AN ABSTRACT OF THE THESIS OF

STEPHEN GRANT LEBSACK for the degree of MASTER OF SCIENCE in FISHERIES AND WILDLIFE presented on March 11, 1977

Title: SELECTED ASPECTS OF THE PHARMACODYNAMICS OF MALATHION IN CANCER MAGISTER DANA

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R. S. Caldwell

The metabolism of malathion in vivo was studied by injection of adult male Dungeness crab, Cancer magister, with 0.75 μg/g wet weight [^{3}H] malathion. At selected times within 24 hours of injection, subsamples of three crabs were taken for analysis of malathion and metabolites. Malathion was extensively activated by oxidative desulfuration to malaoxon, and hydrolyzed to various water soluble metabolic products. Hydrolysis appeared to be promoted by two distinct enzyme types: carboxylesterases and phosphotriesterases.

An attempt was made to study the oxidative desulfuration of malathion in vitro using preparations from crab hepatopancreas and, as a reference, rat liver. Appreciable malathion desulfurase activity was detected in the rat, but no conversion of malathion to malaoxon was observed in crab hepatopancreas. The condition and content of cytochrome P450, a primary component in the desulfurase system, was examined by difference spectrometry. Substantial P450 was detected in crab hepatopancreas, 0.272 nM/mg microsomal protein.

Anomalies in the absorption spectrum, however, indicated that the cytochrome may have partially existed as a nonfunctional derivative, cytochrome P420. It was suggested that contamination of microsomes during preparation by bile salts naturally occurring in hepatopancreas may have been responsible for denaturation of the oxidative enzymes *in vitro*.

The studies reported here indicate that the decapod *C. magister* is capable of the same metabolic conversions of malathion that have been demonstrated for other classes and phyla of organisms.

Selected Aspects of the Pharmacodynamics of Malathion in Cancer Magister Dana

by

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INTRODUCTION

The absorption of foreign organic chemicals into the tissues of fish (Hamelink et al., 1971; Lee et al., 1972) and aquatic invertebrates (Nimmo et al., 1971; Sheridan, 1975) is well documented. The bioactivities of these compounds are dependent, in part, upon the rates at which they are converted to metabolites which may differ pharmacologically from the parent compound, or which are more polar and, therefore, more easily excreted. While purely chemical reactions may occur, e.g., acid hydrolysis in the stomach, foreign compounds in general serve as substrates for a series of enzymes present in the tissues. Oxidative, hydrolytic, and reductive reactions are commonly the first steps in this metabolic process in terrestrial animals.

The predominant pathways in the biotransformation of foreign compounds are the oxidative reactions catalyzed primarily by the mixed function oxidase (MFO) system. The MFO system has been extensively reviewed (Gillette, 1966; Mannering, 1971; Dewaide, 1971). Liver endoplasmic reticulum or its homogenization fragments, the microsomes, contain the enzymes associated with this system. Many reactions, including aromatic hydroxylation, O- and

N-dealkylation, S-oxidation, and oxidative desulfuration, are known to be catalyzed by these enzymes which require NADPH, molecular oxygen, and cytochrome P450 (Gillette, 1966). The sequence, as proposed by Omura et al. (1965) is shown in Figure 1. Cytochrome P450 plays the central role in the oxidation of foreign chemicals, serving as the oxygen activating oxidase (Omura et al., 1965) and locus of substrate activation (Stevens et al., 1975). Microsomal and soluble cytochrome c reductase may also effect certain oxidative and reductive reactions (Buhler and Rasmussen, 1968a; Elmamlouk and Gessner, 1976b).

Until the past decade the necessity of the MFO system in aquatic animals has been questioned (Brodie and Maickel, 1962). Broidie and Maickel (1962) suggested that aquatic animals may dispose of foreign compounds directly by diffusion through the gill or skin into the surrounding water without initial metabolism to more polar derivatives. The extensive accumulation and persistence of many foreign chemicals, however, suggest that this pathway may not be particularly effective. In the dogfish, Squalus acanthias, approximately 0.5, 1.0, and 2.0% per hour of the dose for p-aminobenzoic acid, sulfadiazine, and antipyrene, respectively, were excreted across the gills (Rall et al., 1966). Similarly, the slow rate of elimination of DDT (2, 2-bis(p-chlorophenyl)-1, 1, 1-trichloroethane) from blue crab, Callinectes sapidus (Sheridan,

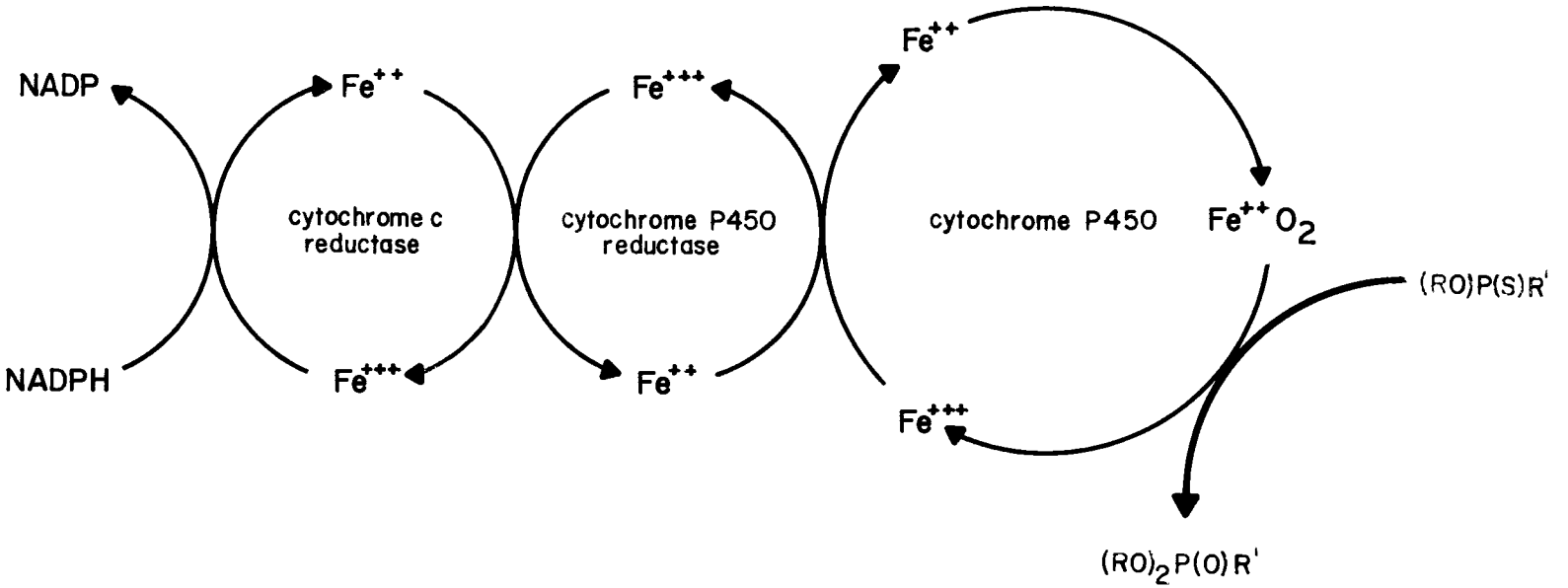


Figure 1. Proposed microsomal electron transport chain involved in the metabolism of foreign compounds (After Omura et al., 1965).

1975), and polychlorinated biphenyls from the shrimp, Paneaus duorarum (Nimmo et al., 1971), indicate that this route may be minor in marine crustacea.

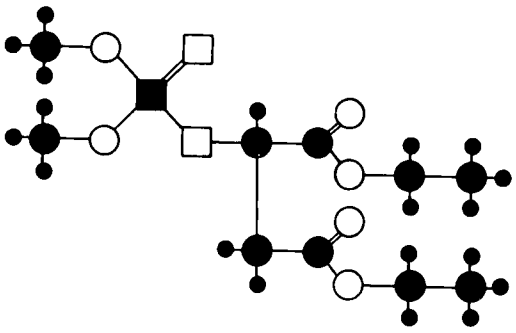
Mixed function oxidase catalyzed reactions have since been described in several fish species and include the epoxidation of aldrin (Chan et al., 1967; Burns, 1976), the hydroxylation of 2-acetyl-animoflourene (Lotikar et al., 1967), the O-dealkylation of p-nitroanisole and p-nitrophenetole (Hansen et al., 1972), and the oxidative desulfuration of parathion (Ludke et al., 1972). Buhler and Rasmussen (1968b) demonstrated that preparations from fish hepatic tissue could catalyze the hydroxylation of aniline and acetanilide, the N-dealkylation of aminopyrene, and the O-dealkylation of phenacetin.

Observations for crustacea are limited and the available data are inconclusive. Several reports indicate that crustaceans metabolize foreign compounds in vivo. Brodie and Maickel (1962) reported that the lobster, Homarus americanus, could metabolize several drugs including aminopyrene, hexobarbital, and chlorpromazine, while Mellet et al. (1969) demonstrated that this species could activate cyclophosphamide. In addition to drugs crustaceans also appear to deal effectively with hydrocarbons. Sheridan (1975) found that the blue crab, Callinectes sapidus, dehydrochlorinated DDT. The spider crab, Maia squinado (Corner et al., 1973) and C.

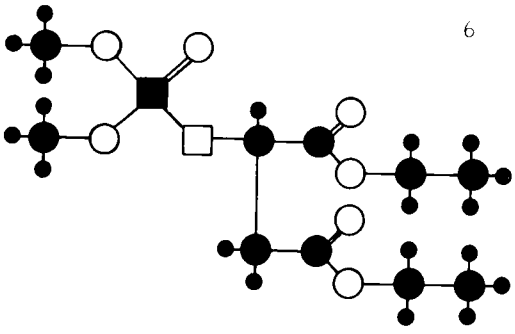
sapidus (Lee et al., 1976) were also shown to hydroylate several polycyclic aromatic and paraffinic hydrocarbons.

