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Dermal Absorption Of A Dilute Aqueous Solution Of Malathion

John E. Scharf MD
University of South Florida

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By

John E. Scharf, MD

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Major Professor: Raymond D. Harbison, Ph.D.
Thomas E. Bernard, Ph.D.
Stuart M. Brooks, M.D.

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ABSTRACT

Malathion is a commonly used organophosphate pesticide on field crops, fruits, nut trees, vegetables, livestock, agricultural premises, and land. The approved uses also include mosquito and medfly control. These uses can result in human skin contact. The purpose of this study is to evaluate the human skin absorption of malathion for the purpose of assessing the risks associated with aqueous solution exposures following applications. Aerial applications can result in solubilized malathion in swimming pools and other waters that may be contacted. Human volunteers were selected and exposed to aqueous solutions of malathion at various concentrations. Participants submerged their arms and hands in twenty liters of dilute malathion solution in either a stagnant or stirred environment. The "disappearance method" was applied by measuring malathion concentrations in the water before and after human subject exposure to the water for various periods of time. Malathion was measured using Gas Chromatography. No measurable skin absorption was detected in 42% of the participants. Measurable skin absorption among the remaining 58% of participants resulted in doses that were more than an order of magnitude less than the minimal dose necessary to cause a measurable change in red blood cell acetylcholinesterase (RBC-AChE). Extrapolation of these results to a mathematical model for recreational swimmers and bathers exposed to contaminated swimming pools and surface waters typically detected after bait application again are an order of magnitude below the doses needed to cause a detectable change in RBC-AChE. These data indicate that exposure to aqueous malathion following usual aerial bait applications is not appreciably absorbed, and therefore, it is not a public health hazard.

Chapter One

Organophosphate Pesticides

Introduction. The use of organophosphate compounds as insecticides began in the 1930s and has increased markedly since many organochlorine insecticides were banned in the 1970s. In contrast to organochlorine insecticides, organophosphate insecticides degrade rapidly in the environment and do not accumulate or concentrate in the food chain. Thus, organophosphates have less potential for chronic health effects or environmental contamination than do organochlorines and pose less risk to consumers of food products. However, organophosphate compounds have a greater potential for acute toxicity in humans than do chlorinated compounds. Even among the organophosphate pesticides, however, a wide spectrum of potency exists. As insects develop greater resistance, the trend is to use more potent, and consequently, more lipid-soluble and longer-lasting insecticides.

Malathion has a generally low mammalian toxicity in spite of its strong insecticidal properties. Malathion itself has little or no cholinesterase activity. Like many other organophosphates, malathion is activated by mono-oxygenase attack to produce the potent anticholinesterase malaoxon. Malathion and malaoxon are rapidly detoxified in mammals by carboxylesterase attack (but not insects) to produce their respective monacids. If the carboxylesterase detoxification pathway is inhibited, mammals may be made almost as sensitive as insects to malathion (Caldwell, 1983).

Malathion is metabolized in human liver by one of two pathways: (1) hydrolysis to non-toxic metabolites; or (2) oxidation to the active metabolite, malaoxon. Malaoxon has **68x** the inhibition of plasma cholinesterase activity as malathion. Furthermore, malaoxon is

generally acknowledged to be the actual inhibitor of plasma cholinesterase and to be the primary compound responsible for the full spectrum of cholinergic symptoms observed in poisoned subjects. Normally, the balance of metabolic reactions in the gut is heavily in favor of oxidation to malaoxon in insects, but favors hydrolysis to non-toxic metabolites in humans; thus the selective toxicity of malathion on insects vs. humans.

Physical Properties. In organophosphate molecules, R_1 and R_2 are usually alkyl groups (typically ethyl or methyl). X is commonly an alkyl group that is replaced by a hydrogen atom during irreversible inhibition, or "aging", of the organophosphate-enzyme complex.

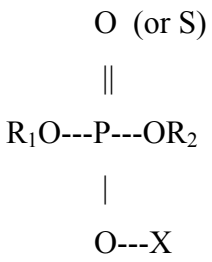

Figure 1: Organophosphate.

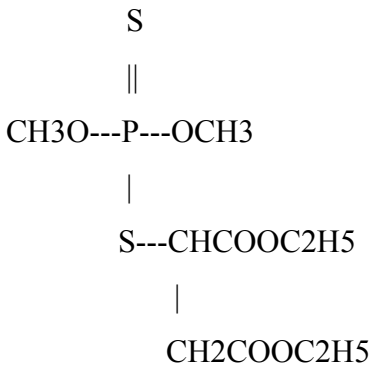

Figure 2: Malathion.

- Appearance: Clear, amber liquid with penetrating garlic odor.
- Chemical Name: diethyl (dimethoxy thiophosphorylthio) succinate
- CAS Number: 121-75-5
- Molecular Weight: 330.36
- Water Solubility: 130 mg/L
- Solubility in Other Solvents: very soluble
- Melting Point: 2.85 C
- Vapor Pressure: 5.5×10^{-6} mmHg at 20°C
- Log K_{ow} Partition Coefficient: 2.75
- Specific Gravity: 1.23

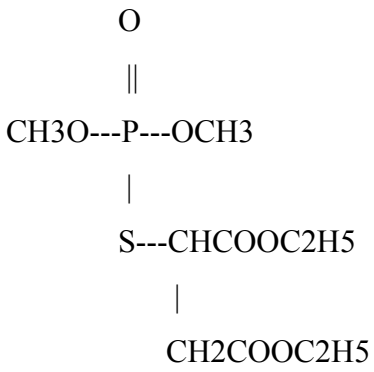

Figure 3: Malaoxon.

- Appearance: Colorless, oily liquid.
- Chemical Name: diethyl ((dimethoxy phosphinyl) thio) butanedioate
- CAS Number: 1634-78-2
- Molecular Weight: 314.36
- Water Solubility: 209 mg/L
- Soluble in ethanol, methanol and acetone.
- Melting Point: < 20 C
- Vapor Pressure: 9.8×10^{-6} mmHg
- Log K_{ow} Partition Coefficient: 2.02
- Specific Gravity: 1.23

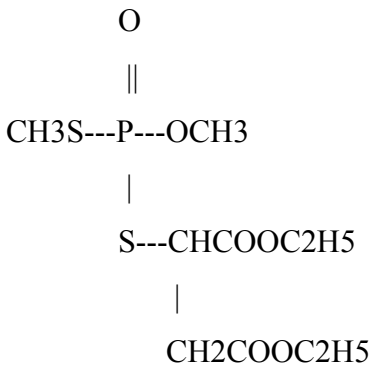

Figure 4: Isomalathion.

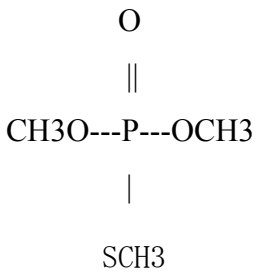

Figure 5: O,O,S-trimethyl phosphorothioate.

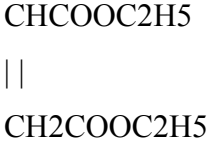

Figure 6: Diethyl Fumarate.

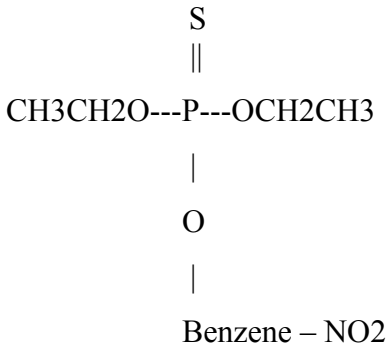

Figure 7: Parathion.

- Appearance: Deep brown to yellow liquid with a faint odor of garlic.
- Chemical Name: O,O-diethyl O-4-nitrophenyl phosphorothioate
- CAS Number: 56-38-2
- Molecular Weight: 291.3
- Water Solubility: 24 mg/l
- Soluble in alcohols, oils, ethers.
- Melting Point: 6 C
- Vapor Pressure: 1.9×10^{-5} mm Hg
- Log K_{ow} Partition Coefficient: 3.83

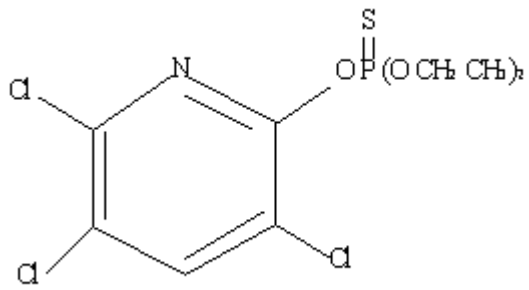

Figure 8: Chlorpyrifos.

