parent compounds (Williams, 1959). The aglycone, which is known to have deleterious effects on liver function (Nakaue et al., 1973; Gandolfi et al., 1974), was thus modified and presumably removed from its site of action.

LITERATURE CITED

Black, J. G., Sprott, W. E., Howes, D., Rutherford, T., *Toxicology* **2**, 127 (1974).
Bollenback, G. N., et al., *J. Am. Chem. Soc.* **77**, 3310 (1955).
Buhler, D. R., Dost, F. N., Rasmusson, M. E., Gandolfi, A. J., *Xenobiotica*, submitted for publication (1977).
Buhler, D. R., Harpootlian, H., Johnston, R., *Biochem. Pharmacol.* **15**, 1507 (1966).
Buhler, D. R., Rasmusson, M. E., unpublished results, 1976.
Buhler, D. R., Rasmusson, M. E., Nakaue, H. S., *Environ. Sci. Technol.* **7**, 929 (1973).
Donaldson, H. H., "The Rat", Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, Pa., 1924, 469 pp.
Gandolfi, A. J., Buhler, D. R., *Xenobiotica* **4**, 693 (1974).
Gandolfi, A. J., Dost, F. N., Buhler, D. R., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **31**, 605 (1972).
Gandolfi, A. J., Nakaue, H. S., Buhler, D. R., *Biochem. Pharmacol.* **23**, 1997 (1974).
Haque, R., Buhler, D. R., *J. Am. Chem. Soc.* **94**, 1824 (1972).
Kimbrough, R. D., *Crit. Rev. Toxicol.* **2**, 445 (1974).
Kimmel, C. A., Moore, W., Hysell, D. K., Stara, J. F., *Arch. Environ. Health* **28**, 43 (1974).
Mahin, D., Lofberg, R., *Anal. Biochem.* **16**, 500 (1966).
Mahler, W., *J. Am. Chem. Soc.* **76**, 3920 (1954).
Nakaue, H. S., Dost, F. N., Buhler, D. R., *Toxicol. Appl. Pharmacol.* **24**, 239 (1973).
Polakova, A., Gwilliam, C. G., Quamme, G. A., Williamson, D. G., Layne, D. S., *Can. J. Biochem.* **49**, 368 (1971).
St. John, L. E., Jr., Lisk, D. J., *J. Agric. Food Chem.* **20**, 389 (1972).
Shaffer, G. W., Nikawitz, E., Manowitz, M., Daeniker, H. U., *Photochem. Photobiol.* **13**, 347 (1971).
Stowe, C., Plaa, G., *Annu. Rev. Pharmacol.* **8**, 337 (1968).
Tashiro, S., Sasamoto, T., Aikawa, T., Tokunaga, S., Taniguchi, E., Eto, M., *J. Agric. Chem. Soc. Jpn.* **44**, 124 (1970).
Udall, V., Malone, J. C., *Proc. Int. Soc. Study Drug Toxicity* **11**, 244 (1970).
Wang, C. H., in "Methods of Biochemical Analysis", Glick, C. D., Ed., Wiley, New York, N.Y., 1967, pp 311–368.
Williams, R. T., "Detoxication Mechanisms", Wiley, New York, N.Y., 1959, 796 pp.
Williams, R. T., in "Fundamentals of Drug Metabolism and Drug Disposition", La Du, B. N., Mandel, H. G., and Way, E. L., Ed., Williams and Wilkens Co., Baltimore, Md., 1971, pp 187–205.
Wit, J. G., Van Genderen, H., *Acta Physiol. Pharmacol. Neerl.* **11**, 123 (1962).
Wotiz, H. H., Smakula, E., Lichtin, N. N., Leftin, J. H., *J. Am. Chem. Soc.* **81**, 1704 (1959).

Received for review April 19, 1976. Accepted September 7, 1976. This work was supported by Grants FD 00041 and ES 00210 from the Food and Drug Administration and the National Institutes of Health, U.S. Public Health Service. Manuscript issued as Technical Paper No. 4215 from Oregon Agricultural Experiment Station.

Metabolism of [^{14}C]Photodieldrin in House Flies

Gunda Reddy and M. A. Q. Khan*

Metabolism of [^{14}C]photodieldrin was studied in male and female house flies. It was found that the rate of metabolism is relatively higher in female flies. The analyses of extracts of whole flies (organic and aqueous) and their feces by TLC followed by autoradiography revealed the presence of three metabolites and photodieldrin. Two of these metabolites were identified as *trans*-photoaldrindiol and another one as a ketone derivative of photodieldrin.