Brodie and Maickel (1962) first suggested that in crustaceans the oxidative enzymes may be located in the hepatopancreas. In C. sapidus, Lee et al. (1976) found that during exposure to polycyclic aromatic hydrocarbons both parent compounds and dihydroxy metabolites were quickly detectable in this tissue. However, with the apparent exception of aldrin epoxidation in crayfish, Procambarus sp. (Khan et al., 1972), and lobster, H. americanus (Carlson, 1974), microsomal preparations from crustacean hepatopancreas have been found incapable of catalyzing many MFO reactions. These include the dealkylation of ethylmorphine (Pohl et al., 1974), the hydroxylation of aniline or biphenyl (Elmamlouk and Gessner, 1976b), or the desulfuration of parathion (Carlson, 1973; Elmamlouk and Gessner, 1976a). The inability to demonstrate oxidative desulfuration in vitro of organophosphorus substrates may be considered unusual since several studies indicate the reaction proceeds rapidly in vivo.

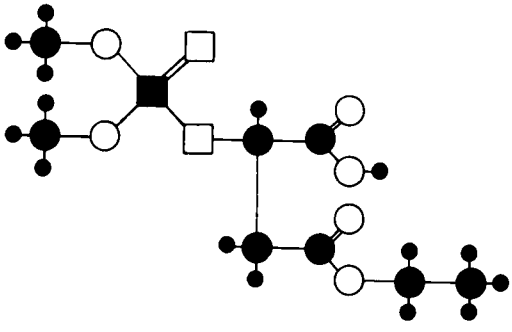
The toxicity of malathion and its desulfuration product, malaoxon (Figure 2), to aquatic animals has been extensively examined (Pickering et al., 1962; Eisler, 1965; Conte et al., 1971; Sanders, 1972; Caldwell et al., 1977). Fish are highly susceptible to malathion: 96-hour LC_{50} values for the bluegill, Lepomis



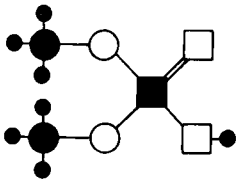
MALATHION
(O, O-dimethyl-S-(1, 2-bis-carbethoxy)ethyl phosphoro-dithioate)



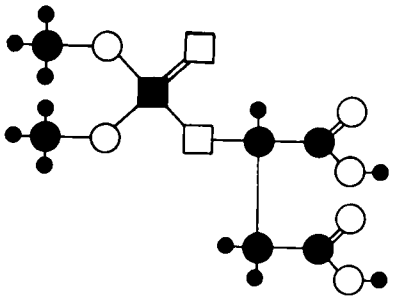
MALAOXON
(O, O-dimethyl-S-(1, 2-bis-carbeth-oxy)ethyl phosphorothiolate)



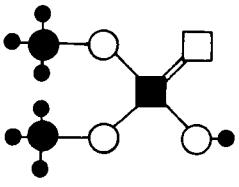
DMCCP
(O, O-dimethyl-S-(1-carboxy-2-carbethoxy) ethyl phosphorodithioate)



DMDPT
(O, O-dimethyl phosphoro-dithioate)



DMCP
(O, O-dimethyl-S-(1, 2-bis-carboxy)ethyl phosphoro-dithioate)



DMPT
(O, O-dimethyl phosphoro-thioate)

- Hydrogen
- Carbon
- Oxygen
- Sulfur
- Phosphorus

Figure 2. Structural representations of malathion and some reported metabolic products.

macrochirus; the goldfish, Carassus auratus; and the guppy, Lebistes reticulatus, were 0.09, 0.45, and 0.84 mg/L, respectively (Pickering et al., 1962). Murphy (1966) has shown in fish, as had been demonstrated earlier for mammals and insects (Krueger and O'Brien, 1959), that malaoxon was responsible for the high mortality associated with malathion intoxication, although malathion may exert a minor effect prior to desulfuration.

Crustacea, in general, show an order of magnitude greater sensitivity to malathion than fish. Eisler (1965) determined 96-hour LC_{50} values for Crangon septemspinosa, Paleomonetes vulgarus, and Pagurus longicarpus, of 0.033, 0.082, and 0.083 mg/L, respectively. Caldwell et al. (1977) demonstrated 96-hour LC_{50} values of 0.0012 and 1.33 mg/L, for zoeal and adult Dungeness crab, Cancer magister, respectively. The greater sensitivity of marine crustacea to malathion may be the result of a more rapid rate of uptake. However, Lee et al. (1972) have shown that with several polycyclic aromatic hydrocarbons uptake rates for crustacea and fish were similar. Thus, these data suggest that crustacea may have the ability to effect a high rate of desulfuration of malathion in vivo or alternatively, the accumulation of malaoxon to critical concentrations may indicate a lesser ability in these animals to enzymatically degrade malathion and malaoxon.

Both malathion and malaoxon are inactivated, in part, by

enzymecatalyzed hydrolyses (March et al., 1956; Krueger and O'Brien, 1959; Bourke et al., 1968; Zayed et al., 1972; Walker and Stojanovic, 1974). The hydrolysis of malathion proceeds at three sites; the carbethoxy, the alkylthionate, or the phosphoryl-thionate linkages, resulting in the formation of several phosphorothionic di- and tri-ester acid anions (Figure 2). The reactions appear to be catalyzed by at least two distinct enzyme types: carboxylesterases (E.C. 3.1.1.1), which preferentially hydrolyze aliphatic esters, and phosphotriesterases (E.C. 3.1.1.2), which preferentially hydrolyze phosphoryl aromatic esters (Hutson, 1970). Malaoxon appears to be hydrolyzed primarily by the carboxylesterases at the carbethoxy linkage, while the phosphotriesterases do not appear to contribute significantly to its metabolism. In contrast to the oxidative enzymes which are only intracellular, a number of hydrolytic enzymes have been shown to be present both intracellularly and in the blood plasma. O'Brien (1967) reported three serum esterases which hydrolyzed malathion in mice. Cohen and Murphy (1972) have shown that malaoxon was hydrolyzed by at least two esterases in mouse liver.

As suggested by the recovery of identical hydrolysis products from mammals, birds, and insects (March et al., 1956), plants (Bourke et al., 1968), and bacteria (Walker and Stojanovic, 1974) exposed to malathion, carboxylesterases and phosphotriesterases

appear to be the primary means by which malathion is degraded in most organisms. Several crustacean species have also been shown to possess hydrolytic esterases (Barlow and Ridgeway, 1971), however, the participation of these enzymes in the metabolism of foreign compounds is uncertain. The toxicity of malathion to crustacea may reflect a low level of enzymatic activity against it and other foreign chemicals susceptible to hydrolysis. The selective toxicity of malathion to many species of insects has been shown to be due to the low activity of the hydrolytic enzymes (Krueger and O'Brien, 1959).

The primary objective of the studies presented here was to examine the metabolism of malathion by C. magister emphasising both hydrolytic and oxidative pathways in order to compare the handling of this compound by the crab with organisms representing other classes and phyla. Also, because of the contradictions regarding the presence of MFO activity in crustaceans (Elmamlouk and Gessner, 1976b; Lee et al., 1976) a second objective was to examine this system with respect to malathion desulfuration in crab hepatopancreas in vitro.

MATERIALS AND METHODS

Chemicals

Tritiated methanol (5.0 mCi) was obtained from New England Nuclear Corporation. Cythion®; and standard reference samples of malathion, O, O-dimethyl-S-(1, 2-bis-carbethoxy)ethyl phosphorodithiote; malaoxon, O, O-dimethyl-S-(1, 2-bis-carbethoxy)ethyl phosphorothiolate; O, O-dimethyl-S-(1-carboxy-2-carbethoxy)ethyl phosphorodithioate (DMCCP); O, O-dimethyl-S-(1, 2-bis-carboxy) ethyl phosphorodithioate (DMCP); O, O-dimethyl phosphorodithioate (DMDPT); and O, O-dimethyl phosphorothioate (DMPT) were kindly supplied in gratis by American Cyanimide Company Agricultural Division. The following chemicals were obtained from Sigma Chemical Company: nicotinamide-adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P), and glucose-6-phosphate dehydrogenase (G6PD). Diethyl maleate was obtained from ICN Pharmaceuticals, Inc. All additional chemicals were purchased from Mallinkrodt Chemical Works.

Animals

Male Dungeness crabs, Cancer magister Dana, of similar weight, 277 $\pm$ 48 g, were collected by trawl from Yaquina Bay,

Oregon during the period 1 January - 31 March, 1976, and held at the Marine Science Center, Newport, Oregon. Crab were housed individually in glass aquaria at near constant temperature, 9-11° C, and salinity, 27-33°/oo, without feeding. Experiments were conducted within 2 to 7 days of the date of capture.

One female rat (OSU-Wistar), 4 months old and weighing 233 g, was a gift of Dr. D. R. Buhler, Oregon State University, Corvallis, Oregon.

Acute Toxicity Bioassays

Preliminary acute toxicity bioassays were conducted in order to establish the dosage of injected malathion to be used in the studies of malathion metabolism in vivo. Cythion® (95.5% malathion) dissolved in 95% ethanol was injected into the ventral venous sinus via the coxal joint of the second left walking leg. Controls were injected with ethanol only. The dosages of malathion tested ranged from 0.75 to 10.0 μg/g wet weight. A total injection volume of 0.5 μl/g wet weight was maintained at all dosages. Mortalities were recorded at 30 minute intervals for the first 6 hours and at 6 hour intervals for the remaining 64 hours. The criterion for death was lack of visible scaphognathite movement during a 2 minute observation period (Caldwell, 1974).

Metabolism of Malathion in vivo

The metabolism of malathion in vivo was studied by injection of whole crabs with 0.75 μg/g wet weight of the insecticide radiolabelled with tritium at the methoxy position. A flow chart of the procedure used for the extraction, separation, and analysis of the insecticide and metabolites is shown in Figure 3.

At selected sampling intervals three crabs were taken for analysis. While homogenates were prepared by homogenizing individual crabs for three minutes in an Osterizor blendor. Five gram subsamples were then rehomogenized for 1 minute in a Sorvall Omnimixer with 10 ml of 10% trichloroacetic acid (TCA) and 25 ml of chlorform (Krueger and O'Brien, 1959). Each homogenate was filtered through Whatman No. 1 filter paper on a Buchner funnel and the residue was washed on another flask with 60 ml of acetone-methanol (1:1). The acetone-methanol filtrate was evaporated to dryness and combined with the initial TCA-chloroform filtrate to which was added sufficient sodium hydroxide to give a final pH of 7.0 to 7.5. The filtrate was then thoroughly shaken in a separatory funnel and the chloroform and water fractions separated. Using this extraction scheme it was possible to selectively partition malathion and malaoxon into the chloroform fraction.

