# Inhibition of Mitochondrial Electron Transport by Guthion, Some Related Insecticides, and Degradative Products*

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In a series of preliminary chronic toxicity investigations various chlorinated cyclodiene pesticides, benzene hexachloride, toxaphene (1), and a series of polychlorinated biphenyls (2) were shown to inhibit mitochondrial electron transport systems in vitro. In similar studies designed to investigate the chronic toxicology of various pesticides compared with their environmental conversion products, DDT and DDE were shown to inhibit mitochondrial electron transport systems whereas dichlorobenzophenone, and p-chlorophenol, two of their photodecomposition products, did not (3). In the same study, a relatively non-persistent pesticide, sevin (carbaryl), did not inhibit mitochondrial electron transport whereas dihydroxy-naphthalene, a photodegradative product, was as inhibitory towards the mitochondrial enzymes as the most potent chlorinated pesticides tested (3). These findings emphasize the necessity to evaluate on a chronic basis, the toxicology of not only pesticides, but their environmental degradative products as well. This is a particularly important consideration for the non-persistent pesticides as they are degraded much faster than the persistent chemicals.

Guthion, one of the more persistent organo-phosphate insecticides, has been shown to undergo photoinduced decomposition (4) to benzazimide, O,O,S-trimethylphosphorodithioate, anthranilic acid and an insoluble resin. Various phosphorodithioates, such as guthion, have also been shown to undergo metabolic desulfuration, which results in their respective oxygen derivatives. These oxygen analogs are more effective inhibitors of acetylcholine esterase than the parent phosphorodithioates; consequently, metabolic desulfuration of some phosphorodithioate pesticides increases their insecticidal action (5) and presumably acute toxicity.

The effect of guthion and its metabolic desulfuration and photo-induced breakdown products on mitochondrial electron transport has not been investigated from a chronic standpoint. Consequently, a comparison of the effects of guthion, its derivatives and decomposition products on beef heart mitochondrial NADH-oxidase and succin-oxidase systems was conducted and the results reported herein.

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## METHODS AND MATERIALS

Heavy beef heart mitochondria (HBHM) were isolated and prepared as previously described (6). The activity of the succinoxidase and NADH-oxidase systems was determined manometrically in the absence and presence of the potential inhibitors (7,8). The various pesticides and electron transport carriers were added in ethanol or water depending on their solubility. The ethanol concentration was kept constant in all of the assay flasks (0.1 ml of ethanol in 3 ml of reaction mixture).

Mitochondrial protein was determined by the Lowry method (9).

Coenzyme $Q_{10}$ was kindly supplied by Dr. Karl Folkers of the Institute for Biomedical Research, University of Texas.

## RESULTS AND DISCUSSION

The data presented in Table I indicate that at a concentration of 138.9 nanomoles of pesticide per milligram of mitochondrial protein, ethyl guthion, guthion, ethyl guthion oxygen analog and guthion oxygen analog depressed mitochondrial NADH-oxidase activity to 32,53,86 and 88% of the uninhibited controls respectively.

TABLE I
The effect of various guthion analogs and breakdown products on the beef heart mitochondrial NADH-oxidase system

| Compound added (138.9 nanomoles/mg protein) | Enzyme specific activity (u atoms oxygen consumed/min/mg protein[1]) | Percent Activity[2] |
|---|---|---|
| No additions | 0.615 ± 0.010[3] | 100 |
| Ethyl guthion | 0.198 ± 0.005 | 32 |
| Guthion | 0.328 ± 0.018 | 53 |
| Ethyl guthion oxygen analog | 0.533 ± 0.028 | 86 |
| Guthion oxygen analog | 0.540 ± 0.044 | 88 |
| Benzazimide | 0.637 ± 0.024 | 104 |
| Anthranilic acid | 0.611 ± 0.064 | 99 |
| 1389 nanomoles/mg protein | | |
| Ethyl guthion | .038[4] | 6 |
| Guthion | .071 | 12 |
| Ethyl guthion oxygen analog | .189 | 31 |
| Guthion oxygen analog | .492 | 80 |
| Benzazimide | .674 | 109 |
| Anthranilic acid | .579 | 94 |

1. Each flask contained 0.58 mg of mitochondrial protein.
2. Average percent of uninhibited controls containing coenzyme $Q_{10}$ (100 nanomoles/flask), rounded off to the nearest percent.
3. Average of from three to five determinations ± standard error of the mean.
4. Average of two determinations.

