LITERATURE CITED
Anglin, C., Mahon, J. H., *J. Assoc. Off. Agric. Chem.* **41**, 177 (1958).
Cheng, L. S., Steffens, G. L., *Tob. Sci.* **20**, 90 (1976).
Davis, D. L., Atkinson, W. O., Smiley, J., *Crop Sci.* **14**, 109 (1974).
Epstein, S. S., Andrea, J., Jaffe, J., Joshi, S., Falk. H., Mantel, N., *Nature (London)* **215**, 1388 (1967).
Epstein, S. S., Mantel, N., *Int. J. Cancer* **3**, 325 (1968).
Haeberer, A. F., Schlotzhauer, W. S., Chortyk, O. T., *J. Agric. Food Chem.* **22**, 328 (1974).
Haeberer, A. F., Chortyk, O. T., *J. Agric. Food Chem.* **22**, 1135 (1974).
Hoffman, I., *J. Assoc. Off. Agric. Chem.* **44**, 723 (1961).
Lane, J. R., *J. Assoc. Off. Agric. Chem.* **48**, 744 (1965).
Lane, J. R., Gullstrom, D. K., Newell, J. E., *J. Agric. Food Chem.* **6**, 671 (1958).
Wood, P. R., *Anal. Chem.* **25**, 1879 (1953).

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Photomirex: Synthesis and Assessment of Acute Toxicity, Tissue Distribution, and Mutagenicity

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Photomirex (8-monohydro mirex), the major photodecomposition product of mirex, was synthesized by reductive dechlorination of mirex and the compound characterized by MS, NMR, and GC. The acute oral toxicity of photomirex was determined in rats given single oral dose of 0, 50, 100, 150, and 200 mg/kg of body weight. The 200 mg/kg dose caused 80% mortality in males and 40% mortality in females. The compound accumulated to high levels in adipose tissue and ovaries and to lower levels in liver, kidney, spleen, heart, brain, and testes. Livers and kidneys were mottled and congested in all animals treated with photomirex. Mirex, photomirex, and kepone were not mutagenic in a standard Ames test including liver microsomal activation.

Photomirex (8-monohydro mirex, 1,2,3,4,5,5,6,7,-9,10,10-undecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane) was recently identified as the fourth highest organochlorine pollutant (after PCBs, DDE, and mirex) in the body lipid and eggs of herring gulls breeding in colonies on Lake Ontario (Hallett et al., 1976). Photomirex was also found to be present at similar ratios to the other major contaminants in coho salmon muscle and liver and in alewives and smelt taken from this lake (Norstrom et al., 1977).

Gibson et al. (1972) showed that approximately 5% of mirex was converted to the 8-monohydro derivative after being exposed to sunlight for 3 months as deposits on silica gel plates. Mirex has also been shown to undergo photolytic dechlorination in cyclohexane and 2,2,4-trimethylpentane (Dilling and Dilling, 1967; Alley et al., 1973; Alley and Layton, 1974) and in egg solids (Lane et al., 1976) when irradiated with UV light. The primary photodegradation product was 8-monohydro mirex with lesser amounts of 5,8-dihydro mirex. Carlson et al. (1976) showed that from 16 to 19.5% of the total mirex-related residues recovered from soil samples recovered 12 years after treatment at 1.12 kg/ha was photomirex. Lesser amounts of kepone (3.1 to 6.3%), 10-monohydro mirex, and two isomers of dihydro mirex were also present. When 4X mirex bait was exposed to intense UV irradiation in a Rayonet-type RS reactor for 19.5 h, similar degradation patterns were found with photomirex being the major degradation product (19.9%), along with lesser amounts of kepone (0.2%), and the other derivatives (Carlson et al., 1976).