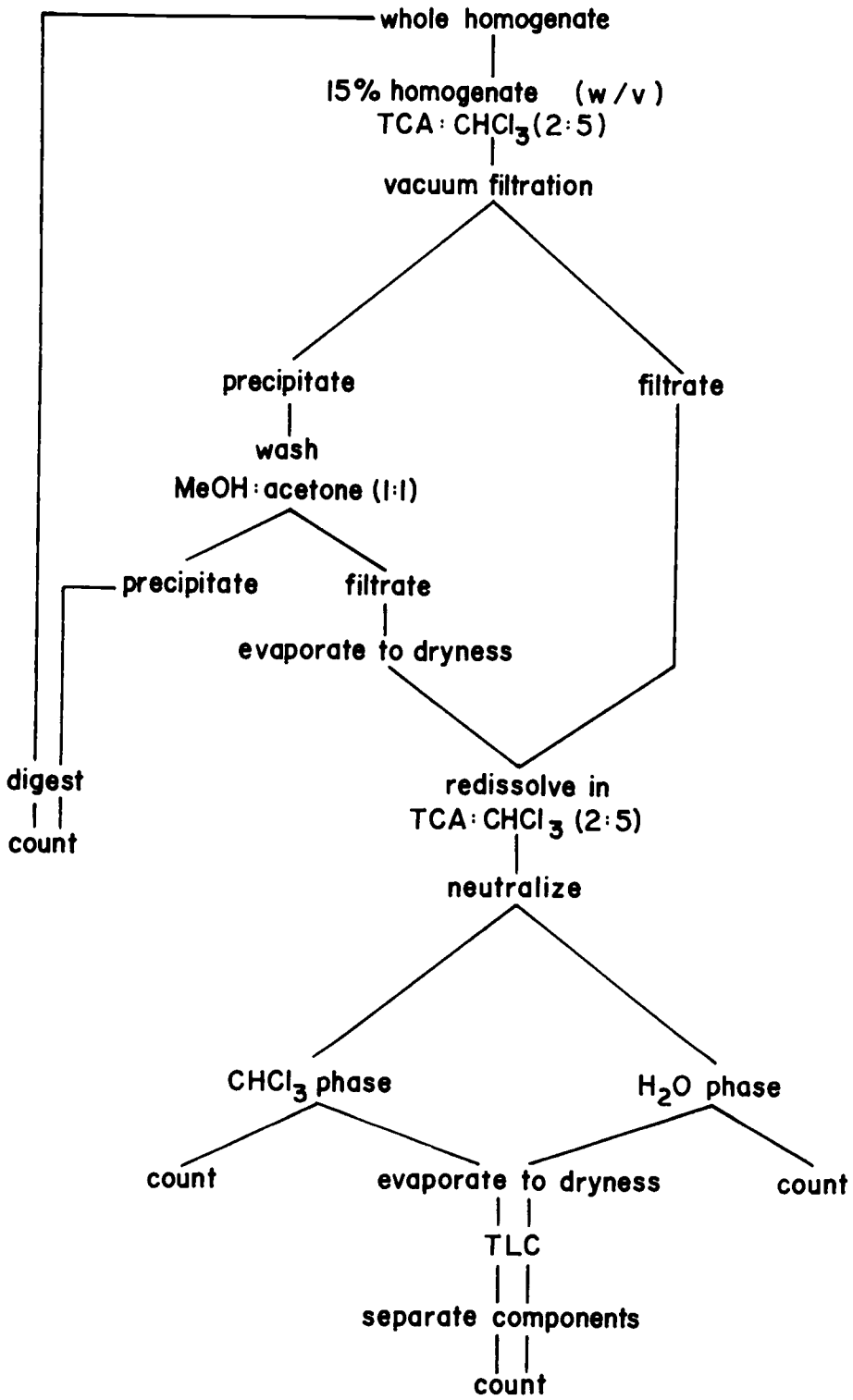


Figure 3. Flow chart of the analytical procedure used in the recovery of [^{3}H] malathion and metabolic products

Total chloroform extractable radioactivity was determined using 10 ml of the chloroform fraction. The chloroform was first evaporated to dryness, then digested to remove color in 0.6 ml of 70% perchloric acid- 30% hydrogen peroxide (1:2) by heating 2 hours at 75°C with slight agitation (Mahin and Loftberg, 1966). The residue was combined with fluor type 1 and counted by liquid scintillation as described below. Total water extractable radioactivity was determined by pipetting 2.0 ml of the water fraction into sufficient fluor type 2 and counted without further treatment. Permanently bound radioactivity was measured as the difference in activity in unextracted minus extracted tissue. Unextracted samples of whole homogenate or extracted tissue residue were placed into tared scintillation vials, dried to constant weight, and then digested in perchloric acid-hydrogen peroxide. Fluor type 1 was added to the vials which were then counted as previously indicated.

Individual radiolabelled components in the chloroform and water fractions were resolved by thin layer chromatography (TLC). Aliquots of the chloroform fraction were evaporated to dryness, redissolved in hexane, and applied across the bottom of thin layer plates of silica gel G. Reference samples of malathion and malaoxon were spotted at the margins of the plates which were then developed in hexane: acetic acid:ethyl ether (75:15:10) (Kadoum, 1970). Following development, plates were partially exposed and

the reference materials visualized by spraying with a solution of 0.5% 2, 6-dibromoquinonimine-4-chloroimide (DQC) in acetone, and heating at 110°C for 10 minutes (Menn et al., 1957). The R_f values were calculated and the bands corresponding to the location of the crab extracts were scraped from the glass into scintillation vials, combined with fluor type 2, and counted. Aliquots of the water fraction were evaporated to dryness and extracted with acetone. The acetone extract was evaporated to dryness, redissolved in hexane, and applied to thin layer plates. Reference samples of DMCCP, DMCP, DMDPT, and DMPT were spotted at the margins of the plates which were then developed in benzene:acetic acid (4:1) (Kadoum, 1970). Samples were located, prepared, and counted as described previously. To recover metabolites for which reference materials were not available samples were prepared from the orgin and remaining portions of the chromatogram.

Malathion radiolabelled with tritium at the methoxy position was synthesized by a modification of the method of Krueger and O'Brien (1959). One hundred milligrams of phosphorus pentasulfide was placed in a 25 ml three-necked reaction flask. Five milliliters of anhydrous toluene and a stirring bar were added to the flask, and 0.07 ml [^{3}H] methanol (2.88 mCi/mM) dissolved in 2 ml of toluene was added dropwise with stirring. The contents were refluxed with stirring at 85°C. After 3 hours the temperature was dropped to

50°C and 0.215 ml diethl maleate was added. The contents were then refluxed with stirring for an additional 5 hours. Toluene was driven off under dry nitrogen, the residue redissolved in chloroform, and partitioned with an equal amount of cold 10% sodium carbonate. The chloroform was evaporated to dryness, the residue redissolved in 2, 2, 4-trimethylpentane (TMP), and chromatographed on a Celite column.

The Celite column was prepared by mixing 25 g Celite with 15 ml methanol, two-thirds saturated with TMP. This mixture was slurried in 125 ml of TMP, saturated with methanol, and packed to form a column of 2.0 x 35.5 cm (Bowman and Cassida, 1957). The residue was placed on top of the column and eluted with methanol-saturated TMP. Chemical purity was determined by gas chromatography using a Hewlett-Packard Model 5700A chromatograph equipped with a [^{63}Ni] electron capture detector, and a 6 ft x 4 mm I.D. glass column packed with 3.0% UCW-98 on 80/100 mesh chromosorb W-HP. The flow rate was 60 ml/minute with an oven temperature of 220°C and a detector temperature of 300°C. The synthesis yielded 70 mg of malathion with an initial specific activity of 5.42 mCi/mM.

All scintillation counting was performed in a Packard Model 3375 TriCarb spectrophotometer, equipped with external standardization. Quench curves were prepared by the addition of variable

small amounts of water. The counting chest was regulated to 9°C. Samples were equilibrated 45 minutes before counting.

Type 1 fluor, used for perchloric acid-hydrogen peroxide digests, consisted of toluene:2-ethoxyethanol (2:1) containing 2, 5-diphenyloxazole (6.0 g l). Type 2 fluor consisted of p-dioxane:methanol: ethylene glycol (88:10:2) containing 2, 5-diphenyloxazole (4.0 g/L), 2, 2-p-phenylene-bis-(5-phenyl)-oxazole (0.6 g/L), and naphthalene (60.0 g/L) (Wang and Willis, 1965).

Estimation of Microsomal Cytochrome P450 Content

To more fully characterize the oxidative desulfuration of malathion by C. magister this system was examined using microsomes prepared from crab hepatopancreas. Prior to the assay for malathion desulfurase the content of cytochrome P450 was first determined. Microsomes were prepared from 16% homogenates of crab hepatopancreas or rat liver in 1.0 M phosphate buffer (pH 7.4) containing 0.154 M KCl. The homogenization was performed on ice using an SDT Tissuimizer for 1 minute at 5000 rpm.

The homogenates were first centrifuged at 9000 x g for 30 minutes in a Sorvall RC-2 refrigerated centrifuge. The 9000 x g supernatant was centrifuged at 105, 000 x g for 60 minutes in a Spinco Model L ultracentrifuge to separate the microsomal fraction from the soluble fraction. The pellet was washed by suspending it

in the homogenization medium and recentrifuging for 60 minutes. The pellet was resuspended to a final volume equal to the original 9000 x g supernatant volume. Protein content was determined by the method of Lowry et al. (1951).

The suspension was divided equally into two 1-cm cuvettes and a baseline of equal light absorbance was established using a Spectronic 505 double beam recording spectrophotometer. To determine the concentration of cytochrome P450 the contents of the sample cell were first gassed for three minutes with carbon monoxide (CO) then reduced with sodium dithionite resulting in the formation of a CO-reduced cytochrome P450 complex (Omura and Sato, 1964). The difference spectrum of the CO-reduced complex minus the oxidized cytochrome P450 was recorded and the concentration determined using the extinction coefficient of 100 for the change in absorbance at 450 nm minus 490 nm (Bend et al., 1972).

Assay for Microsomal Malathion Desulfurase

Malathion desulfurase activity was examined in vitro to document the involvement of the MFO system in crustacean metabolism. The desulfurase system was examined using microsomes prepared from crab hepatopancreas and rat liver as previously described but employing 1.0 M potassium phosphate buffer (pH 8.0) containing 1.0 mM EDTA and 0.25 M sucrose.