- Appearance: Amber to white crystalline solid with a mild sulfur odor.
- Chemical Name: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
- CAS Number: 2921-88-2
- Molecular Weight: 350.62
- Water Solubility: 2 mg/L
- Soluble in benzene, acetone, chloroform, ethers.
- Melting Point: 44 C
- Vapor Pressure: 2.5 mPa
- Log K_{ow} Partition Coefficient: 4.70

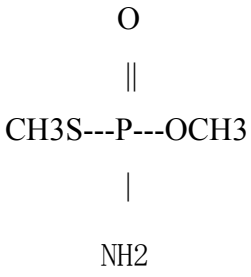

Figure 9: Methamidophos.

- Appearance: Crystalline solid, with off-white color and pungent odor.
- Chemical Name: O,S-dimethyl phosphora-midothiolate
- CAS Number: 10265-92-6
- Molecular Weight: 141.12
- Water Solubility: 90 g/L
- Solubility in solvents unavailable.
- Melting Point: 44 C
- Vapor Pressure: 3 x 10-4 mmHg
- Log K_{ow} Partition Coefficient: NA

(Extoxnet, 2003).

Table 1: Physical Properties and Partition Coefficients (K) of Malathion vs. Malaoxon.

System	Malathion	Malaoxon
Lipid Solubility	More soluble	Less soluble
Log K_{ow}	2.75	2.02
Log K Chloroform-water	64	5.8
Log K Hexane-water	19	0.42
Log K Petroleum ether-ethanol	11	1.8
Log K CCl4-water	39	2.9
Cuticle Penetration	Better	Worse
Alkaline Hydrolysis Stability	More stable	Less stable
AntiAChE activity	Poor	Good
I_{50} Inhibit AChE Conc. (M)	1×10^{-4}	4×10^{-7}

Oxidation of malathion to malaoxon greatly increases partitioning into polar solvents, but less likely to penetrate skin (O'Brien, 1957) (Miller, 1998).

Exposure Pathways. The site of introduction of a xenobiotic into the organism qualitatively governs the initial exposure. An orally administered compound has to traverse the intestines, the liver, and the lung before it reaches the systemic circulation. Due to this 'first-pass effect', the amount that reaches the systemic circulation is much less than the amount administered. In contrast, this 'first-pass effect' is bypassed through dermal absorption. The entire amount systemically absorbed through the skin is passed through the lungs by the venous blood before it is retuned to the heart and distributed by arterial blood to the vital organs (Caldwell, 1983).

Most exposures to organophosphates occur from skin absorption. Skin absorption can occur when dermal contact is made during handling and application, and when contact is made with chemical residues on plants, fruits, and foliage; in soil; and in dust particles after spraying. Organophosphate insecticides such as malathion are applied in the sulfur-containing (-thion) form, but readily undergo desulfuration to form a more toxic oxygen-

containing (-oxon) form. In the field, this conversion occurs slowly under the influence of oxygen and light, producing residues with altered physical properties and potentially more toxic as well.

Biologic Fate. Skin absorption of malathion is incomplete because much of the applied dose is lost from the skin surface by washing, evaporation and gradual exfoliation of the outer layers of the stratum corneum. The amount absorbed into the body depends on a relationship between the speed with which it penetrates the skin and the speed with which it is lost from the skin surface. Importantly, systemic absorption after skin application can be delayed, and the resultant absorption rate across skin is much slower than across mucous membranes. Typically, experimental subjects differ by a factor of five in the amount of malathion percutaneous penetration, with standard deviations up to one half the mean value. Thus, assuming a normal distribution, one person in ten will absorb twice the mean value, while one in twenty will absorb three times that amount.

Dary exposed human forearms to 1% malathion aqueous solution, placing an occlusive patch over the exposure site after 4 hours of observation. This occlusive patch was removed at 24 hours, analysis of which revealed 65% of the applied dose. This amount in the occlusive patch may represent evaporative and mechanical loss potential. Preliminary mass balance over the initial 24-48 hour post-exposure period was as follows:

- 70% of initial applied dose removed by washing skin surface-epidermis;
- 40% of the remaining dose (12% of initial dose) slowly lost through evaporation;
- 60% of the remaining dose (18% of initial dose) retained in dermis;
- 90% of the dermal to systemic dose is eliminated in urine;
- 15% of the initial applied dose is systemically absorbed and eliminated in urine.

The slow rate of dermal absorption and efficient rate of elimination by liver carboxylesterases and kidneys reduce the risk of acute toxicity; however, repeated exposures could burden these same organs of metabolism and elimination (Dary, 1994).

Although the skin is often considered as merely acting as a passive barrier to the entrance of chemicals, it is clearly capable of metabolizing endogenous substances as well as

drugs and other exogenous chemicals. Despite the low activity of metabolic enzymes in skin, it must be appreciated that skin is the largest organ in the body, comprising 5% of total body weight. Thus, the skin can contribute significantly to total metabolism of chemicals. For example, skin is well known to contain cytochrome P-450, NADPH-cytochrome c-reductase and glutathione S-transferase. Cutaneous enzymes with mono-oxygenase activities such as these can be induced by a variety of xenobiotics. Immunohistochemical investigations have documented the epidermis, sebaceous glands, and hair follicles as the sites of xenobiotic activation and detoxification within the skin (Caldwell, 1983).

Parathion, a closely related organophosphate to malathion, has demonstrated unusual percutaneous absorption kinetics and systemic toxicity patterns. For example, in an isolated perfused porcine skin flap model of parathion, almost 70% of the parent compound parathion penetrated the skin into the perfusate as paraoxon. Thus, biotransformation of topically dosed organophosphates by skin-based cytochrome p450 enzymes to the more toxic –oxon form may play a crucial role in risk assessment (Carver 1988) (Kao, 1990).

In the mammal, vigorous hepatic hydrolytic degradation of organophosphates at the carboxylic ester link typically outstrips the process of accumulation caused by oxidation. Hepatic conversion is brought about chiefly by microsomal esterases. This hydrolyzing system of the liver is more active against malathion than malaoxon; ultimately, however, both –thion and –oxon forms are metabolized to non-toxic alkyl phosphate mono-acids and excreted rapidly. In the rat model, after oral malathion ingestion, twelve hour urine samples contained 1% of the dose as malaoxon mono-acid and 67% as malathion acids (Kizer, 1991). On the other hand, in the cockroach insect, both fat body and midgut metabolic systems oxidize malathion to malaoxon, but only the fat body hydrolyzes malaoxon to the non-toxic alkyl phosphate. In the insect, then, oxidation is much more rapid than hydrolysis, thus malaoxon may accumulate steadily to a lethal level (O'Brien, 1957).

Physiologic Effects. Organophosphates combine with and inhibit cholinesterase enzymes, of which acetylcholinesterase (AChE) in nerve tissue is the most important. After conversion of malathion to its oxygen analog, malaoxon, the active site of AChE is phosphorylated (and inhibited) by dimethyl or methyl phosphate. Inactivation of AChE results in accumulation of acetylcholine at the neuroreceptor transmission site, resulting in massive overstimulation of the cholinergic system as follows:

- Accumulation of acetylcholine in the muscarinic autonomic receptors cause miosis, tearing, salivation, perspiration, nausea, vomiting and diarrhea.
- Accumulation of acetylcholine in the nicotinic neuromuscular receptors cause fasciculations, cramps and weakness.
- Rising accumulation of acetylcholine in the central nervous system cause sensory and behavioral disturbances, incoordination, depressed cognition, and respiratory failure.

Biologic Indicators. Detection of intact organophosphate compounds in blood is usually not possible. In general, organophosphates do not remain unhydrolyzed in the blood more than a few minutes. Alkyl phosphate metabolites can often be detected in the urine up to 48 hours after exposure. The appearance of these urinary metabolites can demonstrate pesticide absorption at lower dosages than those required to depress cholinesterase activity. Occupational malathion exposures have resulted in dimethyl-dithiophosphate (DMDTP) serum concentrations ranging to 3.5 mg/L (Shafnik and Enos, 1969). Up to 23% of a single oral dose of malathion is excreted in the 16 hour urine as ether-extractable phosphates, where biological activity was estimated via colorimetry (Mattson and Sedlak, 1960).