The half-life of mirex dispersed in water under intense UV light at 95 °C is 48.4 h, as measured by formation of CO_2. This is similar to DDT (42.1 h) and rather long relative to dieldrin (11.5 h) (Knoevenagel and Himmelreich, 1976). Mirex is very resistant to metabolic attack being slowly dechlorinated to a monohydro derivative by anaerobic microbial action in sewage sludge (Andrade et al., 1975) and likely by enteric bacteria in monkeys as evidenced by the formation of a fecal metabolite (Stein et al., 1976). There has been no reported evidence of metabolic degradation by soil microorganisms (Jones and Hodges, 1976) or in mammals (Gibson et al., 1972). It has been shown to accumulate unaltered in both terrestrial and aquatic ecosystems (Mehendale et al., 1972; Metcalf et al., 1973; Pritchard et al., 1973; Collins et al., 1974). Residues of mirex have been detected in human adipose tissue samples taken in Georgia and Louisiana (Kutz et al., 1974).

An acute oral LD_{50} of mirex was reported as 365 mg/kg for female rats (Gaines and Kimbrough, 1970) and 2400 mg/kg for mallard ducks (Tucker and Crabtree, 1970). Reproductive effects of mirex in the diet of rats include reduced survival rate of progeny and a high incidence of cataracts at 25 mg/kg (Gaines and Kimbrough, 1970). Mirex administered to pregnant female rats on days 6 to 15 of gestation at 6 and 12.5 mg/kg resulted in maternal toxicity, pregnancy failure, decreased fetal survival, reduced fetal weight and increased incidence of visceral anomalies (Khera et al., 1976). Male rats were shown to accumulate mirex from daily oral dosages of 1.5 to 6.0 mg/kg in a dose-related manner. Highest concentrations were found in adipose tissue with lower concentrations in liver and testes although this did not affect reproduction parameters in subsequent mating trials. The distribution

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Table I. Photomirex Acute Toxicity

	Dose, mg/kg				
	200	150	100	50	0
No. dead/5 animals dosed					
Male	4	2	1	0	0
Female	2	1	0	0	0

patterns of mirex-^{14}C and photomirex-^{14}C in rats after a single oral dosage of 0.2 mg/kg were shown to be similar after 7 days (Gibson et al., 1972).

Virtually no toxicological information is available on photomirex. Since photomirex has been found as the major degradation product of mirex in Lake Ontario, and in mirex bait after application, studies were undertaken to synthesize the compound in quantity and to evaluate the acute toxicity and accumulation of the compound in rats. The mutagenicity of the compound was also examined using the Ames bacterial assay.

EXPERIMENTAL SECTION

Reagents and Solvents. Mirex (1,2,3,4,5,5,6,7,8,9,-10,10-dodecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane), 98% pure, was obtained from Hooker Chemical Co., Niagara Falls, N.Y. Kepone (decachloropentacyclo-[5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$]decan-4-one) was obtained from Pesticide Repository, U.S. EPA, Perrine, Fla. Mesitylene was obtained from Baker Chemicals, N.J. Propionic acid and triphenylphosphine were obtained from Fisher Scientific Co., N.J. Corn oil was obtained from Canada Starch Co., Montreal. All solvents used were glass distilled, nanograde quality from Caledon Laboratories, Georgetown.

Synthesis of Photomirex. Photomirex was prepared by the reduction of mirex with triphenylphosphine in propionic acid as described by Kecher et al. (1974). The large quantity of photomirex necessary for this study was produced in ten lots by refluxing mirex (19 g) in mesitylene (150 g) with triphenylphosphine (80 g) and propionic acid (300 mL) at 145 °C for 30 h. The mesitylene was then evaporated, and the remaining reactants were treated with 50 mL of concentrated HNO_3 and refluxed for an additional 3 h. The mixture was then cooled and diluted with 300 mL of H_2O. The dechlorinated mirex was removed by partitioning two times with cyclohexane (200 mL). The cyclohexane was then evaporated to dryness. The photomirex was then purified by repeated crystallization in cold methanol five times. Crystals were then treated with concentrated H_2SO_4 and partitioned into hexane. Yield was 6 g of purified compound/10 g of mirex reacted.