Desulfurase activity was determined by estimation of radio-labelled malaoxon formed during 60 minute incubations under air. Incubation mixtures (3.0 ml) contained the microsomal equivalents of 100 mg wet weight hepatopancreas or liver; 0.4 mM $MgCl_2$; 0.004 mM $MnCl_2$; an NADPH-generating system consisting of 0.1 mM NADP, 6.0 mM G6P, and 1.0 unit G6PD; and the substrate, 3.03 mM [^{3}H] malathion, in 1.0 M potassium phosphate buffer (pH 7.4 or 8.0). The NADPH-generating system was incubated for 10 minutes prior to the addition of tissue to insure complete conversion to NADPH.

Zero time incubations and those without microsomes served as controls. Incubations were carried out on a Warner-Chilcott metabolic shaker at 20°C for crab or 37°C for rat preparations. The reactions were terminated by the addition of 2.0 ml of 10% TCA.

TCA-treated incubations were transferred to separatory funnels and extracted with chloroform containing malathion and malaoxon (nonlabeled) as reference compounds. The chloroform phase was dried over anhydrous sodium sulfate, concentrated under dry nitrogen and chromatographed on silica gel G thin layer plates using hexane:acetic acid:ethyl ether (75:15:10) as the solvent system (Kadoum, 1970). Malathion and malaoxon were located with DQC and prepared for scintillation counting as previously described.

RESULTS

Acute Toxicity Bioassays

Crabs injected with malathion showed classical symptoms of organophosphate intoxication at all dosages tested; initial hyperirritability followed by a progressive loss of motor coordination. There were no indications of recovery in animals surviving the 72 hour test period. In contrast, controls, injected with ethanol only, were alive and active when the experiment was terminated.

A log-probit plot of percent survival vs. time for each dosage is given in Figure 4. Median survival times obtained from these plots were compared directly with the dosage (Figure 5). Median survival time ranged from 50 minutes for crabs injected with 10.0 μg/g malathion to 1100 minutes for those given a 0.85 μg/g dosage. Injection of crabs with 0.75 μg/g of the insecticide failed to kill 50% of the animals during the test period. The resulting sharp break in linearity of the plot of median survival time vs. dosage between 0.75 and 0.85 μg/g indicates that the median lethal threshold for acute toxicity falls between these two concentrations. On the basis of this experiment a dosage of 0.75 μg/g was selected for the studies of malathion metabolism *in vivo*.

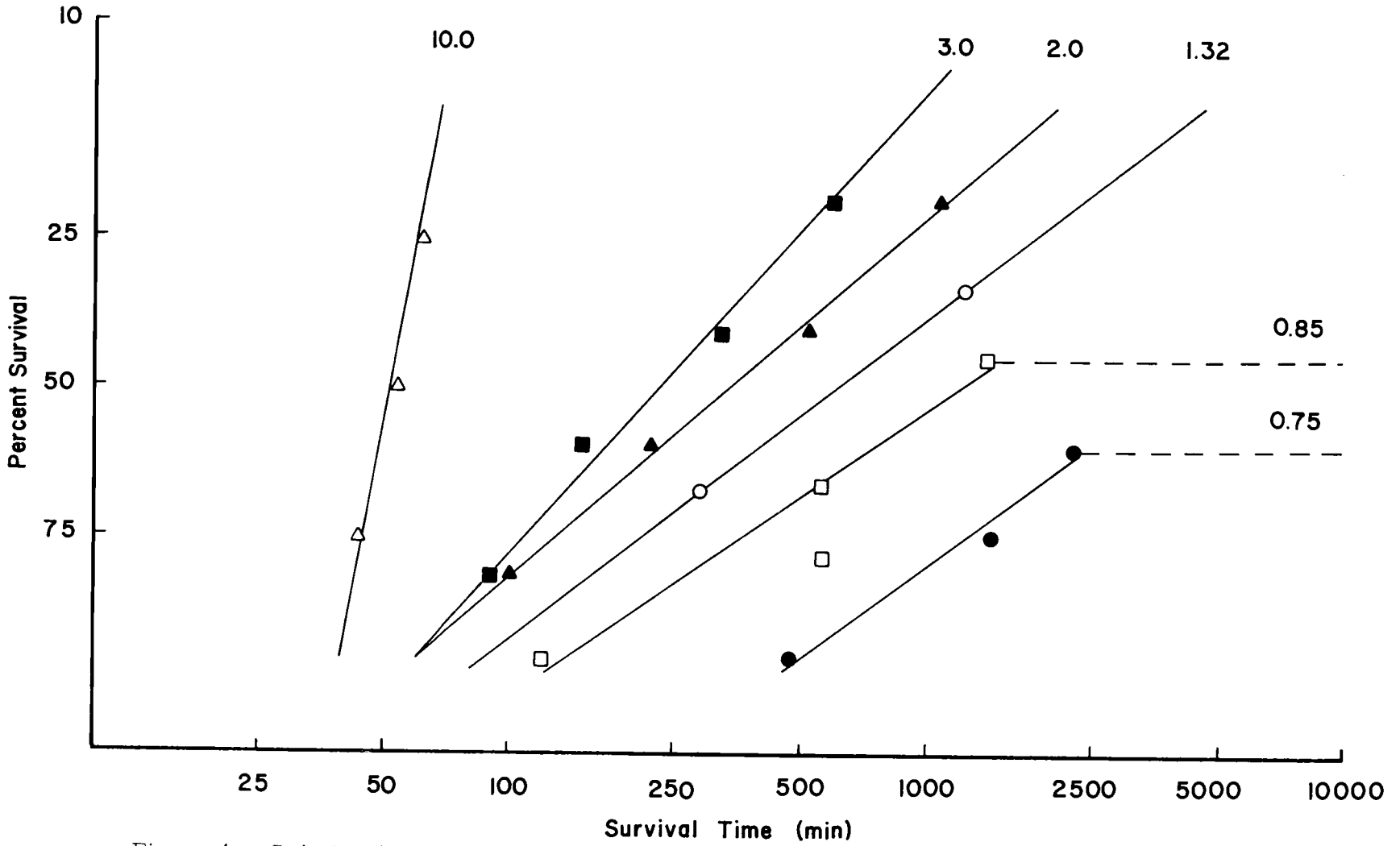


Figure 4. Relationship between percent survival and time for C. magister at six dosages of malathion. Dosages are expressed as μg/g

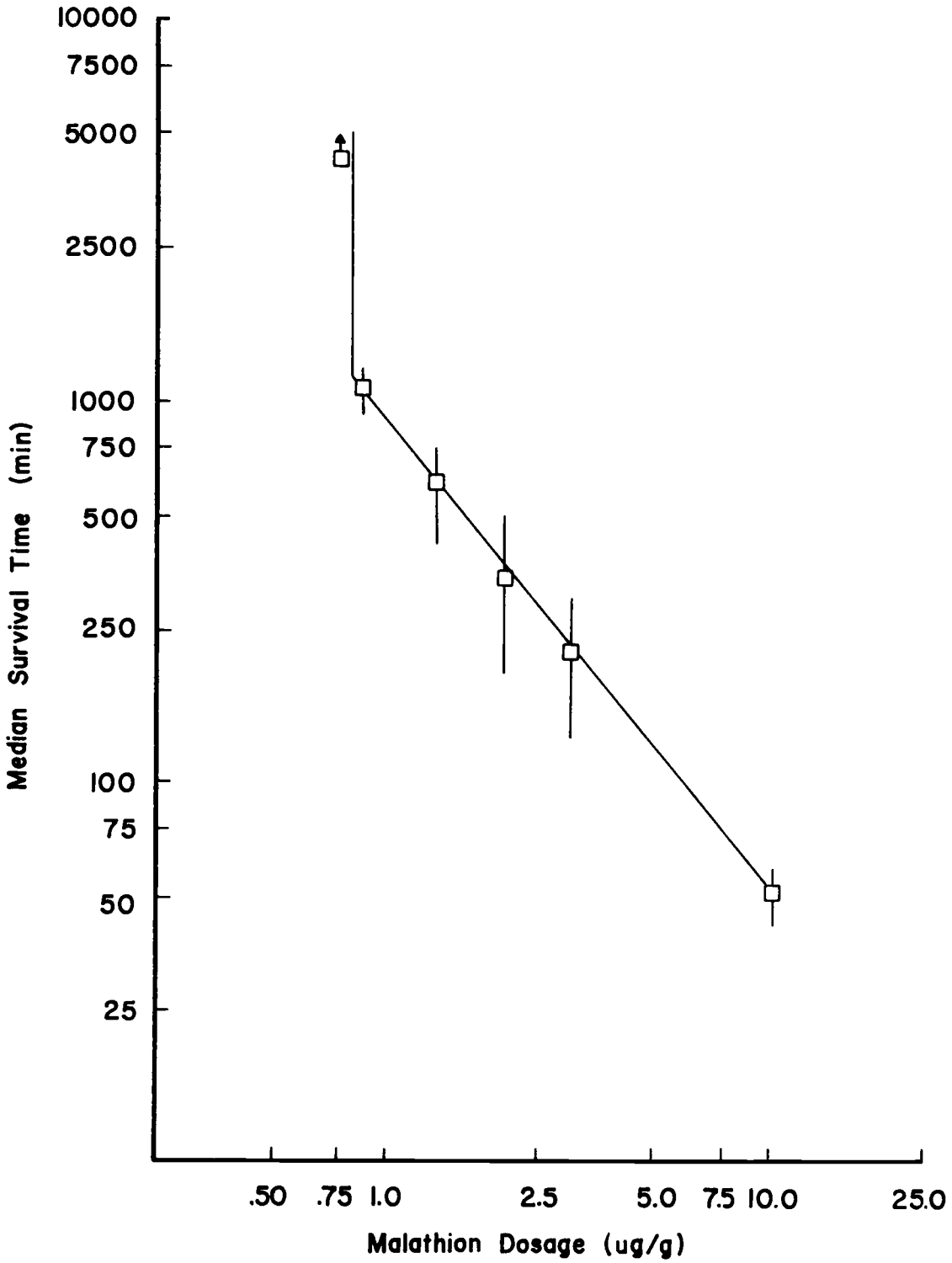


Figure 5. Median survival time of C. magister as a function of injected dose of malathion

Metabolism of Malathion in vivo

The recovery of injected radioactivity from whole crab homogenates was found to decline as a function of time after injection (Figure 6). The rate of loss was rapid during the first 4 hours leaving 50-60% of the original radioactivity after that period of time. During the remaining 20 hours a much slower rate of loss of radioactivity occurred, and at the end of 24 hours 40% of the injected radioactivity was still retained by the crabs. All of the recovered radioactivity could be accounted for in the chloroform and water fractions of the extracted tissues (Figure 6). Using Student's "t" test it was determined that at the 95% probability level no significant difference in percent of injected dose recovered existed between the whole homogenate and the combined chloroform and water fractions at any time after injection. Furthermore, no detectable radioactivity could be demonstrated in the insoluble residue after extraction.