At concentrations of 1389 nanomoles of pesticide per milligram of mitochondrial protein, ethyl guthion, guthion, ethyl guthion oxygen analog and guthion oxygen analog depressed the mitochondrial NADH-oxidase enzyme system to 6,12,31 and 80% of the uninhibited controls respectively.

Benzazimide and anthranilic acid were noninhibitory to the NADH-oxidase system at either of the concentrations employed.

The data presented in Table II indicate that at 1282 nanomoles/mg of mitochondrial protein, none of the chemicals tested depressed mitochondrial succinoxidase activity below 70% of the uninhibited controls. It was concluded that none of the guthion derivatives and breakdown products tested were inhibitory to the mitochondrial succinoxidase system ($I.D._{50}$s above 1000 nmoles/mg protein were arbitrarily considered noninhibitory).

Since ethyl guthion, guthion and ethyl guthion oxygen analog inhibit mitochondrial-NADH oxidase but not succinoxidase activity, it may be interpreted that these chemicals inhibit mitochondrial NADH-oxidase activity in the Complex I region (NADH-coenzyme Q reductase) of the electron transport chain.

It is interesting to note that the decomposition compounds tested, benzazimide and anthranilic acid, did not inhibit either of the mitochondrial electron transport systems investigated.

TABLE II

The effect of various guthion analogs and breakdown products on the beef heart mitochondrial succinoxidase system

| Compound added (1282 nanomoles/mg protein) | Enzyme specific activity (u atoms oxygen consumed/min/mg protein[1]) | Percent Activity[2] |
|---|---|---|
| No additions | 0.498 ± 0.022[3] | 100 |
| Ethyl guthion | 0.356 ± 0.041 | 72 |
| Guthion | 0.501 ± 0.017 | 101 |
| Ethyl guthion oxygen analog | 0.509 ± 0.026 | 102 |
| Guthion oxygen analog | 0.514 ± 0.017 | 103 |
| Benzazimide | 0.494 ± 0.026 | 99 |
| Anthranilic acid | 0.497 ± 0.069 | 100 |

1. Each flask contained 0.78 mg of mitochondrial protein.
2. Average percent of the uninhibited control rounded off to the nearest percent.
3. Average of six determinations ± standard error of the mean.

The data presented in Figure 1 shows the titration curves for inhibition of the mitochondrial NADH-oxidase system by ethyl guthion, guthion and ethyl guthion oxygen analog. These titration curves indicate that the compounds exhibit simple hyperbolic inhibition kinetics in the range tested.

The amount of insecticide per mg mitochondrial protein which results in the loss of one half the activity of the mitochondrial NADH-oxidase system is termed the inhibitor dose$_{50}$ (I.D.$_{50}$). The I.D.$_{50}$ was calculated from the titration curves (Figure 1) and is used in comparing the potency of the various inhibitors toward the mitochondrial NADH-oxidase system. Thus, ethyl guthion (I.D.$_{50}$ of 70 nanomoles/mg protein) is twice as potent an inhibitor of NADH oxidase as guthion (I.D.$_{50}$ of 140 nanomoles/mg protein). Ethyl guthion oxygen analog (I.D.$_{50}$ of 400 nanomoles/mg protein) is only a moderate inhibitor in comparison with ethyl guthion and guthion.