Dieldrin, which is one of the most persistent chlorinated hydrocarbon insecticide chemicals, is considered an environmental contaminant (cf. Edwards, 1970). Its residues are reported to form photoconversion products (Roburn, 1963; Rosen et al., 1966), which are generally more toxic than their parent compound (Khan et al., 1974). Photodieldrin, the photoisomer of dieldrin, is considered as a "terminal residue" (Khan et al., 1974). Therefore, studies of its metabolic fate in various biological systems are of considerable importance. Several studies on the metabolism of dieldrin in mammals, insects (cf. Menzie, 1974), and microorganisms (Matsumura, 1974) have been conducted but there is only a limited number of studies of the metabolism of photodieldrin. The investigations in insects have revealed that photodieldrin is oxidatively metabolized to photodieldrin ketone (Khan et al., 1969). Klein et al. (1969) have shown that photodieldrin is converted to two metabolites in the mosquito, *Aedes aegypti*, and to three metabolites in the cabbage looper; one of the metabolites in both these species is hydrophilic in nature. However, none of these metabolites have been chemically characterized. We, therefore, carried out further investigations to learn more about the metabolic pathways of photodieldrin in house flies and to identify the metabolites.

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois 60680.

MATERIALS AND METHODS

Chemicals. [^{14}C]Photodieldrin (25 μCi/μmol), generously supplied by Shell Chemical Co., was further purified by TLC in benzene–ethyl acetate (3:1) and benzene solvent systems. The final purified product was essentially free of interfering compounds as tested by electron-capture gas chromatography and autoradiography.

Instrumentation. Thin-layer chromatography (TLC) was employed for the separation of metabolites. Samples were applied on 0.25-mm thick silica gel F-254 precoated glass plates (E. Merck, Darmstadt, West Germany) and developed in solvent system and then exposed to X-Ray No-Screen film (Eastman-Kodak Co.) for 15 to 20 days. After developing the film, the darkened areas were noted and R_f values corresponding to them were recorded for radioactivity measurements. The areas of silica gel corresponding to the spots were scraped and extracted with acetone; aliquots of the latter were counted in liquid scintillation solution (Reddy and Khan, 1975).

Analyses of gas–liquid chromatography and liquid scintillation counting were performed as described pre-

viously (Reddy and Khan, 1974). The details of operating parameters of GLC are given in respective tables.

Infrared data were obtained on a Perkin-Elmer grating spectrophotometer, Model 237B, either in $CHCl_3$ solution or in KBr pellet.

Mass spectral data of isolated metabolites were obtained on a Hewlett Packard GC/MS (mass spectrometer 5930A, GC5700A). The GC column was 4 ft × 0.25 in. of 5% SE-30 on Gas-Chrom Q, 28–100 mesh. The column temperature was 240 °C with a carrier gas flow rate of 40 ml/min. The photodieldrin diacetate standard was run using a direct probe inlet system at 250 °C and samples were run with an electron energy of 70 V.

Insects. House flies of resistant strain (YFC) were reared in the laboratory. They were fed on sugar, milk, and water, ad libitum. Five- to six-day old flies were used in all experiments.

Treatment. Male and female flies were separated under cold immobilization and then treated topically on the thorax with insecticide (220 dpm/0.018 μg per fly) in 1 μl of acetone with the help of a Hamilton microsyringe. Batches of 50 treated insects were transferred to clean 500-ml beakers covered with perforated aluminum foil. For 0- and 6-h observations the dosage was 220 dpm/0.018 μg per fly and for longer periods it was 122 dpm/0.01 μg per fly. After the desired lengths of time, all insects were frozen until further analysis.

Extraction. Flies were homogenized with sand using a mortar and pestle in 20 ml of acetonitrile–water (1:1). The homogenate was filtered on filter paper through suction. The residue was reextracted with acetonitrile–water and then filtered. The combined filtrate was concentrated on a rotary evaporator until only water was left, which was then extracted three times with 50 ml of ether. The combined ether extract was dried over anhydrous sodium sulfate and ether was evaporated. The residue was redissolved in acetone. The aliquots of the latter were used for liquid scintillation counting and for TLC analysis. Prior to TLC separation of [^{14}C]photodieldrin or its metabolites, lipids present in the residue were isolated with acetonitrile and hexane (Jones and Riddick, 1952). The aqueous phase of flies extracted was lyophilized and the residue was extracted with methanol. The aliquots of the latter were counted to measure the water-soluble or conjugated ^{14}C radioactivity. The methanol extract was treated with 2 ml of 1 N HCl for 1 h at 90 °C and then cooled and neutralized with NaOH and extracted with ethyl acetate (Dorough et al., 1974). The ethyl acetate extract was used for TLC analysis to check for acid-released photodieldrin or its metabolites present in aqueous phase.