Malathion was rapidly metabolized by the crab to a variety of chloroform and water soluble products. A comparison of the R_f values for thin layer separations of six reference standards with those of unknown spots containing radioactivity is given in Table 1. The R_f values of 0.82 and 0.23 for reference standards of malathion and malaoxon, respectively, are in good agreement with the R_f values of unknown spots resulting from separation of the chloroform

Table 1. A comparison of R_f values for reference standards with R_f values for radioactive unknowns recovered in the chloroform and water fractions of extracted whole crab homogenates.

Reference Standard	R_f of Reference Standard	R_f of Radioactive Unknown
Chloroform Soluble		
Malathion	0.82	0.82
Malaoxon	0.23	0.23
Water Soluble[a]		
DMCCP	0.61	0.63
DMCP	0.37	0.40
DMDPT	0.20	0.20
DMPT	0.12	0.11
none	0	0

[a]Reference standards used were O,O-dimethyl-S-(1-carboxy-2-carbethoxy) ethyl phosphorodithioate (DMCCP); O, O-dimethyl-S-(1, 2-carboxy) ethyl phosphorodithioate (DMCP); O,O-dimethyl phosphorodithioate (DMDPT); and O,O-dimethyl phosphorothioate (DMPT).

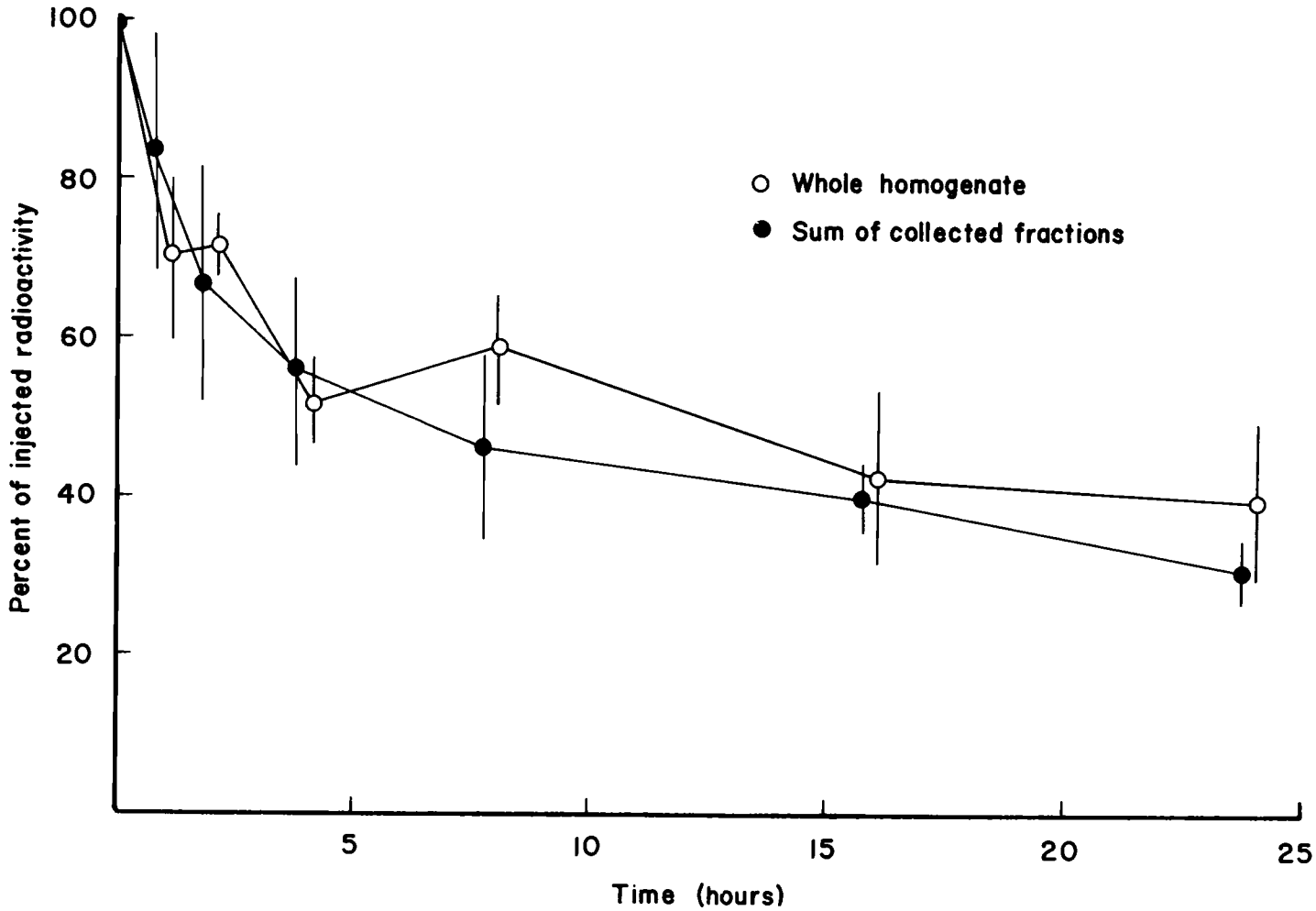


Figure 6. Percent of injected radioactivity recovered in whole crab homogenates and combined chloroform and water fractions as a function of time after injection of C. magister with 0.75 μg/g [^{3}H] malathion

fraction indicating that these unknowns were malathion and malaoxon. Further analysis of the chlorform fraction by gas-liquid chromatography confirmed the identity of malathion. The R_f values of reference standards of DMCCP, DMCP, DMDPT, and DMPT were very similar to four of the five radioactive spots observed on thin layer chromatograms of the water soluble fraction (Table 1). Although further chemical characterization of the unknown spots was not attempted, these identifications were assumed to be valid from consideration of the available cleavage sites on the malathion molecule and had previously been identified as the metabolic products of malathion metabolism in both insects and mammals (Krueger and O'Brien, 1959). A fifth, unidentified, spot was found at the orgin of the thin layer plates.

The percentage of recovered radioactivity associated with malathion and its metabolic products with respect to time after injection of adult C. magister with [^{3}H] malathion is given in Tables 2 and 3, and summarized graphically in Figure 7. Malathion declined consistently throughout the 24 hour period, contributing only 11.1% of the total extractable radioactivity at the end of that time. Malaoxon increased to a maximum of 8.2% of the recovered radioactivity within the first hour, but declined rapidly after that time and was not detected after 8 hours.

Within 2 hours of injection more than 50% of the extractable

Table 2. Composition of chloroform extractable radioactivity recovered from whole crab homogenates as a function of time after injection of C. magister with 0.75 μg/g [^{3}H] malathion.

Time after treatment (hr)	Percentage of total recovered radioactivity[a]	
	Malathion	Malaoxon
1.0	50.0 ∓ 15.4	8.2 ∓ 4.3
2.0	43.8 ∓ 7.3	1.2 ∓ 2.0
4.0	30.8 ∓ 4.1	0.9 ∓ 1.0
8.0	19.7 ∓ 4.7	0.5 ∓ 1.0
16.0	14.0 ∓ 4.7	[b]
24.0	11.1 ∓ 2.5	[b]

[a] Values represent mean ∓ standard deviation of 3 determinations.

[b] Malaoxon not detected after 8 hrs.

Table 3. Composition of water extractable radioactivity recovered from whole crab homogenates as a function of time after injection of C. magister with 0.75 μg/g [^{3}H] malathion.

Time after treatment (hr)	Percentage of total recovered radioactivity[a]				
	Metabolite				
	DMCCP	DMCP	DMPT	DMDPT	Orgin
2.0	27.3 ± 2.1	1.8 ± 0.7	15.2 ± 6.9	5.2 ± 1.2	5.5 ± 1.0
4.0	19.6 ± 6.1	9.9 ± 2.7	22.0 ± 5.1	6.2 ± 4.4	10.4 ± 7.4
8.0	17.5 ± 5.2	16.7 ± 3.2	21.6 ± 4.3	10.7 ± 3.0	13.0 ± 6.0
24.0	11.8 ± 10.0	17.8 ± 6.0	26.0 ± 7.8	13.6 ± 10.1	17.7 ± 3.2

[a]Values represent mean ± standard deviation of 3 determinations.

radio activity was recovered in the water fraction. At that time, the major metabolite observed was DMCCP, which comprised 27.3% of the total recovered radioactivity. After 2 hours, the percentage of the recovered radioactivity associated with DMCCP steadily declined to only 11.8% at the end of 24 hours. An initial lag of 2 hours was observed before the appearance of DMCP, but after this time the percentage of radioactivity associated with DMCP continued to increase, representing 17.8% of the recovered radioactivity at the end of 24 hours. The incorporation of radioactivity into DMPT, DMDPT, and the unidentified radioactivity located at the orgin of the thin layer plates occurred without an initial lag and reached a nearly constant percentage of the recovered radioactivity within 8 hours. The radioactivity associated with DMPT was roughly twice that found with either DMDPT or the unknown throughout the entire 24 hour period. The final percent recovered radioactivity for these components was 26.0, 13.6, and 17.7, respectively.