Acute Toxicity Study. Groups of five male and five female Wistar rats, each rat weighing from 175 to 200 g, were administered a single oral dosage of photomirex dissolved in corn oil by stomach intubation. The doses, expressed in mg/kg, were 0, 50, 100, 150, and 200. The volume of the oil per dose for all experimental groups was 10 mL/kg of body weight, and controls were given corn oil alone in the same amount. The rats were killed on day 28 after treatment and examined for gross pathological changes. Samples of liver, heart, brain, kidney, spleen, fat, ovaries, and testes were removed from surviving animals and frozen.

Mutagenicity Study. The mutagenicity of mirex, photomirex, and kepone was tested by standard Ames bacterial assay including a liver microsomal activation mixture. Mutation was detected by determining the reversion of *Salmonella typhimurium* (strains TA1535, 1537, 1538, 98, and 100, Ames et al., 1975) His^- to His^+ on minimal medium supplemented with biotin and a trace of histidine. The liver microsomal activation mixture was prepared from Aroclor 1254-pretreated male Sprague-

Table II. Tissue Residue of Photomirex in Rats 28 Days after a Single Oral Dosage

Dosage, mg/kg	Sex	No. of animals	Concentration (mg/kg of wet weight)[a] in tissues							
			Liver	Heart	Brain	Kidney	Spleen	Fat	Ovaries	Testes
200	Female	3	40.8 ± 17.6	20.2 ± 12.5	16.9 ± 9.3	18.7 ± 12.0	13.0 ± 3.2	182 ± 91.0	167 ± 104	
	Male	1	58.0	6.0	7.7	15.5	5.3	182		6.9
150	Female	4	30.2 ± 13.2	6.5 ± 3.0	3.5 ± 1.2	7.6 ± 1.0	7.9 ± 5.8	187 ± 114	56.1 ± 22.6	
	Male	3	56.4 ± 22.7	7.2 ± 2.1	5.2 ± 1.2	7.5 ± 2.6	5.4 ± 1.4	125 ± 43		4.3 ± 0.5
100	Female	5	12.7 ± 4.7	4.8 ± 2.0	3.4 ± 1.2	5.1 ± 1.0	4.3 ± 1.4	176 ± 59	47.7 ± 16.4	
	Male	4	28.0 ± 10.6	3.3 ± 1.4	2.2 ± 0.8	3.4 ± 1.6	2.2 ± 1.1	81.6 ± 17		2.1 ± 0.1
50	Female	5	9.7 ± 4.1	5.1 ± 1.2	4.6 ± 1.4	5.7 ± 1.2	3.7 ± 2.4	136 ± 37.9	44.4 ± 14.7	
	Male	5	15.2 ± 4.7	1.4 ± 0.7	1.1 ± 0.8	1.4 ± 0.7	2.5 ± 4.4	39.1 ± 16		0.8 ± 0.2

[a] The average values ±SD.

Table III. Mutagenicity of Mirex, Photomirex, and Kepone for *Salmonella typhimurium* Strains

Treatment	μg/plate	TA1535	TA1537	TA1538	TA98	TA100
Mirex	10-1000	–	–	–	–	–
Photomirex	10-1000	–	–	–	–	–
Kepone	10-1000	–	–	–	–	–
α-Naphthylamine	150					+
Benzidine	50				+	
β-Naphthylamine	2	+				
Benzo[*a*]pyrene	20		+	+		

Dawley rats. Two milligrams of microsomal protein was added to each plate. Since some metabolizing enzymes require anaerobic conditions and reactive intermediates of certain mutagens are oxygen labile, duplicate plates were maintained under nitrogen for the first 12 h of incubation.

Extraction and Cleanup of Photomirex from Tissues. Tissues (1 g) were homogenized in distilled water (9 mL) and an aliquot (1 mL) partitioned for 40 min in acetonitrile (4 mL) and hexane (5 mL). An aliquot of the hexane layer (1 mL) was diluted to 10 mL with hexane and washed with concentrated H_2SO_4 (2 mL). The hexane layer was separated and dried over anhydrous Na_2SO_4.