Feces. Feces excreted in the beakers and the aluminum foil during the observation period were extracted with 20 ml of chloroform–methanol (1:1) (Oonithan and Miskus, 1964) and dried over sodium sulfate. The filtrate was evaporated to near dryness and redissolved in acetone and an aliquot of it was counted by liquid scintillation for excreted radioactivity. After evaporating acetone, the residue was treated with 2 ml of 1 N HCl for 1 h at 90 °C for acid hydrolysis. After cooling, it was neutralized with NaOH and extracted with ethyl acetate which was used for TLC in order to check for the acid-released [^{14}C]-photodieldrin or its metabolites.

To obtain greater quantities of metabolites for identification, 5000 female flies were treated and extracted as described earlier and metabolites were pooled for further analysis. Separation, quantification, and identification of [^{14}C]photodieldrin or its metabolites obtained from flies

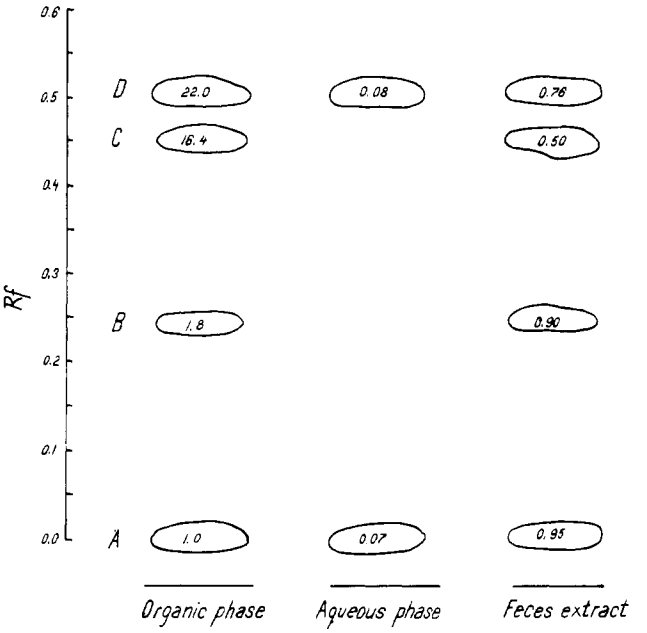

Figure 1. Thin-layer chromatographic presentation of [^{14}C]photodieldrin and its metabolites in house fly extracts of organic, aqueous, and feces. Spots: (A) photodieldrindiol; (B) unidentified metabolite; (C) photodieldrin ketone; (D) photodieldrin. Various fractions of house fly extracts were applied onto a TLC plate and developed in the solvent benzene–ethyl acetate (3:1) and exposed to x-ray film for 10 to 20 days. Numbers in each spot represent the percent of total radioactivity.

extracts were carried out by TLC, GLC, and liquid scintillation as described earlier (Reddy and Khan, 1974).

Acetylation of Metabolite. The metabolite designated as A (Figure 1), which was very hydrophilic, was extracted with acetone from TLC plates. Some of it was saved for infrared analysis and the remaining portion was used for acetylation (Feil et al., 1970). Approximately 3000 to 4000 dpm was treated with 0.3 ml of acetic anhydride and 10 μl of triethylamine. The mixture was allowed to stand at room temperature for 3 days and then poured onto ice (~2 ml) and extracted with methylene chloride. The methylene chloride layer was dried over sodium sulfate and the solvent was then evaporated under a gentle current of air. The residue was used for GLC and TLC with different columns and solvent systems, respectively.