Estimation of Microsomal Cytochrome P450 Content

Prior to the assay for microsomal malathion desulfurase activity microsomes prepared from hepatopancreas were examined for cytochrome P450. Rat liver microsomes were examined at the same time and served as a control on the method. Both preparations contained detectable quantities of cytochrome P450, indicated in the

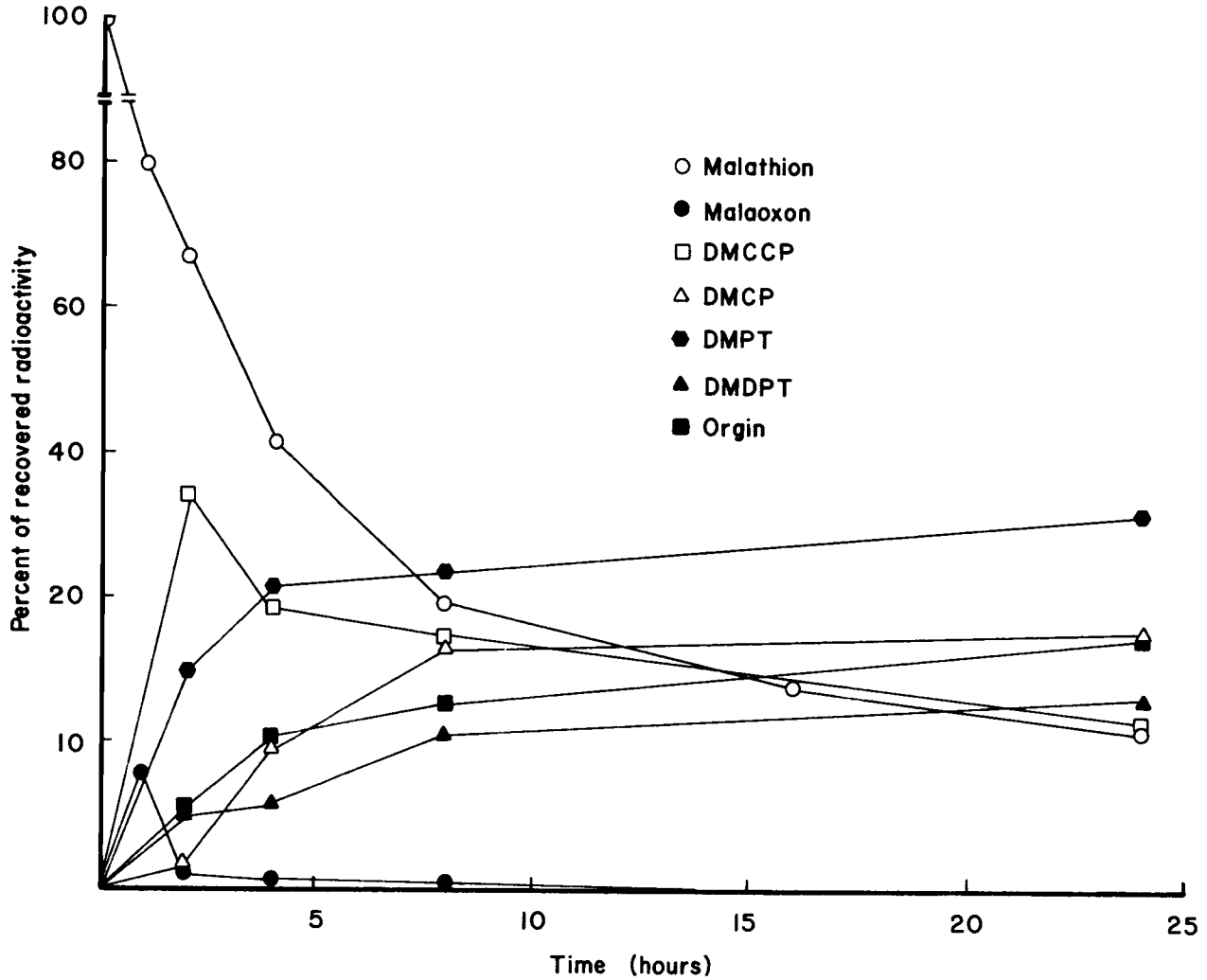


Figure 7. Percent of recovered radioactivity as malathion and metabolic products as a function time after injection of C. magister with 0.75 pg/g [^{3}H]

reduced-CO difference spectrum as a peak with maximum absorbance at 450 nm (Figure 8). In addition to the peak located at 450 nm, a second peak with a maximum absorbance at 420 nm was observed. The concentrations of cytochrome P450 was nmoles/mg microsomal protein for crab and rat were 0.272 and 0.941, respectively (Table 4). The concentration determined for rat was consistent with those previously reported (Elmamlouk et al. 1974).

Table 4. Cytochrome P450 content of microsomes prepared from crab hepatopancreas and rat liver.

Microsomal preparation	nmoles/mg microsomal protein[a]
	Cytochrome P450
Crab	0.272 + .038
Rat	0.941 +

[a]Values for crab microsomes represent mean + standard deviation for three determinations. A single determination was made using rat microsomes.

Assay for Microsomal Malathion Desulfurase

An attempt was made to estimate the rate of malathion desulfuration in vitro using microsomal preparations from crab hepatopancreas. The desulfuration product, malaoxon, was detected in rat hepatic microsomal incubations in appreciable quantities, indicating the validity of the procedure employed (Table 5). In contrast no netabolism of malathion to malaoxon was carried out in

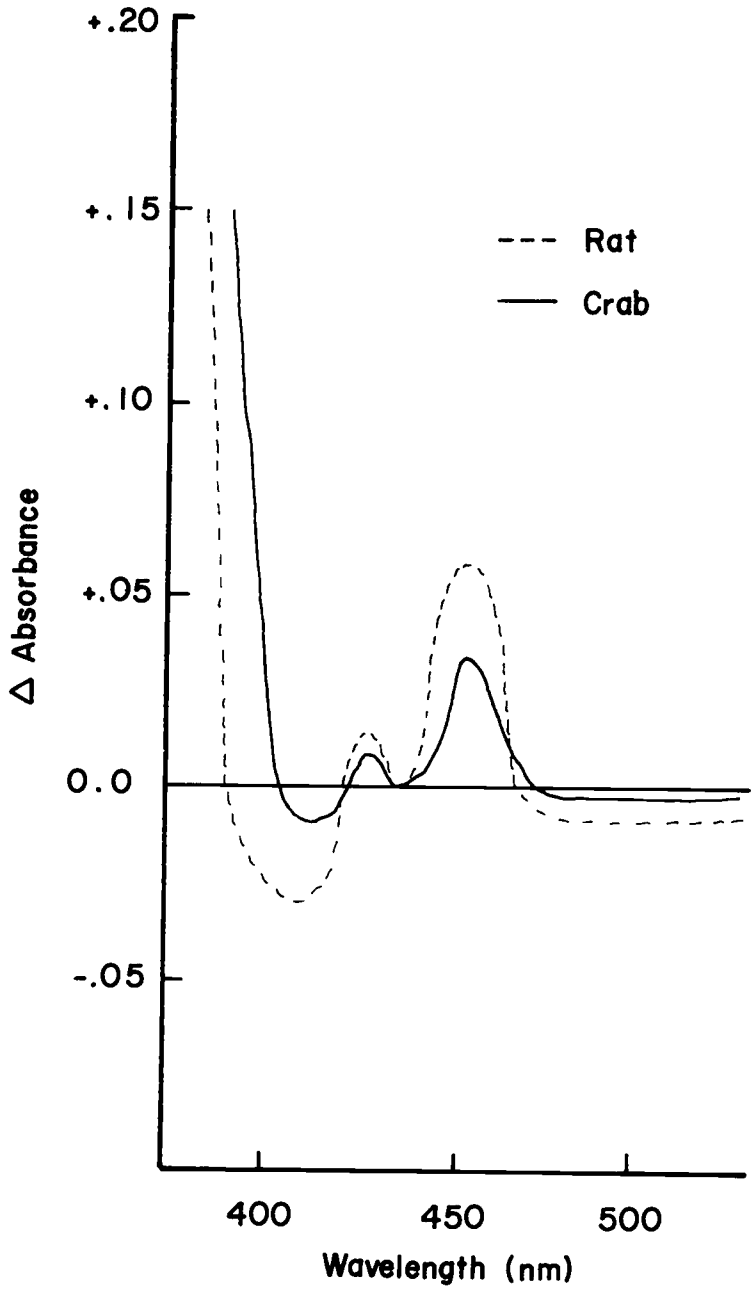


Figure 8. Reduced-CO minus oxidized difference spectrum of microsomal preparations from crab hepatopancreas and rat liver. Microsomes were bubbled with CO for 3 minutes, the sample cell was reduced with sodium dithionite and the difference spectrum was recorded. Protein concentrations in this determination were 1.48 mg/ml for crab and 0.73 mg/ml for rat

preparations of crab hepatopancreas radioactivity recovered in the malaoxon fraction was not greater in hepatopancreas incubations of 60 minutes than in zero time and no enzyme controls (Table 5).

Table 5. A comparison of radioactivity recovered as malathion and malaoxon from NADPH-fortified microsomal preparations of crab hepatopancreas or rat liver.

Enzyme preparation	Recovered Radioactivity[a]	
	Malathion	Malaoxon
Crab[b]		
No enzyme-control	14784	153
Zero time-control	14750	115
60 minutes	14555	146
Rat[c]		
No enzyme-control	14650	157
Zero time-control	14453	170
60 minutes	13444	1156

[a] Radioactivity as counts per minute was determined after chromatographic separation of the chloroform fraction as described under Methods. Individuals values represent averages of four trials.

[b] Preparations with crab microsomes were incubated at 20°C.

[c] Preparations with rat microsomes were incubated at 37°C.

DISCUSSION

Data presented in this report demonstrate that the decapod, Cancer magister, possesses the capability to metabolize malathion in vivo. As indicated by the products recovered, malathion underwent both oxidative desulfuration and hydrolysis, suggesting the involvement of both the mixed function oxidase (MFO) system and class-action hydrolases.

Following injection of radiolabelled malathion, the whole body concentration of malaoxon increased rapidly, reaching a maximum level within 1 hour (Figure 7). The rapid conversion to malaoxon observed in C. magister is consistent with that seen in other arthropods, e.g. maximum levels were reached with 1 and 2 hours in the American cockroach, Periplaneta americana, and the German cockroach, Blattella germanica, respectively (Krueger and O'Brien, 1959). It is noteworthy that the level of malaoxon observed in C. magister after 1 hour, 6.7% of the injected dose, was much higher than that found in either species of insect, 0.7 and 1.0% of the injected dose, respectively, while the LD_{50} for C. magister, 0.80 µg/g (Figure 4), was an order of magnitude less than for either insect, 8.0 and 7.2 µg/g, respectively (Krueger and O'Brien, 1959). The high level of conversion of malathion to malaoxon observed in C. magister compared to certain insects may explain the differential toxicity of

malathion to these animals. It has been shown in various vertebrates that the rate of malathion to malaoxon conversion in vitro is inversely correlated with the LD_{50} (Murphy, 1966; O'Brien, 1967). Murphy (1966) suggested that high malaoxon concentrations could be obtained through either of two mechanisms; high specific activity in the desulfurase system or a lesser ability to enzymatically degrade malaoxon and malathion.