It is interesting to note that the two most potent inhibitors of mitochondrial NADH-oxidase are the phosphorodithioates ethyl guthion and guthion (Figure 2). In addition, the data presented in Figure 1 demonstrate that di-syston, which is an aliphatic phosphorodithioate, inhibited the NADH-oxidase system (I.D.$_{50}$ = 375 nanomoles/mg protein) whereas dylox, which is an aliphatic phosphonate, did not. These data suggest that the phosphorodithioate structure may be important in the inhibition of mitochondrial NADH-oxidase enzyme systems. Further investigations are required to elucidate the precise nature of the structure-inhibition relationships.

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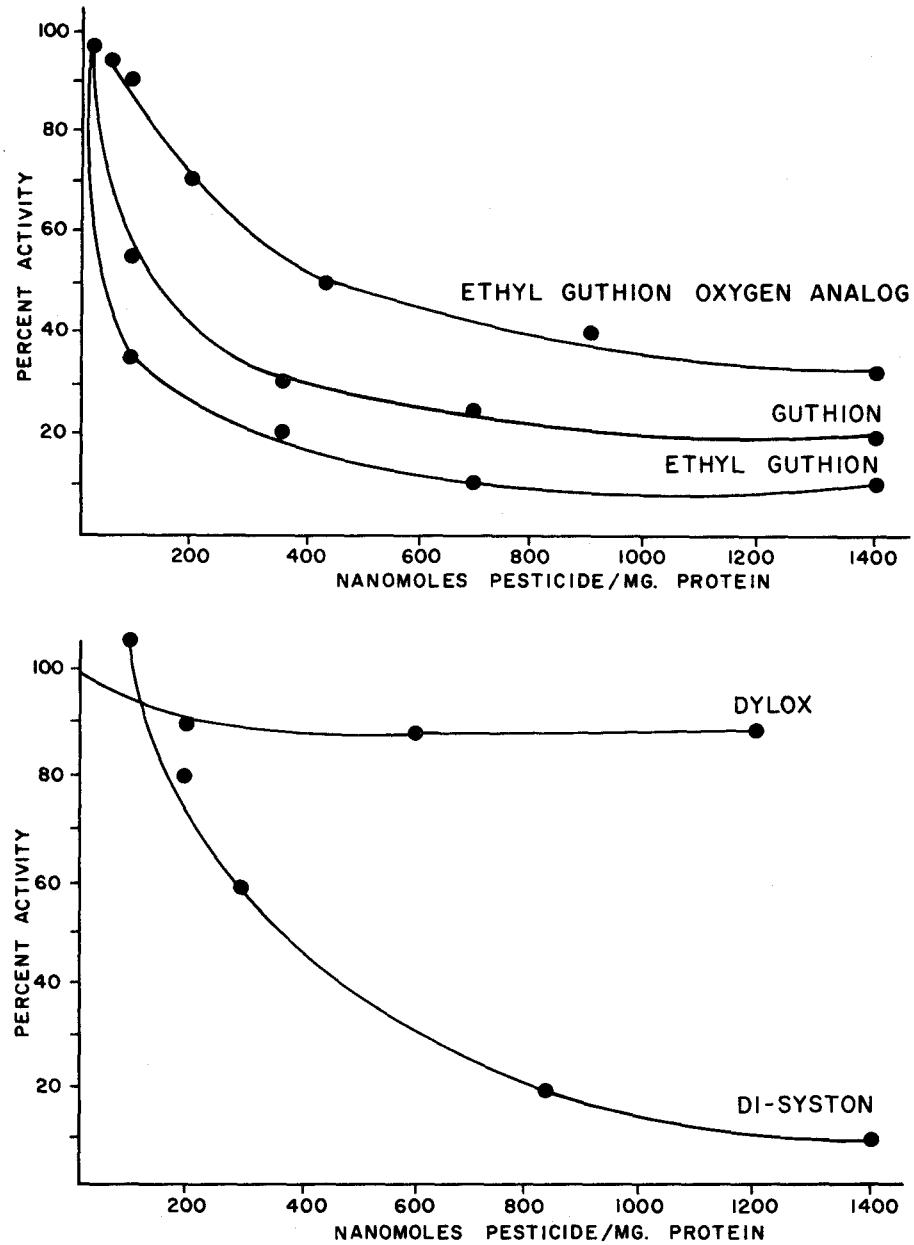
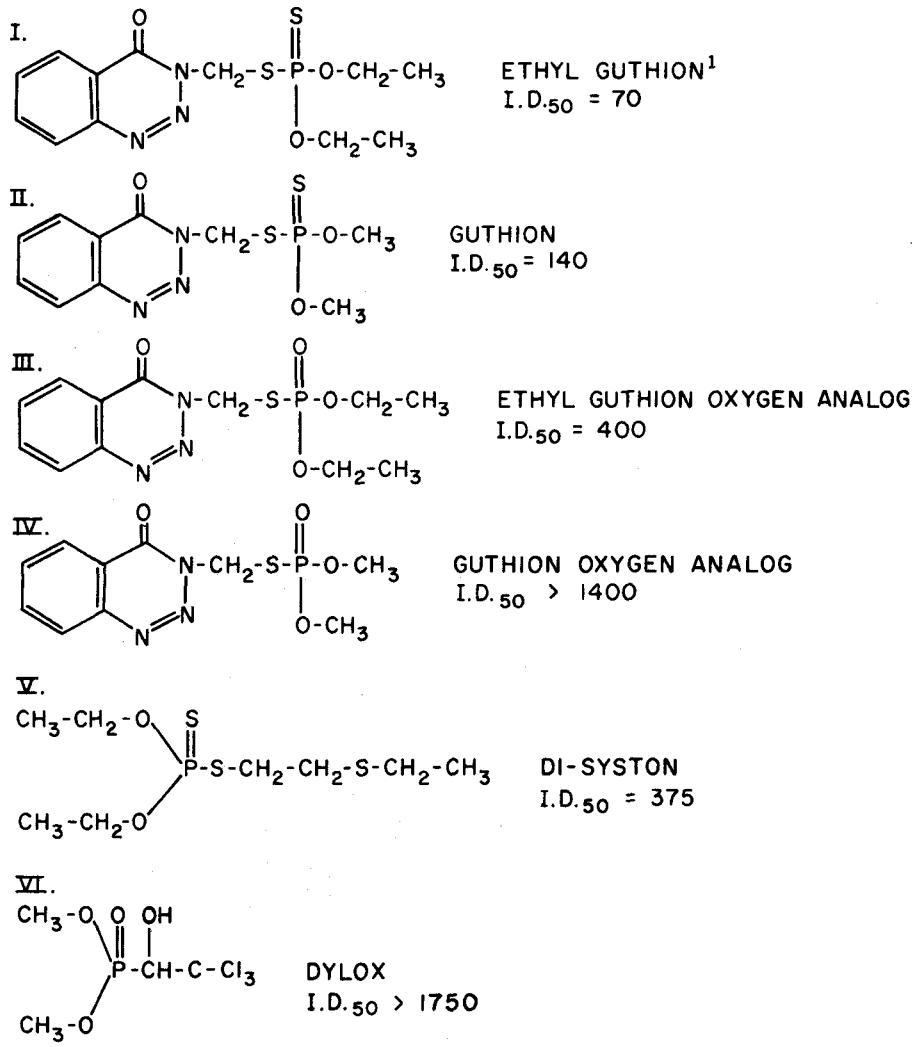



Figure 1. Titration curves for inhibition of mitochondrial NADH oxidase by various pesticides.




1 Inhibitor dose$_{50}$ for mitochondrial NADH oxidase system.

Figure 2. Pesticides checked for inhibition of mitochondrial electron transport.