RESULTS

Initial experiments on the toxicity of photodieldrin to house flies showed that males are more susceptible than female flies. A dose of 0.018 μg/fly when applied topically produced 90 and 70% mortality in males and females, respectively, in 6 h. The analysis of male and female flies extract (organic and aqueous phase) by liquid scintillation counting showed that total recovery of applied photodieldrin was about 100% in both sexes soon after treatment, but after 6 h, about 2 and 10% loss of recovery of total ^{14}C activity in males and females, respectively, was observed (Table I). About 10 and 20% loss of recovery of total ^{14}C activity from male and female flies, respectively, was also observed in 24 h. This clearly suggests that photodieldrin or its metabolites are bound to the tissues or are water soluble, which were not easily extractable with the methods employed. The difference in the loss of total ^{14}C radioactivity in the organic phase and consequently the presence of radioactivity in the aqueous phase and feces further lends support to the observation that photodieldrin is indeed metabolized to hydrophilic metabolites which may be present in conjugated or free form, in the

Table I. Radioactivity Extracted from House Flies after Topical Treatment with [^{14}C]Photodieldrin

Expt	Time after applctn,[a] h	% recovery of the applied dose: Org phase	Aq phase[b]	Feces	Total
Male	0	95.0	7.0		102
Male	6	87.0	9.0	2.0	98.0
Male	24	80	6.0	3.0	89.0
Female	0	93.0	5.0		98.0
Female	6	78.0	8.0	3.0	89.0
Female	24	67	4.0	6.0	77.0[c]

[a] 220 dpm/0.018 μg per fly for 0 and 6 h; 120 dpm/0.01 μg per fly for 24 h. [b] Aqueous phase lyophilized and extracted with methanol, aliquot of it counted. [c] Low total recovery might be due to water-soluble and conjugated [^{14}C]photodieldrin and its metabolites in aqueous phase and feces.

aqueous fraction of tissue as well as in feces. The results (Table I) suggest an explanation for the relative resistance of the female flies to the toxic effects of photodieldrin since the rate of metabolism in females is more rapid than in males.

Analysis of different fractions of flies (organic and aqueous) and of feces extracts, by TLC followed by autoradiography, revealed the presence of a number of metabolites in various fractions (Figure 1). They were designated A, B, and C as metabolites, and D as photodieldrin. There was no qualitative difference in number of metabolites formed in both sexes. Since males produced very low levels of metabolites, these data are not included in the figure and it was not pursued further. The metabolites produced by female flies were collected and purified by TLC using various solvent systems and then used for further characterization. No breakdown products were observed in control flies experiments soon after treatment (at 0 h).

Metabolite A was more polar than other compounds as seen by TLC analysis. About 1% of the administered dose was present in organic extracts of flies and in feces. A very low level (0.07%) of metabolite A was detected in the aqueous phase (after acid hydrolysis). Infrared spectroscopic analysis showed a peak at 3325 cm^{-1} indicating the presence of an OH group in the compound. The metabolite was further confirmed by acetylation to form the acetate derivative. The R_f values of this acetate derivative were found to be the same as those of the authentic standards of *cis*- and *trans*-photodieldrin diacetate (Table II). The cis and trans isomers showing the same R_f values, however, could be separated by GLC on different columns. The relative retention time of metabolite A acetate derivative was close in agreement with *trans*-photodieldrin diacetate (Table III). This clearly showed that metabolite A is a *trans*-photodieldrin diacetate derivative. The GC–MS spectrum of metabolite A diacetate derivative was identical with an authentic photodieldrin diacetate standard. Both compounds showed the highest m/e value, 445, *p*-Cl.

The metabolite designated as B obtained from different experiments was pooled. Our attempts to analyze by GLC the metabolite from 5000 flies (purified on TLC and dissolved in 2 ml of hexane and injected into the gas chromatograph) were unsuccessful. This suggested that the quantity of the metabolite was either too low to be detected or was nondetectable.

Analysis of metabolite C by GLC showed that its relative retention time was the same as that of an authentic standard of photodieldrin ketone (Table III).

Table II. R_f Values for Photodieldrin and Its Metabolites Obtained from House Flies of the Authentic Reference Compounds

Compd or metabolites	R_f values in indicated solvent system[a]: Benzene–ethyl acetate (3:1)	Chloroform–methanol (9:1)	Benzene
Photodieldrin	0.51	0.77	0.29
Photodieldrin *cis*-diacetate	0.42	0.68	0.00
Photodieldrin *trans*-diacetate	0.42	0.68	0.00
Metabolite A	0.00	0.24	0.00
Metabolite A diacetate derivative	0.42	0.68	0.00
Metabolite B	0.25	0.61	0.10
Metabolite C	0.46	0.74	0.21
Metabolite D	0.51	0.77	0.39

[a] Average values of two to three experiments.