Red Blood Cell (RBC) cholinesterase, the same enzyme found in the nervous system, is a useful surrogate indicator for AChE activity at neuroreceptor sites. RBC cholinesterase activity is generally only restored as new red blood cells are formed at 1% per day. However, it is notoriously difficult to interpret cholinesterase inhibition without baseline values because normal values vary widely. The laboratory normal range is not useful because upper and lower limits typically differ by a factor of four. RBC cholinesterase

inhibition from 25-50% of the individual's baseline is generally regarded as evidence of toxicity, or the Lowest Observable Adverse Effect Level (LOAEL).

In the best available No Observable Adverse Effect Level (NOAEL) study, volunteers ingested 16 mg of malathion daily for 47 days without effect on RBC Cholinesterase. Doses of 24 mg, however, when administered for 56 days caused 25% depression of RBC cholinesterase, with maximal effects occurring three weeks after administration. No clinical signs or symptoms were noted in spite of the RBC cholinesterase depression (Moeller and Rider, 1962).

Demyelination and Phospholipid Peroxidation. In humans and other vertebrates, reaction of organophosphates with neuropathy target esterase (NTE) may initiate events which culminate in axonal degeneration. Organophosphates inhibit biosynthesis of phospholipids which in turn may cause peroxidation of the myelin sheath. Thus, toxic organophosphate exposure may result in demyelination-induced neurotoxicity. For example, toxic malathion exposure in rats has induced spinal cord phospholipid peroxidation (Haque, 1987).

Chlorpyrifos is heavily used for agricultural and domestic purposes due to its persistence and relative safety compared to other organophosphate insecticides. Unlike parathion, chlorpyrifos evokes delayed neuropathies only with very high exposures. Animal studies confirm that chlorpyrifos has an order of magnitude higher systemic toxicity in neonates. Perhaps more alarmingly, recent studies in rats have shown that chlorpyrifos, in doses below the threshold for symptoms of systemic toxicity, nevertheless can compromise the basic cellular processes of brain development.

In the neonate, programming of brain cell reactivity and synaptic connections are at their peak and are thus most vulnerable. In the neonate, the adverse effects of cholinesterase inhibition are exacerbated by poor synaptic adaptability to cholinergic hyperstimulation. In a neonatal rat animal model after chlorpyrifos exposure at doses that do not cause

systemic toxicity, there was alteration in coordination skills and locomotor activity (Dam, 2000).

In a similar neonatal animal model, chlorpyrifos exposure during the period of brain cell acquisition and axonogenesis resulted in neuronal loss and abnormalities of synaptogenesis. The adverse effects on cholinergic neurotransmission are a particular concern since cholinergic pathways are critically important for cognitive function. In fact, neonatal exposure in rats produced lasting reductions in hippocampal cholinergic presynaptic activity. Short-term behavioral studies in this animal model indicated alternations in several activity measures, and additional effects on activity emerged well after the end of cholorpyrifos administration (Levin, 2001).

These results (e.g. alterations in behavior and locomotion) stress the importance of looking beyond the acute period of organophosphate exposure for adverse effects. Indeed, developmental deficits may appear well after organophosphate exposure and cholinesterase inhibition have disappeared.

Delayed Neuropathy. Shortly after organophosphate exposure, a weak reversible bond is formed with AChE. With time, however, a more permanent AChE-phosphate bond forms ("aging") that inactivates the enzyme and requires an antidote to break. If an antidote such as pralidoxime [2-PAM] is not given within 24-48 hours after exposure, the AChE-phosphate bond becomes so strong that physiologic recovery depends on de-novo synthesis of AChE. At the nerve junction, AChE is restored in an average of two weeks; in the body as a whole, it may require up to three months to restore enzyme activity to near normal levels (Pope and Rall, 1995).

Symmetric distal axonal degeneration is a systemic health effect caused by some organophosphates that is not due to AChE inhibition. The degeneration is a dying back of large diameter axons and their myelin sheaths in distal parts of the peripheral nerves and in long spinal cord tracts. This degeneration is caused by inhibition of a neuronal, nonspecific carboxylesterase known as neuropathic target esterase (NTE), essential for

lipid metabolism in neurons. The resulting clinical syndrome, organophosphate-induced distal neuropathy (OPIDN), typically begins one to two weeks after exposure, and consists of flaccidity, paralysis and paresthesias of the lower extremities.

All organophosphates inhibit acetylcholinesterase (AChE), but only a subset of these inhibit neuropathy target esterase (NTE), the enzyme definitively associated with OPIDN. In essence, a sufficient amount of NTE must be irreversibly inhibited by the organophosphate before the OPIDN syndrome may occur. In vitro (neuroblastoma cells) and in vivo (hen, cat, and sheep) testing have established organophosphate-specific NTE / AChE inhibition ratios, and correlated this ratio precisely with the ability to produce the OPIDN syndrome. Previous work has shown that if AChE / NTE Inhibition Ratio >> 1, the dose required for producing OPIDN >> LD50. Accordingly, malathion, parathion, chlorpyrifos, dichlorvos and trichlorfon have not been linked to OPIDN.

Table 2: Organophosphates, OPIDN, and AChE / NTE Inhibition Ratio.

Organophosphates linked to OPIDN:	AChE / NTE Inhibition Ratio:
• DFP [diisopropyl fluorophosphonate]	5.3
• TSP [cyclic tolyl saligenim phosphate]	1.5
• PSP [cyclic phenyl saligenim phosphate]	0.12
• Mipafox	1.1
• DBVP	0.81
• DOVP	0.56
• EPN [ethyl nitrophenyl phenylphosphonate]	
• Leptophos	
• Merphos	
• Tri-ortho-cresyl phosphate [TOCP]	
• Tri-ortho-tolyl phosphate [TOTP]	
• Triaryl phosphate	
• Methamidophos	

Organophosphates not linked to OPIDN:	AChE / NTE Inhibition Ratio:
• Parathion	700,000
• Malathion	76,000
• Isomalathion (1R, 3R)	49000
• Isomalathion (1R, 3S)	150000
• Isomalathion (1RS, 3RS)	9100
• Isomalathion (1S, 3R)	2500
• Isomalathion (1S, 3S)	1500
• Chlorpyrifos	200
• Dichlorvos [DDVP]	42
• Trichlorfon	68

(Rosenstock, 1991) (Jianmongkol, 1996) (Ehrich, 1997).

Although there are no known case reports of OPIDN specifically linked to malathion, malathion exposure has been listed at least as a contributory factor in a significant OPIDN case series examination. In this series, exposed patients had moderate increases in vibrotactile thresholds in the lower extremities. These results suggest that previously reported OPIDN cases may represent only the worst disease in a spectrum of polyneuropathy impairment, a sequelae of organophosphate exposure that may be much more common than previously thought (McConnell and Rosenstock, 1994).

To evaluate the latent neurological effects of organophosphate poisoning, an epidemiological study matched 100 pairs of individuals with previous acute organophosphate poisoning and non-poisoned controls. Ten different organophosphates were listed as the cause of primary poisoning, most being parathion, but of which six were due to malathion. In the neurological examination component of the study, clear abnormalities were demonstrated in the organophosphate exposed group in memory, abstraction, mood, motor reflexes and simple motor skills. In the psychological examination component, clear abnormalities were demonstrated in intellectual functioning, academic skills, abstraction and flexibility in thinking. Minnesota Multiphasic Personality Inventory analysis revealed greater distress and complaints of disability for the poisoned subjects. Twice as many cases as controls had Halstead-Reitan Battery summary scores in the range characteristic of individuals with cerebral damage or dysfunction (Savage EP, 1988).

Delayed polyneuropathy (DP) is typically caused by organophosphate induced inhibition of NTE, the enzyme essential for normal neuronal and axonal processes. DP is characterized as a distal sensori-motor axonopathy, beginning about two weeks after toxic exposure, with recovery beginning six to twelve months later. The best available case report of malathion-induced delayed polyneuropathy (DP) occurred in 1990 as follows:

A 65yo female was admitted to the hospital in cholinergic crises with muscarinic, nicotinic and central effects after deliberate ingestion of 100 ml of malathion (235 mg/kg)

dissolved in isoproponal. Artificial ventilation, atropine and pralidoxime were initiated immediately. The patient was found to have complete and total depression of serum cholinesterase. The patient regained consciousness and strength a few days later, and was noted to have complete normalization of serum cholinesterase on day 12. Her hospitalization was further complicated by pneumonia and acute respiratory distress syndrome. At day 10, her muscle strength began to deteriorate again with loss of deep tendon reflexes. Distal muscles were more involved than proximal ones. On day 35, a gastrocnemius muscle biopsy showed evidence of denervation and reinervation. A sural nerve biopsy showed degenerating axons, moderate loss of myelinated fibers and clusters of small regenerating axons. Six weeks after admission, muscle strength began to recover and was normalized after three months. This patient was found to have exposure to a significant pesticide residue as the key DP causative factor: isopropyl-malathion (Argiles, 1990).