Malathion was extensively degraded by C. magister, with greater than 40% of the recovered radioactivity present in the water extractable fraction of the crabs after 1 hour (Table 3). Several observations indicate that water soluble products may have been excreted as well. Throughout the 24 hour incubation period there occurred a significant loss of radioactivity from the crabs; approximately 15% of the injected radioactivity during the first hour and greater than 60% by the end of 24 hours (Figure 6). The rate of loss approximated zero order kinetics for the first 2 hours following injection then tended to become first order. Such patterns are typical in animals whose execretory mechanisms are initially saturated (Dewaide, 1971). Secondly, an analysis of the holding water used during preliminary experiments with non-labelled malathion revealed the presence of the same metabolic products later recovered from the whole crab homogenates.

In mammals and insects the enzymatic degradation of malathion

and malaoxon is primarily accomplished by a series of class-action hydrolases (O'Brien, 1967; Cohen and Murphy, 1972). As indicated by the products recovered, hydrolysis was also the major route of detoxification in the crab (Table 3), and could be differentiated into carboxylesterase and phosphotriesterase components which catalyzed the hydrolysis of the carbethoxy and the phosphorylthionate and alkylthionate linkages, respectively (Figure 9). Greatest initial activity was observed in the carboxylesterase component as suggested by the rapid accumulation of DMCCP (Figure 7), but these enzymes appeared to have become inactive within 2 hours of injection of the dose. This latter conclusion is indicated by the relative constancy of the proportion of recovered radioactivity found in the combined carboxylesterase products, DMCCP and DMCP (Table 3) after this time. Continued activity of carboxylesterases should have been accompanied by a continual increase in the proportion of these products in the total recovered fraction unless excretion was unusually rapid or they were subjected to secondary metabolism, e.g. by the phosphotriesterases.

Carboxylesterase inactivation appears to be a common response during malathion intoxication. Murphy (1967) was the first to investigate in detail the reduction of malathion-carboxylesterase activity during malathion intoxication. In studies with guinea-pigs given a dosage of malathion of 200 μg/g, a 70% inhibition of liver

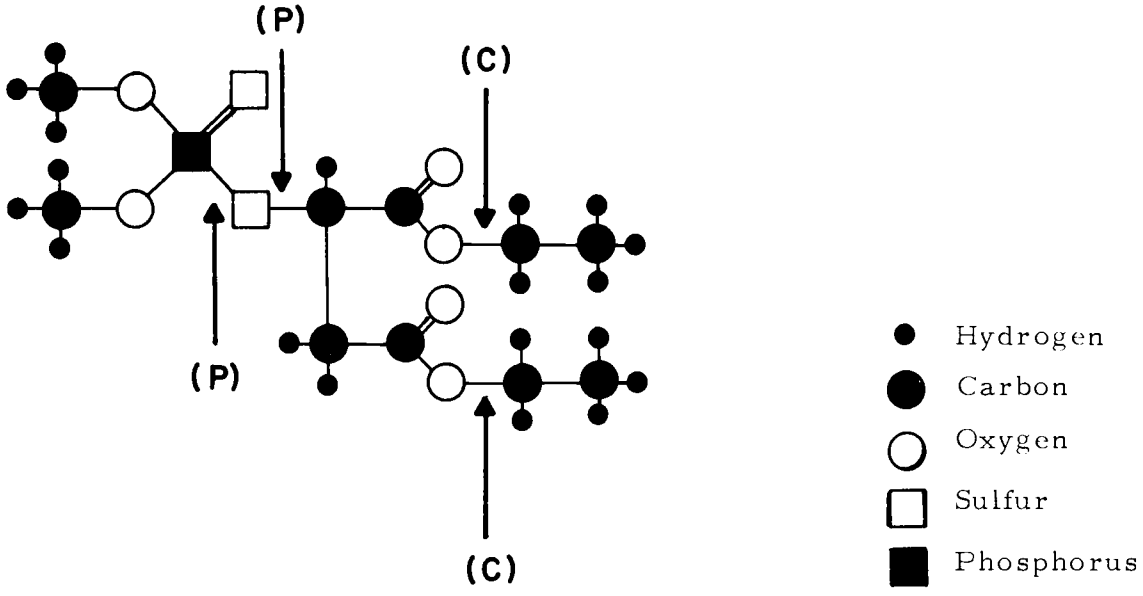


Figure 9. Probable sites of hydrolysis of malathion by carboxylesterases and phosphotriesterases.

carboxylesterase was observed after 1 hour, while brain and erythrobyte cholinesterases were not significantly affected. It has since been shown that the inactivation results from the irreversible finding of caboxylesterase with malaoxon (Cohen and Murphy, 1971). The process appeared to be temperature independent between 1° and 38°C and non-specific; pretreatment with several organophosphates including paraoxon, DFP (diisopropylphosphorofloridate) and EPN (O-ethyl-O-p-nitrophenyl phenyl phosphorothioate) reduced malaoxon binding, but still inhibited carboxylesterase (Cohen and Murphy, 1972).

Carboxylesterase inhibition during malathion intoxication has also been reported for several species of insects. Krueger and O'Brien (1959) found in P. americana that the combined carboxylesterase products declined from 50.2 to 44.0% of the total water-soluble radioactivity during a 24 hour period. A 10-fold increase

in the dosage of [^{32}P] malathion used further reduced the combined carboxylesterase products to 28% at the end of that same time, indicating the decline in carboxylesterase activity was not due to the depletion of malathion or to differences in substrate affinity relative to the phosphotriesterases. In C. magister, assuming that DMCCP and DMCP were excreted in proportion to their whole body concentration, the contribution of combined carboxylesterase products to the total water-soluble radioactivity was 52.8% after 2 hours and 33% at the end of 24 hours. Thus the pattern of carboxylesterase inactivation as seen in P. americana is similar to that seen in C. magister.

It was noteworthy that the remaining carbethoxy linkage (DMCCP) was hydrolyzed throughout the 24-hour incubation period, although there was an indication of carboxylesterase inactivation. The occurrence of DMCP as a major metabolic product has been reported in numerous insect (Krueger and O'Brien, 1959) and mammalian species (O'Brien, 1967). Either of two mechanisms may explain the continued carboxylesterase activity at the second carbethoxy site. Dauterman and Main (1966) have suggested that a functionally different esterase may catalyze this second deesterification on the basis of work with hydrolysis rates of several carbalkoxy homologs of malathion. Several carboxylesterase isozymes have been identified in both mammalian (Holmes and Masters, 1967) and lobster

(Barlow and Ridgeway, 1971) tissues, although the participation of specific isozymes in malathion metabolism is presently undocumented. Substantial differences may exist with respect to substrate and inhibitor specificities among this group of enzymes. Alternatively, carboxylesterase components catalyzing the second deesterification may be located in tissues whose partition characteristics are unfavorable for the carboxylesterase inhibitor malaoxon. One such possible tissue is serum; the contribution of serum esterases to the detoxification of malathion in mammals is well documented (Murphy and DuBois, 1957; Murphy, 1967).

The second type of hydrolytic reaction observed in C. magister was that catalyzed by phosphotriesterases which hydrolyze the alkylthionate and phosphorylthionate linkages of malathion resulting in the production of DMDPT and DMPT, respectively (Figure 9). In contrast to the carboxylesterases, the phosphotriesterases did not appear to become inactivated during the 24-hour incubation period; steady increases in the percent of recovered radioactivity for both products was observed throughout the entire 24 hours (Table 3). If both compounds had been excreted under similar constraints as assumed for the carboxylesterase products the combined phosphotriesterase products would have accounted for 37.5% after 2 hours, and 59% at the end of 24 hours, of the total water-soluble radioactivity. After 24 hours the combined phosphotriesterase products

accounted for 50, 47, and 67% of the total water-soluble radioactivity for P. americana, B. germanica, and the house fly, Musca domestica, respectively (Krueger and O'Brien, 1959).

Based on the recovery of metabolic products from the cotton leaf worm, Spodoptera littoralis, 24 hours after topical treatment with 500 μg/g [^{32}P] malathion, Zayed et al. (1972) suggested that the carboxylesterase products underwent extensive secondary hydrolysis, probably by phosphotriesterases. Their data, however, do not preclude the possibility that the low recovery of carboxylesterase products, 11.2 and 3.0% of the total water-soluble radioactivity for DMCCP and DMCP, respectively, may have been due to low specific activity of the carboxylesterases, and consequently, a low rate of formation in the first place. Secondary metabolism was not apparent in the crab, based on the pattern of appearance of metabolic products from C. magister as a function of time. The carboxylesterase products appear to be excreted. The pattern as seen in C. magister may be an accurate reflection of that seen in most organisms. Electronic and inductive effects resulting from the ionization of the carboxylic acid group(s) predicts a much greater resistance to nucleophilic attack by phosphotriesterases of the alkylthionate and phosphorylthionate linkages of these products than seen for malathion or malaoxon (O'Brien, 1967).

Although further hydrolysis of the carboxylesterase products

by phosphotriesterases appears improbable, secondary metabolism has been shown for many terrestrial animals by the recovery of demethylated metabolites, i.e. phosphoric, methylphosphoric, dimethylphosphoric, and thiophosphoric acids (March et al., 1956; Krueger and O'Brien, 1959; Zayed et al., 1972). These products generally comprise a small percentage of the total water-soluble products in insects, e.g. 4.2 and 3.9% for phosphoric acid in P. americana and B. germanica, respectively (Krueger and O'Brien, 1959), yet may account for a major portion of the products recovered from mammals (O'Brien, 1967). Demethylation did not contribute significantly to the metabolism of malathion in C. magister. While the use of the [^{3}H] methoxy label precluded the detection of phosphoric and thiophosphoric acid products, both dimethylphosphoric acid and the monomethyl analogs would have been detected by the methods employed unless present at very low levels. Furthermore, Elmanlouk and Gessner (1976a) have noted that lobster lacks the ability to deethylate parathion in vitro.