Table III. Relative Retention Times of Photodieldrin, Metabolites of Photodieldrin, and Authentic Reference Compounds on GLC Columns[a]

Compd or metabolite	Rel retention time[b]: Column A	Column B
Dieldrin	1.00	1.0
Photodieldrin	1.93	2.28
Photodieldrin ketone	1.18	1.23
Photodieldrin *cis*-diacetate	6.00	7.04
Photodieldrin *trans*-diacetate	4.68	5.33
Metabolite A diacetate derivative	4.68	5.33
Metabolite B	ND	ND
Metabolite C	1.18	1.23
Metabolite D	1.93	2.28

[a] (Column A) 5 ft × 1/8 in. glass column packed with 6% DC-200 Varaport-30 (80–100 mesh); (Column B) 6 ft × 1/8 in. glass column packed with 5% SE-52 Chromosorb W, acid washed DMCS treated (80–100 mesh). [b] Relative retention time calculated by considering dieldrin as one. Average values of two to three experiments. Operating conditions were: temperature, inlet 220 °C, column 210 °C, detector 220 °C; N_2, 25 ml/min; sensitivity, 1×10^{-9}; photodieldrin on column A, 0.47 μg/cm^2; column B, 0.2 μg/cm^2; ND, not detectable.

The infrared spectrophotometric analysis showed the absorption peak at 1720 cm^{-1}, indicating the presence of a carbonyl group, and the peak at 875 cm^{-1}, indicating the presence of an epoxy group.

The metabolite designated D was found to be the parent compound photodieldrin (by TLC and GLC analysis). About 22% of the applied dose was recovered in a free state in the organic phase (Figure 1). Very low levels of photodieldrin were recovered in the aqueous fraction of fly homogenate and feces extracts, indicating that it was either water soluble or conjugated in nature.

DISCUSSION

The present results of toxicity and metabolism of photodieldrin in house flies confirmed the previous findings (Khan et al., 1969). The analysis of different fractions (organic, aqueous, and feces) by TLC indicated that photodieldrin is metabolized to hydrophilic and lipophilic metabolites. The tentative identification of metabolites shows that photodieldrin is metabolized oxidatively to ketone and hydroxylated to photoaldrindiol.

Weisgerber et al. (1975) also identified photoaldrindiol, one of the products from the soil treated with [^{14}C]photodieldrin. Khan et al. (1970) showed that the antioxident sesamex affects the toxicity and metabolism of photodieldrin in insects. The formation of ketone and photodieldrindiol in the present study shows the involvement of oxidative and hydroxylating detoxication mechanisms. That both of these types of enzyme systems are involved in the metabolism of dieldrin in insects was also shown by other authors (Oonithan and Miskus, 1964; Tomlin, 1968; Sellers and Guthrie, 1972; Nelson and Matsumura, 1973). It was also observed that about 1% of the applied photodieldrin was excreted unchanged in feces of house flies. Similar excretion of unchanged dieldrin through feces has been reported in flies (Sellers and Guthrie, 1972) and locusts (Cohen and Smith, 1961). However, no dieldrin was detected in the feces of cockroaches treated with [^{14}C]dieldrin (Nelson and Matsumura, 1973).

ACKNOWLEDGMENT

We are deeply indebted for the metabolite standards to F. Matsumura (for photodieldrin ketone) and J. D. McKinney and H. B. Matthews (for *cis*- and *trans*-photodieldrin diacetate derivatives). We are grateful to D. M. Whitacre and B. A. Schwemmer of the Velsicol Chemical Corporation for infrared and GC–MS spectral analysis and to K. G. Srinivasan (Department of Chemistry) for useful suggestions. Excellent technical assistance by Audry P. Waszak is acknowledged.