Organophosphate intoxication will cause cholinergic stimulation acutely, but delayed neuropsychiatric sequelae may also result in a subacute paralytic syndrome known as the intermediate syndrome. Intermediate syndrome is characterized by deterioration of muscle strength and mental status about 48 hours after the acute presentation. Typically weakness manifests itself within the neck and proximal muscles, which may include the cranial nerves and the diaphragm. Motor recovery may take 5-20 days and most patients will not develop polyneuropathy. Behavioral manifestations include depression, psychosis, aggression, irritability, and memory and concentration problems. The best available case report of malathion-induced intermediate syndrome occurred in 1998 as follows:

A 44yo female was found in cholinergic crises with muscarinic, nicotinic and central effects after deliberate ingestion of 125 ml of malathion (50% solution). Artificial ventilation, atropine and pralidoxime were initiated immediately. Her hospitalization was further complicated by bronchorrhea requiring glycopyrollate and pneumonia. She was eventually transferred out of intensive care to the medical ward on day 13. On day 23, she began displaying agitation, disorientation, hallucinations and somatic delusions. She

became aggressive and inappropriate, voiding on the floor and throwing feces, and was transferred to a psychiatric ward. Her delirium resolved over 48 hours with psychiatric treatment, and she was discharged from the hospital on day 35 (Choi, 1998).

Racemic Isomalathion. Commercial malathion is supplied as a racemic (RS) mixture of the R and S forms arising from an asymmetric carbon in its diethyl thiosuccinate group. The corresponding steroisomers of malaoxon differ 8.6-fold in their inhibitory potencies against rat brain AChE. Thermal or photochemical isomerization may occur during storage of racemic malathion to yield racemic isomalathion, which is 1000-fold more potent than the parent compound.

Shelf storage of malathion at 38C increases both its isomalathion content and its toxicity. For example, unusually high levels of isomalathion were linked to 2800 poisonings including 5 fatalities among 7500 Pakistani spraymen using malathion for malaria control (Baker, 1978). Contamination of malathion by isomalathion acts synergistically to increase toxicity by inhibiting the detoxification potential of liver carboxylesterase enzymes.

Isomalathion contains two asymmetric centers, one at phosphorus, and the other at the carbon in the diethyl thiosuccinate moiety, yielding four stereoisomers. Inhibitory potencies of these isomers against AChE differ by as much as 29-fold. Individual stereoisomers each form Michaelis complexes with this enzyme prior to the expulsion of the primary leaving group and organophosphorylation of the active site serine. Subsequently, the inhibited enzyme could undergo either reactivation (displacement of the entire organophosphoryl group) or aging (scission of a ligand from the inhibitor, leaving a negatively charged organophosphoryl group).

Aging to yield a negative charge in the active site leaves the enzyme intractable toward reactivation. Diethyl thiosuccinate is the primary leaving group for R-isomalathion, while thiomethyl is the primary leaving group for S-isomalathion. This unexpected stereochemically determined switch in the inhibition mechanism potentially extends the

range of toxicological consequences arising from exposures to the isomalathions. Aging of both electric eel AChE and equine seurm butyrylcholinesterase (EBChE) by S-malathion via an SN2 reaction with P-S bond cleavage to yield an O-methyl phosphate adduct has recently been reconfirmed (Doorn, 2001).

Apart from cholinergic toxicity elicited by AChE inhibition, some organophosphate compounds inhibit and age >70% NTE, producing the OPIDN syndrome. Certain structural requirements have been elucidated for organophosphate inhibitors of NTE. For a given primary leaving group, longer-chain dialkyl ligands on phosphorus are preferred over dimethyl or diethyl analogs, and small leaving groups are favored among longer-chain dialkyl-substituted organophosphates. Considering these structure activity relationships, malaoxon isomers and the isomalathions would be expected to be poor inhibitors of NTE if diethyl thiosuccinate were the primary leaving group. However, if thiomethyl were the primary leaving group, as found in the S-isomalathion configuration, then inhibition of NTE would appear to be a reasonable possibility.

In view of unexplained reports of neuropathic sequelae associated with malathion intoxication in humans, and the fact that isomalathions can arise as impurities in malathion preparations, and the fact that isomalathions are potent inhibitors of esterases other than NTE, the possibility exists that these compounds could be potentiators, rather than initiators, of neuropathic processes (Healy, 1959) (Harell, 1978) (Rivett, 1987) (Komori, 1991) (Jianmongkol, 1996).

Chapter Two

Skin Absorption

Introduction. Interest in skin permeability arises mainly from the capacity of the skin to limit the body's accumulation or elimination of substances by percutaneous transport. Although a complex process, dermal uptake of compounds occurs mainly through passive diffusion, involving a selective mechanism in the lipid and protein structures of the stratum corneum. This epidermal skin barrier is the major factor in this process and can reduce overall rates of accumulation by several orders of magnitude. For example, water-soluble, low molecular weight, non-electrolytes can diffuse into the bloodstream 1000 times more rapidly if the epidermis is diseased, damaged, or removed. Even with the skin intact, there are still 10,000 fold differences in the rates of penetration of different substances.

Stratum Corneum. The principal barrier function of the epidermis resides almost entirely in the stratum corneum, the thin coherent membrane of keratinized, epithelial cells that comprise the "dead" surface layer of the epidermis. The phenomenon of percutaneous absorption is essentially one of adsorption onto the stratum corneum (10 um thickness), diffusion through it and through the viable epidermis (100 um thickness), and finally through the papillary dermis (100-200 um thickness) and into the microcirculation.

The composite skin layer is pierced by two types of diffusion shunts: hair follicles and sweat glands. Sebaceous glands are attached to follicular walls at distances of 500 um below the surface, and for this reason their effectiveness as shunts is probably negligible. Hair follicles (40-70/cm2) and sweat glands (200-250/cm2) do act as diffusion shunts, but their role is more subtle than one might think. Although the fractional area covered by

these appendages is only 0.1%, diffusion constants of low-molecular weight molecules moving through the appendages are greater than through stratum corneum. Before steady-state diffusion is established, and particularly during the first few minutes after the application of a substance, the follicles and ducts cannot always be ignored even when the ultimate steady-state flux through these shunts is small.

The slow rate of malathion dermal absorption and its efficient rate of elimination reduces the risk of acute toxicity for all exposures except when involving substantial portions of unprotected skin. Water as a common vehicle may play an important role in increasing the permeability of malathion in the stratum corneum. If penetrants are easily dissolved in their oil vehicles applied to the skin, penetration is more difficult. For example, carbon disulfide and analine are absorbed through the skin about 100 times faster from aqueous solutions compared with oil solutions. The comparatively high rate of skin absorption of compounds from their aqueous solutions seems to be a rule that could be related to the hydration of stratum corneum (Scheuplein, 1965) (Baranowska-Dutkiewicz, 1982). If the skin is hydrated, or the compound is in solution, diffusion and penetration will be enhanced. On the other hand, stratum corneum may be subject to compaction and dehydration when in contact with pure solvents. In addition, the necrotizing effects of concentrated solvents in contact with skin act to limit absorption. Such data indicate that skin absorption rates from dilute aqueous solutions may be seriously underestimated.

The entire skin and not just the stratum corneum can interact in important ways with solvent mixtures. One might conceptualize percutaneous absorption as a process of diffusion through the stratum corneum, followed by systemic uptake at the epidermal-dermal junction. Furthermore, evaporation from the skin surface, reservoir formation in the dermis, and percutaneous penetration are interrelated processes that are important in determining the fate of topically applied compounds. Rapid penetration into the dermis may occur with pesticides even after attempted soap and water decontamination (Reifenrath, 1991).

Intercellular spaces in the stratum corneum are normally filled and mechanically coherent. The conversion of aqueous epidermal cells into dried, compact, keratin-containing stratum corneum cells is the crucial event in the continuously developing epidermis that largely determines the low permeability of skin. The viable skin tissue layers and the capillaries are relatively permeable, and the peripheral circulation sufficiently rapid so that for the great majority of substances diffusion through the stratum corneum itself is rate limiting. The usual diffusion laws of physics pertaining to passive diffusion processes can therefore be applied to skin permeability phenomena and greatly aid in its description.