Gas Chomatography. Samples were analyzed with a Hewlett Packard 5830A gas chromatograph fitted with a Ni-63 electron-capture detector. GC column: 1100X 4 mm i.d. glass column packed with 10% OV-1 on 80–100 mesh chromosorb W(HP). GC conditions: detector temperature, 300 °C; injector temperature, 250 °C; oven temperature, 250 °C; carrier gas, nitrogen; flow rate, 39 mL/min.

Mass Spectrometry. The mass spectrometer was a Hitachi Perkin-Elmer RMU-6L interfaced with a Varian MAT 620L computer.

Nuclear Magnetic Resonance Spectrometry. NMR spectra were performed on a Varian T-60 Spectrometer. Optimal spectra were obtained with a 10% solution of compound in deuterated chloroform.

RESULTS

Characterization of Photomirex. The dechlorinated mirex produced exhibited an MS with strong ion abundances at *m/e* 270, 236, and 201 and weak ion fragments at *m/e* 508 and 510 and at *m/e* 471 for the M – 35 loss. This supported a molecular composition of $C_{10}HCl_{11}^+$. The mass spectrum was consistent with that of a monohydro mirex formed by irradiation of mirex in cyclohexane and 2,2,4-trimethylpentane (Alley and Layton, 1974) and that of photomirex found in adult herring gull lipid and eggs from Lake Ontario (Hallett et al., 1976). ^1H NMR showed a singlet at 3.87 ppm. GC of the compound gave one peak on EC, FID, and Hall electrolytic conductivity detectors. By these techniques the compound was determined to be 95 ± 1% pure.

The retention time of the compound relative to mirex was 0.75 on an OV-210 column at 180 °C, identical with that of photomirex described by Gibson et al. (1972), different from the other two isomers possible for monohydromirex. The retention time relative to mirex on a 3% OV-1 column at 190 °C was 0.66, similar to that found for photomirex in Lake Ontario Herring Gulls (Hallett et al., 1976).

The monohydro derivative synthesized was assigned the structure 1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo-[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane, 8-monohydro mirex, or photomirex.

Toxicity. A dose–response relationship was established for rats given a single oral dose of photomirex (Table I). The 200 mg/kg dosage likely approaches an acute oral LD_{50} value for photomirex. The compound appeared to be more toxic to males than females at the upper three dosage levels. Gross pathology on the surviving animals of all dosage groups showed mottled and congested livers and kidneys, more accentuated with higher dosage. Hemorrhagic ovaries were noted in surviving females at and above the 100 mg/kg dose.

Tissue Distribution Study. Photomirex accumulated in the tissues of rats after a single oral dose in a dosage-related manner except in fat of females (Table II). After 28 days highest concentrations were found in adipose tissue, followed in decreasing order by ovaries, liver, kidney, spleen, heart, brain, and testes. This was similar to the distribution of [^{14}C]mirex and [^{14}C]photomirex reported by Gibson et al. (1972) after a single oral dosage of 0.2 mg/kg.

Mutagenicity. Mirex, photomirex, and kepone were not mutagenic to the five bacterial strains tested with liver microsome activation mixture (Table III). The TA1535 and TA100 strains are sensitive to DNA base substitution mutations, TA1537, TA1538, and TA98 are sensitive to DNA frameshift mutagens. Although reductive dehalogenations of DDT, CCl_4, and halothane have been reported to require low O_2 tension (Van Dyke and Gandolfi, 1976), there was no evidence of O_2-labile mutagenicity with the three similar organochlorine compounds tested.