To summarize, the pattern of metabolism observed for malathion in C. magister tends to parallel that seen in insects. In both classes of organisms malathion is desulfurated to malaoxon or degraded to various water-soluble products. Furthermore, degradation is hydrolytic and can be differentiated into carboxylesterase and phosphotriesterase components. Both components contribute

equally to the initial hydrolysis of malathion, but the carboxylesterase pathway appears to become inactivated at some time after administration of the dose, and the major portion of hydrolysis is subsequently performed by the phosphotriesterases. In many insects a small percentage of the water-soluble products are initially metabolized by an O-demethylative mechanism prior to hydrolysis, while this activity was not observed in C. magister. In contrast, mammals and birds appear to rely much less heavily on the phosphotriesterases; more than 75% of the water-soluble fraction is recovered as carboxylesterase products in these animals (March et al., 1956; O'Brien, 1967).

The differences in toxicity of injected malathion observed between C. magister (Figure 4) and certain species of insects (Krueger and O'Brien, 1959) may be due to the relative rates at which malaoxon accumulates in these animals. This explanation is consistent with that offered for the selective toxicity of malathion to insects and mammals (Krueger and O'Brien, 1959). The rate at which malaoxon accumulates is influenced, in part, by the level and activity of the carboxylesterase enzymes, which, in addition to detoxifying malathion, both hydrolyze and irreversibly bind to malaoxon (Murphy, 1967; Cohen and Murphy, 1972). Mammals and birds are relatively unsusceptible to malathion and possess much greater carboxylesterase activity than do most insects. Furthermore,

differences in the intraspecific resistance to malathion observed in many insects appears to be due entirely to differences in carboxylesterase concentration: carboxylesterase activity was 13 times greater in resistant mosquitos, Culex tarsalis, than in sensitive individuals (O'Brien, 1967). Low concentrations of carboxylesterase may also be present in the crab, as indicated by the low dosage of malathion required to inhibit this pathway. By analogy, a low level of carboxylesterase activity in C. magister could account for the substantially higher levels of malaoxon in this species than in resistant insects.

It is difficult, nevertheless, to interpret the overall significance of carboxylesterase inactivation without a more complete understanding of the oxidative system responsible for the production of malaoxon. The data presented above does not preclude the possibility that the efficiency of the oxidative desulfurase system was much greater in the crab than in certain species of insects, e.g. P. americana or B. germanica, and that the tissue level of carboxylesterase in these animals were comparable. To more completely evaluate oxidative desulfuration an attempt was made to examine the system in vitro using preparations from hepatopancreas.

Several observations have indicated that in crustacea the MFO system is located in the hepatopancreas. Lee et al. (1976) observed in blue crab exposed to hydrocarbons, a rapid formation of polar

metabolites which were first detected in the hepatopancreas. In preliminary studies with C. magister in which specific tissues were examined, large accumulations of malathion were detected in the hepatopancreas shortly after injection. Secondly, the oxidative desulfuration of malathion is known to involve cytochrome P450 (Stevens, et al., 1975) and the crab hepatopancreas was found to contain appreciable concentrations of this cytochrome (Table 4); concentrations equal to or greater than in several marine fish (Pohl et al., 1974) and twice that reported for lobster (Elmamlouk et al., 1974). Little correlation was apparent, however, between the presence of P450 and the capacity of microsomal preparations from hepatopancreas to effect oxidative metabolism in vitro. The absence of detectable conversion of malathion to malaoxon in vitro in C. magister (Table 5) was consistent with reports of Carlson (1973) and Elmamlouk and Gessner (1976a) who found negligible parathion desulfurase activity in lobster hepatopancreas. Pohl et al. (1974) and Elmamlouk and Gessner (1976b) have also shown that lobster hepatopancreas preparations lack the ability to O-demethylate d-benzphetamine and ethylmorphine, respectively.

A possible explanation for the poor correlation observed in C. magister between in vivo and in vitro studies of malathion desulfuration was that the reaction medium employed may have been deficient in some respect or that damage to the tissue may have occurred

during preparation of microsomes. Elmamlouk and Gessner (1967a) examined the possibility that parathion metabolism in lobster had a different cofactor requirement than the mammalian NADPH-dependent system using various combinations of cofactors and electron donors. None of the combinations that they employed resulted in any appreciable activity, however. Other studies have suggested that naturally occurring components in crustacean hepatopancreas may adversely affect the microsomal enzymes. Contamination of reaction media with 2-4% by volume of lobster or rock crab, Cancer borealis, digestive fluid completely inhibited aniline hydroxylase, d-benzphetamine demethylase, and 7-ethoxycoumarin deethylase activity in the skate, Raja erinacea (Pohl et al., 1974). Taurochenoxycholic acid and acylarcosyltaurine, strong surface active agents, have been identified in the hepatopancreas of crayfish, Procambarus clarkii, and crab, Cancer pagurus, respectively (Yamasaki et al., 1965; van den Oord, 1966). The treatment of mammalian hepatic microsomes with similar chemicals, e.g. sodium deoxycholate, converts P450 to an inactive derivative, cytochrome P420. Imai and Sato (1967) concluded that the conversion resulted from the disruption of the association between the cytochrome and microsomal lipid.

The reduced-carbon monoxide (CO) cytochrome P420 complex is detected by difference spectrometry as a peak with maximum absorbance at 420 nm. In both lobster (Elmamlouk et al., 1974) and

C. magister (Figure 8) the breakdown of P450 and P420 was evident. Furthermore, the relationship between the spectral characteristics and functional state of P450 is not completely understood. Although substantial P450 was observed in C. magister it may not have been functional. Also, it is not possible to assess the condition of the remaining electron transport components (Figure 1) which are intimately associated with the microsomal lipid.

In the few instances where MFO activity has been reported in crustacean hepatopancreas in vitro, e.g. aniline hydroxylation (Elmamlouk and Gessner, 1976b) and aldrin epoxidation (Khan et al., 1972; Carlson, 1974), greatest activity was found in the soluble rather than the microsomal fraction although no soluble cytochrome P450 was detected. Activity levels in the soluble fraction for both reactions were less than in the combined microsomal and soluble fractions suggesting that the microsomal fraction, though inactive, potentiated the activity in the soluble fraction by an as yet unexplained mechanism. Juchau and Symms (1972) have shown that aniline hydroylation in the soluble portion of placenta is due to the hemoproteins hemoglobin and methemoglobin. While NADPH was the preferred cofactor both NADH and FMN were capable of catalyzing the reaction. Aldrin epoxidation has been less thoroughly investigated. The presence of these reactions in the absence of detectable cytochrome P450 may indicate that decapod crustaceans

capable of utilizing alternative oxidative mechanisms in addition to the MFO system to effect specific reactions.

In conclusion, data presented in this report demonstrate that C. magister metabolized malathion in a manner similar to that observed in other classes and phyla of organisms. As indicated by the rapid accumulation of malaoxon and corresponding low LD_{50} value, however, C. magister appears to have a limited capacity to metabolize the insecticide when compared to terrestrial animals. Comparisons of the ability of C. magister and fish to metabolize malathion are not possible, although some preliminary work on the rates of malaoxon degradation in vitro suggest that in several marine species of fish this capacity is about the same as that found in most insects (Murphy, 1966).

The failure to fully characterize the desulfurase system prevents a meaningful evaluation of the role of this enzyme complex in the relative toxicity of malathion to crab and other animals. Some conclusions, however, may be drawn from the concentration of cytochrome P450 observed in C. magister hepatopancreas. Burns (1976) has determined that the rate of aldrin epoxidation in Fundulus heteroclitus, an estuarine fish, was directly proportional to the cytochrome P450 concentration. The concentration of P450 measured in crab was not significantly different from that reported for several species of fish and insects (Pohl et al., 1974), thus

it is unlikely that the specific activity of crab desulfurase differs substantially from either type of animal. Moreover, the available data suggests that a more positive correlation exists between the level of carboxylesterase activity and malathion toxicity than between malathion desulfurase activity. This interpretation is consistent with the apparent conditions in C. magister as indicated by the occurrence of metabolic products as a function of time after injections of the dose. Within 2 hours of injection the carboxylesterases, whose hydrolysis of malathion was initially rapid, appeared to have become inactivated. Carboxylesterase inactivation could allow the rapid accumulation of malaoxon to a lethal concentration.

This study and others (Corner et al., 1973; Sheridan, 1975; Lee et al., 1976) demonstrate that many species of decapod crustacea possess the ability to oxidize foreign organic chemicals and therefore possess an MFO system. With respect to malathion, C. magister was shown to produce malaoxon at high rates in vivo. The failure to demonstrate malathion desulfurace activity in hepatopancreas in vitro may have been related to problems with the preparation of tissue rather than the lack of enzymatic activity.

SUMMARY

The metabolism of malathion by Cancer magister was studied by the injection of adult crabs with 0.75 μg/g [^{3}H] malathion. Examination of the metabolic products as a function of time revealed the presence of two major metabolic pathways: oxidative desulfuration and hydrolysis. The hydrolytic pathway could be further differentiated as consisting of carboxylesterase and phosphotriesterase components. These pathways appeared to be identical to those utilized by most organisms which metabolize malathion, but, the capacity of crab to hydrolyze malathion may be less than that found in many terrestrial species. The limited capacity to hydrolyze malathion, particularly by carboxylesterases, correlated well with the greater sensitivity to injected malathion observed in crab relative to certain species of insect.

An attempt was made to study the crustacean MFO system with respect to cytochrome P450 and malathion desulfurase in vitro using preparations of crab hepatopancreas. Substantial microsomal P450 was detected; concentrations equal to or greater than those reported for several species of marine fish, and twice that reported for lobster. Despite the presence of P450, however, attempts to demonstrate the presence of malathion desulfurase in crab hepatopancreas in vitro were unsuccessful. On the basis of spectral

analysis it was suggested that cytochrome P450 may have existed in a partially non-functional state, and probably causes for the denaturation are discussed.

Despite earlier reports that crustacea may lack the ability to deal enzymatically with foreign chemicals (Elmamlouk and Gessner, 1976a; 1976b) data presented in this report clearly show that C. magister possesses competent oxidative and hydrolytic mechanisms which result in typical patterns of malathion activation and hydrolysis.

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