The rate-limiting barrier within the stratum corneum is typically the hydrated intercellular keratin. This keratin has an affinity for both water-soluble and lipid-soluble compounds. Keratin's bi-functional solubility arises from its inherently mosaic, filament-matrix ultra-structure which allows aqueous and lipid regions to exist separately. For this reason, attempts to predict permeability constants from correlations with oil-water partition coefficients (K_{ow}) have proved only marginally successful. For larger molecules and molecules with several polar groups, the relative importance of the stratum corneum increases still further (Scheuplein, 1971).

Fick's Law. Many investigators have reported on the toxicity and unexpectedly high penetration rates of volatile organic compounds. Through relatively simple analysis of absorption from specific compounds, it is possible to estimate potential absorption using Fick's Law. Dose may then be calculated using experimentally-derived skin absorption rates and constants (K_p). Fick's Law essentially describes the behavior of dilute aqueous solutions, such that absorption of the solute will be directly proportional to concentration. In more detail, Fick's Law may be used to determine the permeation rate of solvents in an aqueous solution, and is expressed by the formula:

$J_s = K_p * \Delta C$

Where:

Js = permeation rate (flux) of the solute expressed as mcg/cm2;

Kp = permeability constant expressed as liters / cm2 / hr;

ΔC = concentration difference of the solute across the specified tissue in mcg/L.

For example, using Fick's Law, permeability constants for the following compounds in weak aqueous solutions were experimentally derived:

Table 3: Reference Permeability Constants (Kp).

Compound	Aqueous Solution Concentration: (mg/L)	Permeability Constant K_p (L / cm2 / hr)
Ethylbenzene	112	.00100
Toluene	180	.00090
Styrene	66	.00060
Carbon Disulfide	1000	.00050
Trichloroethylene	100	.00020
Chloroform	0.5	.00012
Aniline	10000	.00004

(Dutkiewicz and Tyras, 1967, 1968).

Skin absorption rates are typically derived from experimental situations where the hands and wrists are immersed in test solution. Rates obtained in this manner, however, will underestimate actual absorption in cases of whole-body immersion during swimming. The epidermis of the hand represents a relatively greater barrier to penetration than many other parts of the body, especially hair-follicle-rich areas. For example, the scrotum has an imperfect stratum corneum because of the enormous demands made on it in terms of flexibility, and is well known to absorb almost all of several pesticides. In addition, the face, forehead, scalp and neck absorb 2-6 times more than the forearm. Hands and feet absorb about as much as the forearm, but absorption is slower. Larger areas of the body,

such as the back, allow greater absorption than the forearm (Feldmann and Maibach, 1974).

Table 4: Malathion Anatomical Dermal Absorption Rates and Ratios in Humans.

	Ratio	Rate: %Dose Recovered in Urine at 0-5 days
Palm	0.9	
Forearm	1.0	6.8 ± 2.3%
Foot	1.0	6.8 ± 3.2%
Abdomen	1.4	9.4 ± 7.9%
Hand, Dorsum	1.8	12.5 ± 4.0%
Hand, Palm		5.8 ± 2.9%
Scalp	3.7	
Jaw Angle	3.9	
Forehead	3.4	23.2 ± 9.1%
Ear	4.0	
Axilla	4.2	28.7 ± 13.7%
Scrotum	11.8	

Malathion concentration at 4 mcg/cm2 in a non-occlusive acetone vehicle.
Subjects requested not to wash the site of application for 24 hours.
(Maibach, 1971).

Quick reference to a Lund and Browder Chart shows that forearm and hand surface area compared to total body surface area is 11:100, or Fs = 0.11. Adjusting this figure for Maibach's relative absorption characteristics in different body surface areas yields an adjusted factor of 11:142, or Fs_{adj} = .0775.

Table 5: Body Surface Area by Relative Absorption.

Anatomic Location	Surface Area	Relative Absorption	Multiplication Factor
Head	7%	3.7	25.9
Neck	2%	2.0 (est)	4
Ant Trunk	13%	1.4	8.9
Post Trunk	13%	1.4 (est)	8.9
Buttocks	5%	1.0 (est)	5
Genitalia	1%	11.8	11.8
Both Upper Arm	8%	1.0 (est)	8
Both Lower Arm	6%	1.0	6
Both Hands	5%	1.0	5
Both Thighs	19%	1.0 (est)	19
Both Lower Legs	14%	1.0 (est)	14
Both Feet	7%	1.0	7
Total	100%		142.1

(Brown, 1984).

Rate of Absorption. The entire skin, not just the stratum corneum or epidermis, can interact in important ways with topically applied compounds. Evaporation from the skin surface, reservoir formation in the dermis, and percutaneous penetration are interrelated processes that are important in determining the fate of topically applied compounds. Rapid penetration into the dermis may occur with organophosphates and may account for percutaneous absorption even after one minute of decontamination with soap and water. (Reifenrath, 1991).

Percutaneous penetration rates are determined by the physiochemical properties of a compound such as molecular weight, solubility, charge distribution, partition coefficients, and various other parameters. Correlations are consistent for compounds having closely related chemical structures. However, meaningful results are often inconsistent where

chemicals of diverse structure are compared. The rate of dermal uptake of malathion compared to other insecticides was assessed in a shaven mouse skin model (upper shoulder). As shown in Table 6, and compared to the 13 other insecticides investigated, malathion exhibited the longest half-life and the least penetration.

Table 6: Pesticide Rates of Dermal Absorption in Mice.

Compound:	Half-life:	Penetration:		Excretory	GI	Carcass
		1 hr	8 hr	8 hr	8 hr	8 hr
Carbaryl	13 min	72%	88%	73%	8%	6%
Chlordecone	41 min	54%	66%	4%	20%	25%
Parathion	66 min	32%	85%	50%	10%	17%
Dieldrin	72 min	34%	83%	4%	12%	62%
DDT	105 min	34%	71%	4%	15%	41%
Malathion	130 min	25%	67%	30% (urine)	2%	33%

Penetration is disappearance of radioactivity from 4 cm^2 shaved skin, upper shoulder. (Shah, 1981).

It is notable that malathion dermal penetration rates are quite variable in nature among different mammalian species. In Yorkshire swine, after non-occlusive malathion application to the ventral abdominal skin, corrected systemic absorption was only 5% at 6 days. Systemic absorption for malathion as a percentage of the original dermal dosage was less than all other compounds tested (benzoic acid > progesterone > caffeine > testosterone > parathion > malathion) in Yorkshire swine (Carver, 1989). In rats, after non-occlusive malathion application to 10 cm^2 shaven dorsal back for 8 hours, autoradiograms demonstrated 28% of the dose concentrated at the application site, 29% in the skin at large, and 23% contained in the urinary bladder (Saleh, 1997).

Complex mathematical models for the dermal absorption of a dilute malathion solution in humans are available. A physiologically-based pharmacokinetic (PBPK) model has been created that utilizes tissue volume compartments, air and blood flows, and chemical-specific partition coefficients. PBPK incorporates seven body compartments (skin

surface, skin perfused, fat, muscle, kidney-vessel rich group, intestine, and liver) and four external compartments (air, urine, feces, and acid metabolites). Skin evaporation and oxidative metabolism of malathion to malaoxon are incorporated into this model as well. This adult PBPK model, which notably assumes $K_{p\text{-malathion}}$ = 0.001 L/cm2/hr, predicts during total immersion scenarios that 7-31% of the applied dose will be systemically absorbed. Furthermore, 70% of that systemically absorbed dose is metabolized and excreted within 24 hours (Kizer, 1991).

PBPK rate constants were chosen to approximate experimental data. The model's skin permeability constant ($K_p = 1 \times 10^{-5}$ /min to 5×10^{-4} /min, skin surface to viable epidermis) was less than the skin evaporation constant ($K_{evap} = 8 \times 10^{-5}$ /min to 5×10^{-4} /min, skin surface to air)). This important ratio, $K_p/K_{evap} \leq 1$, was based upon several classic studies. Maibach demonstrated in three studies that 4%, 8%, and 7% of [C^{14}]-malathion applied to human forearm skin was excreted in 0-7 day urine samples. Peak excretion was 8 hours post-application. As shown in Table 4, up to 29% urinary recovery came from more absorptive (but smaller surface area) parts of the body (Webster and Maibach, 1983) (Feldmann and Maibach, 1974) (Maibach, 1971). Increasing air flow over the surface of excised pig skin undoubtedly increases evaporative loss for malathion. Significant evaporative loss for malathion occurred from both the skin surface-epidermis layer and the dermis layer. In fact, increased air flow decreased malathion dermal residues but not parathion, DDT, nor DEET dermal residues. Penetration of the applied malathion dose at 48 hours in the excised pig skin model decreased from 21% to 9% as air flow was increased from 60 to 600 ml/min (Reifenrath, 1991).