DISCUSSION

Photomirex (8-monohydro mirex) as found in adult Lake Ontario herring gulls and their eggs, coho salmon, and alewives and smelt (Hallett et al., 1976; Norstrom et al., 1977), and as the major photoproduct of mirex bait exposed to natural sunlight (Gibson et al., 1972; Carlson et al., 1976) was synthesized pure and in quantity by reductive dechlorination of mirex. Photomirex appeared to be more acutely toxic to male rats than female rats causing 80% mortality in males and 40% mortality in females after a single oral dosage of 200 mg/kg. The compound exhibited strong lipophilicity accumulating to high levels in adipose tissue and ovaries and to lower levels in other major organs including brain and testes. It was not mutagenic in the Ames bacterial assay, neither was mirex nor kepone, although mirex has been reported to be a hepatocarcinogen (Innes et al., 1969; Ulland et al., 1977).

Further searches should be made for photomirex in areas where mirex residues have been prevalent, particularly in the Lake Ontario region and in the southern U.S. where mirex bait has been applied. The cumulative toxicity of all mirex degradation products, particularly photomirex and kepone, should be considered in assessing the environmental and health hazards due to mirex contamination.

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LITERATURE CITED

Alley, E. G., Dollar, D. A., Layton, B. R., Minyard, J. P., *J. Agric. Food Chem.* **21**, 138 (1973).

Alley, E. G., Layton, B. R., "Mass Spectrometry and NMR Spectroscopy in Pesticide Chemistry", Hague, R., Biros, E. J.,

Ed., Plenum Press, New York, N.Y., 1974, pp 81–90.
Ames, B. N., McCann, J., Yamasaki, E., *Mutat. Res.* **31**, 347 (1975).
Andrade, P. S. L., Wheeler, W. B., Carlson, D. A., *Bull. Environ. Contam. Toxicol.* **14**, 473 (1975).
Carlson, D. A., Konyha, K. D., Wheeler, W. B., Marshall, G. P., Zaylskie, R. G., *Science* **194**, 939 (1976).
Collins, H. L., Markin, G. P., Davis, J. P., *Pestic. Monit. J.* **8**, 125 (1974).
Dilling, W. L., Dilling, M. L., *Tetrahedron* **23**, 1225 (1967).
Gaines, T. B., Kimbrough, R. D., *Arch. Environ. Health* **21**, 7 (1970).
Gibson, J. R., Ivie, G. W., Dorough, H. W., *J. Agric. Food Chem.* **20**, 1246 (1972).
Hallett, D. J., Norstrom, R. J., Onuska, F. I., Comba, M. E., Sampson, R., *J. Agric. Food Chem.* **24**, 1189 (1976).
Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petracelli, L., Fisbein, E. R., Hart, A. J., Pallo Ha, R. R., Bates, R. R., Falk, H. G., Gart, J. J., Klein, M., Mitchell, I., Peters, J., *J. Natl. Cancer Inst.* **42**, 1101 (1969).
Jones, A. S., Hodges, C. S., *J. Agric. Food Chem.* **22**, 435 (1976).
Kecher, R. M., Skibinskaya, M. B., Gallai, O. S., Zefirov, N. S., *Zh. Org. Khim.* **10**, 411 (1974).
Khera, K. S., Villeneuve, D. C., Terry, G., Panopio, L., Nash, L., Trivett, G., *Food Cosmet. Toxicol.* **14**, 25 (1976).
Knoevenagel, K., Himmelreich, R., *Arch. Environ. Contam. Toxicol.* **4**, 324 (1976).
Kutz, F. W., Yobs, A. R., Johnson, W. G., Wiersma, G. B., *Environ. Entomol.* **3**, 882 (1974).
Lane, R. H., Grodner, R. M., Graves, J. L., *J. Agric. Food Chem.* **24**, 192 (1976).
Mehendale, H. M., Fishein, L., Fields, M., Matthews, H. B., *Bull. Environ. Contam. Toxicol.* **8**, 200 (1975).
Metcalfe, R. L., Kapoor, I. P., Lu, P. Y., Schuth, C. K., Sherman, P., *Environ. Health Perspec., Exp. Issue* **No. 4**, 35 (1973).
Norstrom, R. J., Hallett, D. J., Sonstegard, R., personal communication, 1977.
Pritchard, J. B., Guarino, A. M., Kinter, W. B., *Environ. Health Perspec., Exp. Issue* **No. 4**, 45 (1973).
Stein, U. B., Pittman, K. A., Kennedy, M. W., *Bull. Environ. Contam. Toxicol.* **15**, 140 (1976).
Tucker, R. K., Crabtree, D. G., "Handbook of Toxicity of Pesticides to Wildlife", U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication No. 84.
Ulland, B. M., Page, N. P., Squire, R. A., Weisburger, E. K., Cypher, R. L., *J. Natl. Cancer Inst.* **58**, 133 (1977).
Van Dyke, R. A., Gandolfi, A. J., *Drug Metab. Dispos.* **4**, 40 (1976).

