmol (g of liver)$^{-1}$ (3 min)$^{-1}$	nmol (mg of protein)$^{-1}$ (3 min)$^{-1}$	nmol (g of liver)$^{-1}$ (3 min)$^{-1}$	nmol (mg of protein)$^{-1}$ (3 min)$^{-1}$
reductase[b]	6892 ± 341	182 ± 12	15973 ± 750*[d]	334 ± 14*
dehydrogenase[c]	503 ± 54	13.4 ± 1.2	1036 ± 78*	21.7 ± 1.8*

[a] Details of treatment are given in Table I. Results are means ± SE for four animals assayed individually in duplicate. [b] Measured according to Williams and Kamin (1962). [c] Measured according to Gillette et al. (1957). [d] (*) Significantly different from control at the $p < 0.05$ level.

The major product of photodegradation was identified as the 8-monohydro derivative of mirex, now termed photomirex. The study described here deals with the effect of dietary photomirex on the hepatic mixed function oxidase system of the male rat.

MATERIALS AND METHODS

Chemicals. Photomirex, prepared by the reduction of mirex (Hallett et al., 1978), was a gift from Dr. D. C. Villeneuve of the Canadian Environmental Health Directorate. The purity was specified as >96%.

Animals. Male Sprague-Dawley rats (275–300 g) purchased from Charles River Breeding Laboratories (Wilmington, MA) were held in the central animal facilities away from known inducers. The animals were provided with powdered rat chow diet containing 0 or 50 ppm photomirex and water on an ad libitum basis for 15 days. The photomirex diet was prepared by dissolving an appropriate quantity of photomirex in acetone and applying the solution to the chow. Following evaporation of the acetone under a hood, portions of approximately 200 g were homogenized in an electric blender. These individual portions were then thoroughly admixed. Control diet was prepared in this manner, omitting the addition of photomirex to the acetone. Two animals were maintained per cage. Daily food consumption was recorded.

Following the 15-day pretreatment regimen the rats were sacrificed by decapitation. The livers were excised, weighed, and transferred to ice-cold isotonic (1.15 M) KCl solution. All preparative and centrifugation procedures were carried out in an ice–water bath. The microsomal fraction was isolated according to a standard centrifugation procedure used previously (Mehendale et al., 1977). Four animals from each group were assayed in duplicate incubations. Aniline hydroxylase (Brodie et al., 1953), aminopyrine demethylase (LaDu et al., 1955), and ethylmorphine demethylase (Nash, 1953; Cochin and Axelrod, 1959) were measured. The concentrations of cytochromes b_5 and P-450 were determined according to Omura and Sato (1964). The activities of NADPH oxidase (Gillette et al., 1957) and NADPH-cytochrome *c* reductase (Williams and Kamin, 1962), and aniline, pentobarbital, and hexobarbital interactions with cytochrome P-450 (Schenkman et al., 1967) were assayed according to previously described methods. Protein was measured according to Lowry et al. (1951). Data were analyzed using Student's *t* test for statistical differences between control and treated groups.

RESULTS AND DISCUSSION

No overt signs of toxicity were noted in the rats treated with 50 ppm photomirex. Food consumption and rate of weight gain over the 15-day treatment did not differ significantly from control. The total average dose of photomirex consumed by the treated rats was calculated to be approximately 50 mg/kg body weight. The liver-to-body weight ratio was increased 67.5% by photomirex treatment.

The effect of photomirex on aromatic and aliphatic hydroxylation was measured using aniline, pentobarbital, and hexobarbital as substrates (Table I). These data show 3-

Table IV. Effect of Photomirex on Hepatic Microsomal Content of Cytochrome P-450 and Cytochrome b_5[a]

	control		50 ppm photomirex	
	nmol/g of liver	nmol/mg of protein	nmol/g of liver	nmol/mg of protein
cytochrome P-450[b]	21.5 ± 1.8	0.57 ± 0.04	85.1 ± 7.4*	1.77 ± 0.10*
cytochrome b_5[b]	14.8 ± 0.8	0.39 ± 0.01	22.4 ± 0.60*	0.47 ± 0.01*

[a] Details of treatment are given in Table I. Results are means ± SE for four animals. [b] Measured according to Omura and Sato (1964). [c] (*) Significantly different from control at the $p < 0.05$ level.

to 1.8-fold increases in activities when expressed on a per gram of liver basis, and 2.4- to 1.4-fold increases on a per milligram of protein basis. Pentobarbital hydroxylation was stimulated most strongly and aniline hydroxylation the least, with the hydroxylation of hexobarbital being intermediate. Also presented in Table I is the effect of photomirex on *N*-demethylase activity using aminopyrine and ethylmorphine as substrates. Here demethylation is increased 3.5- and 1.5-fold on a per gram liver basis, and 2.8 and 1.2 fold on a per milligram of protein basis for aminopyrine and ethylmorphine, respectively. All of the aforementioned increases in enzyme activity were significantly different from control except ethylmorphine demethylase when expressed on a per milligram of protein basis.

As biotransformation was increased by the photomirex pretreatment, it was of interest to measure substrate interactions with cytochrome P-450; aniline, pentobarbital, and hexobarbital were chosen for this purpose. Binding of all three substrates was significantly increased by photomirex exposure when the data were expressed either on a per gram of liver basis or on a per milligram of protein basis (Table II). Aniline binding was most strongly enhanced, while the degree of increase was second for hexobarbital and least for pentobarbital. That the order of extent of these interactions with P-450 is opposite to that observed for hydroxylation of these substrates is worthy of note. The apparent inverse relationship of metabolism of these substrates to their binding to the microsomal fraction may be due to alteration of lipid composition, and nonspecific binding to proteins or lipoproteins. For example, alterations in the lipid and/or protein composition of the microsomes may result in increased binding to substituents not necessarily related to the P-450–substrate interactions. Enhanced lipid accumulation within liver after exposure to photomirex is known to occur (Curtis et al., 1979).

The effect of photomirex preexposure on the NADPH-dependent electron-transport system was also studied. The activities of NADPH oxidase and NADPH-cytochrome *c* reductase were increased 2- and 2.3-fold on a per gram of liver basis and 1.6- and 1.8-fold on a per milligram of protein basis, respectively (Table III). The concentrations of cytochrome P-450 and cytochrome b5 were increased 4- and 1.5-fold on a per gram liver basis and 3.1- and 1.2-fold on a per milligram of protein basis, respectively (Table IV). The amount of microsomal protein per gram of liver tissue was found to be 26% higher in photomirex exposed rats than controls.

These data provide evidence that photomirex is indeed an inducer of hepatic mixed function oxidase system in the male rat. The magnitude of induction is consistent with that obtained with similar doses of mirex (Mehendale et al., 1973) and Kepone (Mehendale et al., 1977). The fact that the major photodegration product of mirex retains the capacity to act as a potent enzyme inducer is cause for interest as to possible interactions of this environmental contaminant with other endogenous or exogenous chemical agents. In fact, preliminary work from our laboratory (Curtis et al., 1979) has shown increased hepatotoxicity of carbon tetrachloride following preexposure to photomirex.

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