Total Immersion. Swimming pool and bathing immersion models show that skin absorption represents a significant route of exposure. In fact, dermal absorption can contribute from 29-91% of the total daily dose, for an average contribution of 64%. This information suggests that when doses from dilute aqueous solutions are calculated through Fick's Law, and carefully considered, margins of safety may be significantly more narrow than anticipated. On this basis, regulatory guidelines and policies may need to be reconsidered (Brown, 1984).

In another swimming pool exposure model, chlorinated pools were found to contain an average of 156-500 mcg/L (156-500 ppb) chloroform contamination. A worst case dermal exposure model was built utilizing Fick's Law kinetics. Swimmers were assumed to be totally immersed for three hours daily. During immersion, some absorption of the aqueous chloroform was presumed to occur through the dermal and aural routes. Accelerated dermal absorption would be expected from skin that has been freshly wounded, abraded or sunburned. During some of the time the swimmer was immersed, the internal tissues of the nose, mouth and eyes would also be exposed. For example, about five milliliters of water is taken into the mouth and squirted out with each breath when a child swims. In summary, dermal absorption from the dilute aqueous solution represented 60% of the dose, as shown in the table below:

Table 7: Skin Absorption Rates of Chloroform in 500 ppb Aqueous Solution.

Route	Relative Absorption
Dermal	60%
Oral	3%
Buccal / Sublingual	3%
Orbital / Nasal	3%
Aural	3%
Inhalation	28%

(Beech, 1980).

An improved methodology to predict in vivo Kp values for dilute aqueous solutions based upon molecular weight and oil-water partition coefficients was recently derived by (Bogen, 1994) as follows:

$Log_{10}K_p$ = $-0.812 - 0.0104 * MW + 0.616 * \log_{10}K_{ow}$ cm/hr

K_p = $(10^{(-0.812 - 0.0104 * MW + 0.616 * \log_{10}K_{ow})) / 1000$ L/cm2/hr

$K_{p\ Toluene}$ = $(-0.812 - 0.0104 * \underline{92.1} + 0.616 * \underline{2.73}) / 1000$ = .0008200 L/cm2/hr

$K_{p\ Chloroform}$ = $(-0.812 - 0.0104 * \underline{119.4} + 0.616 * \underline{1.97}) / 1000$ = .0001400 L/cm2/hr

$K_{p\ Parathion}$ = $(-0.812 - 0.0104 * \underline{291.3} + 0.616 * \underline{3.83}) / 1000$ = .0000300 L/cm2/hr

$K_{p\ Chlorpyrifos}$ = (-0.812 – 0.0104 * 350.62 + 0.616 * 4.70) / 1000 = .0000300 L/cm2/hr

$K_{p\ Malathion}$ = (-0.812 – 0.0104 * 330.36 + 0.616 * 2.75) / 1000 = .0000030 L/cm2/hr

$K_{p\ Malaoxon}$ = (-0.812 – 0.0104 * 314.36 + 0.616 * 2.02) / 1000 = .0000015 L/cm2/hr

Vertebrate Models. Vertebrate behavioral studies after exposure to dilute malathion solutions are effective indicators of contamination and reflect sub-lethal toxicity. Because behavior is an integrated result of endogenous and exogenous processes, behavioral studies provide a way to address the question of effect in contaminant work. When using fish as a bio-indicator, it has been suggested that a stressful condition is detected when one or more physiological variables are altered to the point where long-term survival may be impaired. Swimming behavior in fish is frequently assessed as a response in toxicity investigations because altered locomotor activity can indicate effects to the nervous system.

Fish were exposed to 20-50 mcg/L (20-50 ppb) dilute aqueous malathion solutions for 24 hours. Malathion exposure caused dramatic decreases in distance swam and swimming speed as well as pronounced changes in complexity of swimming paths. A highly positive correlation was found between 26% decreased brain cholinesterase activity and decreased swimming speed. Significant fish mortality was found when the dilute aqueous malathion solution concentration was increased to an 50 ppb exposure level and as a result caused 50% brain acetylcholinesterase inhibition (Cook 1976) (Brewer, 2001).

The sensitivity of vertebrate brain cholinesterase to malaoxon is as follows: sculpin fish > chicken > sparrow > flounder > sunfish > bass > rat > mouse > monkey > duck > bullhead > guinea pig. In summary, as far as brain cholinesterase sensitivity is concerned, there is no clear grouping according to vertebrate class. However, sensitivity to poisoning by malathion is as follows: fish > chickens > mice. Mouse liver hydrolyzes more than ten times as much malathion as sunfish liver and about three times as much as chicken liver. Therefore, the greater susceptibility of fish to poisoning by malathion can best be accounted for on the basis of their relative inability to detoxify malaoxon (Murphy, 1968).

Chapter Three

Medfly Eradication Exposure Data

Background. Certain malathion formulations are registered by EPA for aerial spraying over urban areas in mosquito-control programs. The use of malathion in these programs provides an important public health benefit by controlling mosquitoes that transmit human disease (e.g. encephalitis, dengue fever, malaria). However, the spraying of malathion bait over urban populations for Medfly eradication has generated controversy in part because these applications are directed not at preventing human illness but at eradicating an agricultural pest.

Federal law does not permit spraying malathion bait over urban areas without an emergency EPA exemption. If and when the EPA grants said exemption, the responsible government authorities post advance notification of spray schedules, schedule malathion spraying episodes at night, and advise the public to stay indoors during spraying events and wash any skin surface that may contact the malathion bait (Shafey 1999).

Aerial malathion bait may be used as a regulatory control method to establish freedom of nursery or orchard premises from living fruit fly stages, as a condition for movement of produce. To accomplish this, the establishment undergoes a series of treatments at intervals, designed to provide continued freedom from fruit flies during the quarantine period. Bait spray applications normally are limited to locations producing regulated commodities within a quarantined area, but located outside the infested core area. Treatments must start at a sufficient time, at least 30 days, before harvest (to span the interval that normally would include the completion of egg, larval, and pupal

development), then continue throughout the harvest period. The required pre-harvest treatment makes this option useful for only those commodities remaining in the field for more than 30 days after an area is quarantined (Smith, 2001).

Full foliar coverage bait spray of host trees and other plants immediately reduces fruit fly populations by 90 percent or more and reduces subsequent reproduction. This decreases fruit fly numbers in the succeeding generation and reduces the risk that gravid female fruit flies will move to uninfested areas. In this manner, the malathion bait applications reduce wild fruit fly populations to a level of infestation where mating thresholds are not achieved or where continued releases of sterile fruit flies can be effective in reducing the rest of the emerging pest population.

Technical Malathion. Numerous compounds are found in technical grade formulations of pesticides. These co-products occur from breakdown or conversion of the active ingredient, and from unintentional reactions occurring during pesticide synthesis.

Isomalathion is one of the largest potentiators of malathion toxicity, and is equipotent to malaoxon. The in vivo mechanism of potentiation is inhibition of B-type carboxylesterases (CEBs), the group of liver enzymes responsible for the metabolism of malathion and malaoxon into their non-toxic alpha and beta monacids. Technical malathion mixtures stored for extended periods of time under warm, humid conditions may contain isomalathion at concentrations as high as 7%. Each one percent increase in isomalathion content increases mammalian toxicity by an order of magnitude (Baker, 1978).

The concentration of technical malathion co-products varies from batch to batch, but the major co-products are typically as shown in the following table.

Table 8: Technical Malathion.

- Isomalathion 0.20%
- Malaoxon 0.10%
- Diethyl fumarate 0.90%
- O,O,S-trimethyl phosphorothioate [OOS(O)] 0.04%
- Diethyl methylthiosuccinate 1.00%

Concentrations of isomalathion in technical mixtures are generally quite low, rarely exceeding 0.5% of the nominal malathion content. This conversion to isomalathion is most prone to occur in water dispersible powders, where levels exceeding 3.0% are common. Isomerization is a function of storage temperature, relative humidity, and storage time. Increasing relative humidity from 41 to 65% increased the conversion rate, as did storage for six months at 40C. The World Health Organization recommends that technical malathion mixtures exceeding 1.8% isomalathion be discarded and not used.

Trimethyl phosphorothioates [OOS(O)] are present as impurities, reaction products and breakdown products in technical grade malathion. OOS(O) is known as a potent inhibitor of cholinesterases, beta-carboxylesterases, and neuropathy target esterases, especially when reaching concentrations of 0.2-1.0% in the technical grade malathion mixture.