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Insecticidal Aminothio Derivatives of the Pesticidal Carbamate Methomyl

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The insecticidal activities of a series of aminothio derivatives of methomyl were investigated. In general, the spectrum of activity of the compounds closely paralleled that of the parent, methomyl, often being equitoxic to the southern armyworm (*Spodoptera eridania* Cramer), cabbage looper (*Trichoplusia ni* Hübner), cotton bollworm (*Heliothis zea* Boddie), tobacco budworm (*Heliothus virescens* Fabricius), and boll weevil (*Anthonomus grandis* Boheman) but less active against the house fly (*Musca domestica* L.) and house cricket (*Acheta domesticus* L.). Additional biological parameters of a single compound, U-46,855, methyl [[[methyl-(4-morpholinothio)amino]carbonyl]oxy]ethanimidothioate, were compared to those of methomyl in the laboratory. U-46,855 demonstrated a marked increase in foliar residual life, lower mammalian toxicity, and greater crop selectivity while methomyl was much more resistant to mechanical loss due to rain. Evaluation of chemicals for the control of the bollworm complex in Alabama demonstrated that U-46,855 produced significantly higher cotton yields than methomyl.

In recent years certain substituents attached to the carbamate nitrogen of *N*-methylcarbamate insecticides have resulted in the production of compounds with improved ancillary properties over the parent. Certain arylthio and acyl derivatives of carbofuran, propoxur, carbaryl, aldicarb, and other *N*-methylcarbamates exhibit reduced mammalian toxicity and, in some cases, increased insecticidal activity (Reay and Lewis, 1966: Brown and Kohn, 1972; Black et al., 1973a). The selective toxicity was initially attributed to differential metabolism (Black et al., 1973b) but upon further examination these authors were unable to substantiate this (Chiu et al., 1975).

A consideration of carbamate insecticides reveals that few are highly effective against lepidopterous insects. Methomyl, an oxime carbamate, proves to be an exception. However methomyl suffers shortcomings with regard to its mammalian toxicity, crop safety, and residual action. Sulfenylated derivatives of methomyl are reported in the patent literature (Durden and Sousa, 1976; Union Carbide Corp., 1976) as showing improvements in these undesirable properties. The main thrust of this article is to report the results of our investigations of aminothio derivatives of methomyl.

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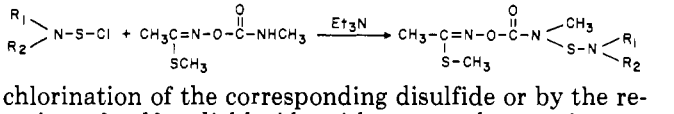
SYNTHESIS OF COMPOUNDS

The aminothio derivatives (Table I) were prepared by the reaction of an appropriate chlorothioamine with methomyl. The chlorothioamines were obtained by the



chlorination of the corresponding disulfide or by the reaction of sulfur dichloride with a secondary amine according to standard procedures (Kühle, 1970; Farbenfabriken Bayer A.G., 1958).

CAUTION: N-Chlorothioamines have been reported to detonate on attempted distillation (Davis and Skibo, 1976); a severe explosion resulting in personal injury and fire has occurred in these laboratories on attempted distillation of *N*-chlorothiopiperidine. Care in assuring purity of starting

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