LITERATURE CITED

Cohen, A. J., Smith, J. N., *Nature (London)* **189**, 600 (1961).
Dorough, H. W., Cardona, R. A., Lolmaugh, S. E., *Drug Metab. Disp.* **2**, 129 (1974).
Edwards, C. A., "Persistent Pesticides in the Environment", CRC Press, Cleveland, Ohio, 1970, 170 pp.
Feil, V. J., Hedde, R. D., Zaylskie, R. G., Zachrison, C. H., *J. Agric. Food Chem.* **18**, 120 (1970).
Jones, L. R., Riddick, J. A., *Anal. Chem.* **24**, 569 (1952).
Khan, M. A. Q., Khan, H. M., Sutherland, D. J., "Survival in Toxic Environments", Khan, M. A. Q., Bederka, J. P., Ed., Academic Press, New York, N.Y., 1974, p 333.
Khan, M. A. Q., Rosen, D. J., Sutherland, D. J., *Science* **164**, 318 (1969).
Khan, M. A. Q., Sutherland, D. J., Rosen, D. J., Carey, W. F., *J. Econ. Entomol.* **63**, 470 (1970).
Klein, W., Kaul, R., Parlor, Z., Zimmer, M., Korte, F., *Tetrahedron Lett.*, 3197 (1969).
Matsumura, F., "Survival in Toxic Environments", Khan, M. A. Q., Bederka, J. P., Ed., Academic Press, New York, N.Y., 1974, p 129.
Menzie, C. M., "Metabolism of Pesticides: An Update", 1974, 486 pp.
Nelson, J. O., Matsumura, F., *Arch. Environ. Contam. Toxicol.* **1**, 224 (1973).
Oonithan, E. S., Miskus, R., *J. Econ. Entomol.* **57**, 425 (1964).
Reddy, G., Khan, M. A. Q., *J. Agric. Food Chem.* **22**, 910 (1974).
Reddy, G., Khan, M. A. Q., *J. Agric. Food Chem.* **23**, 861 (1975).
Roburn, J., *Chem. Ind. (London)* **38**, 1555 (1963).
Rosen, J. D., Sutherland, D. J., Lipton, G. R., *Bull. Environ. Contam. Toxicol.* **1**, 133 (1966).
Sellers, L. G., Guthrie, F. E., *J. Econ. Entomol.* **65**, 378 (1972).
Tomlin, A. D., *J. Econ. Entomol.* **61**, 855 (1968).
Weisgerber, I., Bieniek, D., Kohli, J., Klein, W., *J. Agric. Food Chem.* **23**, 873 (1975).

Received for review May 10, 1976. Accepted September 23, 1976. This study was supported by Grant No. ES-FD-01479 from the National Institute of Environmental Health Sciences.

In Vitro Inhibition of Lactate Dehydrogenases by Kepone

Bruce M. Anderson* and Carter Noble, Jr.

Lactate dehydrogenase catalyzed reactions, both the oxidation of lactate and the reduction of pyruvate, are effectively inhibited by low concentrations of the polychlorinated hydrocarbon pesticide, Kepone. The previous report [Hendrickson, C. M., and Bowden, J. A., *J. Agric. Food Chem.* **23**, 407 (1975)] of Kepone inhibition of rabbit muscle lactate dehydrogenase catalyzed reduction of pyruvate was confirmed; however, a lower K_i value and a different type of inhibition were observed in the present study. The extension of these studies to the investigation of homologous isozymes of lactate dehydrogenase revealed Kepone to be an effective inhibitor of several M_4 isozymes while showing no inhibition (within limits of solubility) of several H_4 isozymes. When detailed studies of the Kepone inhibition were made, mixed inhibition with respect to both substrates and noncompetitive inhibition with respect to the pyridine nucleotide coenzymes were observed. Changes in pH, solvents for Kepone solubilization, and types of buffer had little effect on the Kepone inhibition. The inhibition was observed through dilution experiments to be a fully reversible type of inhibition and no time-dependent inactivation of lactate dehydrogenases was observed in these studies. Studies were carried out with homologous isozymes from rabbit, beef, pig, and chicken.

The polychlorinated hydrocarbon pesticide, Kepone (decachloro-1,3,4-metheno-2*H*-cyclobuta[6*d*]pentalen-2-one), has been observed in in vitro studies to inhibit at least two pyridine nucleotide dependent dehydrogenases, rabbit muscle lactate dehydrogenase (EC 1.1.1.27) (Hendrickson and Bowden, 1975) and beef liver glutamate dehydrogenase (EC 1.4.1.3) (Freedland and McFarland, 1965). In the latter study, some specificity of the glutamate dehydrogenase inhibition by Kepone was suggested by the lack of effective inhibition by other chlorinated hydrocarbons. The inhibition of rabbit muscle lactate dehydrogenase by Kepone was reported to be competitive with respect to pyruvate although no structural analogy to this substrate was apparent. In these studies (Hendrickson and Bowden, 1975), the closely related, fully

Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.