Diethyl fumarate is known as the causative agent of non-immunologic contact urticaria (NICU) in humans. Skin irritation and rashes have been reported following malathion-bait applications, both of which are symptomatic of NICU.

(Kizer, 1991).

Environmental Fate. Most organophosphate insecticides are used for crop spraying in commercial agriculture. Approximately 75% of all insecticides are used for cotton, corn and soybeans. Malathion is the most widely used organophosphate insecticide. Organophosphate compounds degrade in the environment at varying rates: half-lives

range from days to months, although generally half-lives are longest in dry climates and low temperature.

Soil. Malathion is of low persistence in soil with field half lives of up to 25 days. Degradation in soil is rapid and related to the degree of soil binding. Breakdown occurs by a combination of biological degradation and non-biological reaction with water. If released to the atmosphere, malathion will breakdown rapidly in sunlight, with a reported half-life in air of about 1.5 days. It is moderately bound to soils, and is soluble in water, so it may pose a risk of groundwater or surface water contamination in situations which may be less conducive to breakdown.

Water. Malathion when dissolved in bodies of water is readily oxidized to malaoxon by a variety of mild oxidizing reagents. Thus, it is generally recognized that malathion is easily oxidized to malaoxon by swimming pool chlorine concentrations. For example, malaoxon has greatest persistence when pool water is acidic, and malathion is stable in oxygen saturated water at acidic pH for up to two weeks. Sunlight shortens malathion and malaoxon half-lives in pools to 3 days. These data suggest that little accumulation of malathion or malaoxon in swimming pools occurs, but does indicate that they can persist at low levels for a considerable period of time.

In river water, the half-life of malathion is generally less than one week. For example, in the Suwanee River with large amounts of tannins, malathion was 50% degraded by sunlight within 16 hours. However, malathion may remain stable in distilled water for three weeks and its photolysis half-life is 41 days. Applied at up to 6 pounds per acre in log ponds for mosquito control, it is generally effective for 2.5-6 weeks. In seawater, degradation increases with salinity. Breakdown products in acidic water are mono- and dicarboxylic acids such as dimethyl phophorothionic acid and 2-mercaptodiethyl succinate (Howard, 1991) (Wolfe, 1975).

Hydrolysis of malathion in basic pH aqueous solution yields primary breakdown products diethyl fumarate and dimethyl phophorodithioic acid (DMPTA). At pH 8.0, diethyl

fumarate remains stable for 1.5-4 days. In the fathead minnow animal model, two-way and three-way interactions of the breakdown products caused large synergistic increases in toxicity. In particular, diethyl fumarate could be produced as a breakdown product in significant quantities in basic pH aqueous solutions to produce synergistic toxic effects with malathion in vivo (Cook, 1976).

Air. Malathion bait is oxidized rapidly in the atmosphere by ozone and dinitrogen tetraoxide to malaoxon, but not by molecular oxygen. Thus, two days post-spray application the outdoor air concentration of malaoxon generally exceeds that of malathion. Air concentrations of malathion also increase at two days post-spray application by volatilization of the bait droplets. Outdoor particle diameter is ~100 um with particles ranging up to 350 um. Indoor particle diameter is ~3 um and lower, evenly distributed on walls, ceilings and floors. Malathion bait found indoors is 3% of the amount found in the adjacent outdoor area (Caplan PE, 1956) (Kizer, 1991).

Hillsborough County, Florida, 1997. Medfly eradication commenced via aerial malathion protein bait application on June 5, 1997. About 2.4 ounces by weight of malathion with 9.6 ounces of corn syrup protein bait was targeted to each acre in the quarantine area. About 2.6 mg/ft^2 of malathion was targeted into nine square mile areas centered around each Medfly find. During the ten week time period under study, thru August 1997, vintage military aircraft sprayed 31,000+ gallons of malathion bait over 400+ square miles of Hillsborough County landscape. One million people lived and worked in the aerial spray zone areas (Smith, 2001).

This malathion bait concentration transferring from the air into a one meter deep body of water typically yields a 22 ppb dilute aqueous solution. Importantly, however, bait spray applications are made at 5-10 day intervals until eradication is achieved. Certain areas in Lakeland, for example, were sprayed at least 8 times. As a result, large fluctuations in concentration are known to have occurred in “Medfly hot zones”, DC-3 flight pattern overlapping zones, and bodies of water subject to storm-water runoff.

A registry for people reporting increased health problems associated with the malathion spray program was established. The "Malathion Health Survey" is a cooperative effort between the USF College of Public Health, Poison Control Center, and Hillsborough County Health Department. As of 10/6/97, more than 700 calls from people reporting exposure to malathion had been received. Most reported some kind of symptoms, including nausea and headaches.

The Tampa Tribune has featured possible victims with adverse reactions temporally related to the application of malathion bait:

(1) A 7yo diver and swimmer had 3 days of headache, nausea and sore throat when practicing the day after a DC-3 sprayed malathion in and around Brandon Swim and Tennis Club (BSTC) pool. The swimming pool was found the day after spraying to have 12 ppb malaoxon contamination;
(2) An elementary school teacher sprayed with malathion on campus had a rash;
(3) A restaurant worker caught in the path of a DC-3 had nausea and chest pain;
(4) Three children had blisters/rashes on shoulders/arms and diarrhea after playing in a Riverview lime tree the day after malathion spraying;
(5) A man's arm had angry red rash when immersed in family pool 3 hours after malathion spraying.

Only limited environmental sampling has been accomplished during the aerial application of malathion spray in Hillsborough County. The Environmental Protection Commission (EPC) of Hillsborough County has published a limited number of water sample results as follows:

Table 9: Tampa Swimming pool sampling by FL-EPC (ppb).

- Malathion-range 0- 1; mean 0.
- Malaoxon- range 1-19; mean 5.
- Malaoxon at BSTC on Day 0=24 ppb; Day 1=12ppb; Day 2=5ppb; Day 3=2.5ppb.
- BSTC Swim and Diving Team (n=15-20): 33-50% became ill when swimming the day after aerial application of malathion bait to the swimming pool area.

Table 10: Tampa Pond sampling by FL-EPC (ppb).

- Malathion-range 21-79; mean 49.
- Malaoxon-range 00-01; mean 01.
- Malathion city water treatment plant = 0.7, and malaoxon = 0.5.
- Malathion at Riverview apartment pond = 25.
- Malathion at Alafia River = 12.
- Malathion at six fish kill sites = 2.3-25.0.

Dade, Lake, Marion, Manatee and Highland County, Florida, 1998. During the spring and summer of 1998, aerial malathion and ground diazinon application was used by federal and state agriculture authorities to eradicate Medfly infestations that had been detected in portions of five Florida counties. All insecticide application was complete on September 6 with an estimated 132,000 persons residing in the pesticide treatment area.

Reports of potential adverse health effects attributed to the Medfly Eradication Program were solicited by state health and agriculture authorities. During the Spring and Summer months, 230 reports of illness were investigated and classified by the Florida Department of Health as outlined in the following table.

Table 11: Medfly Spray-related Adverse Events, Florida Department of Health.

- Probable: 34 (15%), based upon abnormal medical signs observed by a licensed health-care professional;
- Possible: 89 (39%), based upon abnormal symptoms compatible with organophosphate toxicity reported to health-care workers or a state health authority.
- Probable plus Possible: 123 (54%), or crude incidence rate of 9 / 10,000 residents;
- Eight reports involved children < 5yo;
- Twenty reports involved elderly > 65yo;
- Four reports involved work-related illnesses such as pesticide applicators;

Of the signs and symptoms reported in the "Probable plus Possible" group:

- 71% were respiratory (dyspnea, wheezing, coughing);
- 63% were gastrointestinal (cramping, nausea, vomiting, diarrhea);
- 60% were neurologic (headache, vertigo, ataxia, paresthesia, confusion);
- 23% were dermatologic (erythema, rash, pruritis);
- 23% were ophthalmic (lacrimation, conjunctivitis, blurred vision).

In the case reports, specific exposures were reported while:

- Conducting lawn maintenance business, where grass trimmings stuck to skin;
- Working on roof of house;
- Removing pool cover, which was folded and carried under sleeveless arm.

(Shafey, 1999).

Los Angeles, California, 1990. In California, more extensive environmental sampling has shown the amount of malathion bait actually sprayed on some occasions was 40-350% more than would be predicted from uniform coverage calculations:

Table 12: Los Angeles Swimming pool sampling by CA-EPA (ppb).

- Malathion-range 4-90; mean 18.
- Malaoxon- range 0-46; mean 16.
- Malaoxon was found in two pools one week after application at 7 ppb.

Table 13: Los Angeles Pond sampling by CA-EPA (ppb).

- Malathion-range 21-79; mean 49.
- Malaoxon- range 00-01; mean 01.

Table 14: Los Angeles Storm-water runoff by CA-EPA (ppb).

- Malathion-range 0-703; mean 151.
- Malaoxon- range 0- 18; mean 2.

In summary, there is solid evidence for potential toxic exposure to malathion and its co-products while swimming in outdoor bodies of water during and after aerial malathion bait application. Furthermore, Los Angeles County exposure data has found swimming malathion pool contamination as high as 90 ppb. Many malathion bait spray scenarios may converge to account for high environmental concentrations: e.g. physical-chemical-biological reactions; over-lapping spray areas; repeat spray areas, human error; technical limitations; and weather fluctuations.

Chapter Four

Hazard Index

The aerial application of malathion bait results in exposure by three routes: inhalation, ingestion, and dermal absorption. Exposure may be acute, sub-acute, or chronic in nature. Exposure to malathion and/or its three significant co-products, isomalathion, malaoxon, and diethyl fumarate, may occur depending upon the specific scenario under study. The scope of this analysis will focus only upon an adult and child's acute dermal exposure to dilute aqueous concentrations of malathion and one of its co-products, malaoxon, while immersed in a contaminated swimming pool after a typical aerial malathion bait spray episode.

In presenting exposure and dosage scenarios, the concept of reference exposure level (REL) is utilized: the level at which no adverse health effects are anticipated. Therefore, health protection is achieved if the estimated dosage is below the relevant REL. In order to make REL data easier to interpret, a Hazard Index (HI) format is chosen, where HI = (estimated dosage / REL). Note that hazard index > 1 represents possible exposure scenarios greater than the relevant REL. Estimated dosages > REL or hazard indexes > 1 are not necessarily hazardous, and do not absolutely result in significant health effects; however, further analysis may be warranted.

Several independent studies and guidelines are available to define the "incipient toxicity" level for malathion exposure. In the best available study, depression of cholinesterase activity was observed two weeks after the oral administration of malathion 24 mg to five human subjects for 55 consecutive days. Maximum depression of plasma cholinesterase activity (25%) and red blood cell (RBC) cholinesterase activity (30%) occurred three

weeks after cessation of malathion. In spite of the depression in both plasma and RBC cholinesterase activity, no clinical side effects were observed. In summary, this report agrees with the State of CA assessment of about 0.2 mg/kg/day as the preliminary REL.

The best available approach for estimating dermal exposure to malathion in a swimming pool is the model utilized for chloroform (CF) and trichloroethylene (TCE) for baths and showers. This type model is specific for dilute aqueous solutions of lipophilic, organic contaminants.

In vitro and in vivo measurements of CF and TCE uptake by human skin from dilute aqueous solution indicate that short-term, non-steady-state, dermal uptake kinetics are consistent with a first-order partition model. The first-order partition coefficient estimation assumes that the stratum corneum is the effective skin compartment volume of distribution, as it likely is with most lipophilic, organic compounds such as malathion.

Blood perfusion to skin is likely to cause relatively rapid equilibrium of chemical concentrations within the epidermis, and perhaps within the underlying dermal tissue as well. This scenario is expected particularly in the shower, bath and swimming pool scenarios, involving relatively high water temperatures known to elicit a ten fold increase in local dermal blood perfusion rates (Bogen, 1995).

For example, Bogen's method can be utilized, with some key assumptions, to derive an exposure estimate for a child in a contaminated swimming pool model as follows:

Ds = Kp*Dc*De*Fs*As*Sys / Mb

Where:

- Ds = systemic dose rate in mg/kg/day

And:

- $K_{p\text{-malathion}}$ = permeability constant across the skin = 0.001 L/cm^2/hr;
- Dc = concentration of malathion in water = 30 mcg/L or 30 ppb;
- De = duration of contact with contaminated water = 3 hr;
- Fs = fraction of skin in contact with water = 1.0;
- As = exposed skin surface area = 12 000 cm^2;
- Mb = body mass = 34.5 kg;
- Sys = Dermal absorption to Systemic Absorption = 0.10.

The dermal absorption to systemic absorption conversion factor (Sys = 0.10) is an important parameter that deserves further explanation. This factor is derived from Maibach's three human studies of non-occlusive, concentrated dermal malathion absorption versus Dary's contrasting occlusive study of human forearms utilizing dilute malathion aqueous solution exposures (Webster and Maibach, 1983) (Feldmann and Maibach, 1974) (Maibach, 1971) (Dary, 1994). Dary determined that 15% of the initial applied dose is systemically absorbed and eliminated in urine, but this figure may be adjusted downward towards Maibach's figures (4%, 8%, and 7%) to account for the non-occlusive setting of the swimmer. That is, it is assumed that swimmers first shower with soap and water after three hours of swimming, in effect mechanically removing malathion from the skin surface-epidermis layer, then secondly move into environmental settings conducive to dermal layer evaporation over the remainder of the day.

Assuming $K_{p\text{-malathion}}$ is reasonably estimated from similar Fick's Law studies of ethylbenzene, toluene, styrene, and xylene in dilute aqueous solutions, the specific dose rate in a swimming pool with contamination of 30 ppb malathion to a 7 yo child during a 3 hour work-out is about: 0.003 mg/kg/day, or 0.1 mg total. Thus, swimming pool exposure to malathion in this model is about 70 times less than the REL, yielding a hazard index of 0.015. These preliminary data, with key assumptions, indicate that exposure to aqueous malathion at this concentration in this model is not appreciably absorbed, and therefore, it is not a public health hazard.

Chapter Five

Significance of Research

It is interesting to note that many factors in a swimming pool exposure model may actually enhance Kp over published values. For example, increased water retention in the stratum corneum layer, increased skin surface temperature and increased skin blood flow from exercise all tend to increase Kp. For lipid soluble substances like malathion, lowered viscosity of tissue lipids at higher temperatures reduces activation energies for diffusion. In addition, sunburned or wounded skin accelerates dermal absorption. Finally, skin sites in children appear to be more permeable than skin sites in adults. Most of these factors are operational in the swimming pool model in a way that tends to increase the dermal absorption of malathion.

Nevertheless, the main area of uncertainty in the swimming pool exposure model is within the estimate for $K_{p\text{-malathion}}$. Unfortunately, specific experimental data for $K_{p\text{-malathion}}$ do not exist. Fortunately, Kp has been determined for similar compounds in dilute aqueous solutions such as ethylbenzene, styrene, and toluene, all of which were determined to have similar values to the Kp used for malathion above. Further experimental determination of $K_{p\text{-malathion}}$ as used in Bogen's dermal exposure equations is necessary before models for toxic exposures in dilute aqueous solutions of malathion while bathing are convincing.

The balance of malathion and malaoxon exposure in humans may be swayed by concomitant exposure to exogenous malaoxon. Swimmers may be exposed to malaoxon in addition to malathion as the aerial bait lands in the swimming pool and is oxidized by its chlorine to this much more potent active metabolite. Let's recalculate the dermal

absorption for the 7yo swimmer, except this time assuming exposure to 30 ppb malathion + 30 ppb malaoxon.

Ds = Kp*Dc*De*Fs*As *Sys/ Mb

Note that the chosen concentrations of malathion and malaoxon are within one standard deviation of the mean actual California swimming pool data, and are both well within the UCL values. In addition, let's use potency of malaoxon = 68 * malathion.

- $Kp_{malathion}$ = permeability constant across the skin = 0.0010 L/cm2/hr;
- $Kp_{malaoxon}$ = permeability constant across the skin = 0.0005 L/cm2/hr;
- Dc = concentration of malathion in water = 30 mcg/L or 30 ppb;
- Dc = concentration of malaoxon in water = 30 mcg/L or 30 ppb;
- De = duration of contact with contaminated water = 3 hr;
- Fs = fraction of skin in contact with water = 1.0;
- As = exposed skin surface area = 12 000 cm2;
- Mb = body mass = 34.5 kg;
- Sys = Dermal absorption to Systemic absorption = 0.10.

Assuming $K_{p\text{-}malathion}$ and $K_{p\text{-}malaoxon}$ are reasonable, the specific dose rate in a swimming pool with contamination of 30 ppb malathion and 30 ppb malaoxon to a 7 yo child during a 3 hour work-out is about: 0.11 mg/kg/day, or about 3.8 mg total. Important assumptions in this first pass analysis include swimmers first showering with soap and water after three hours of swimming, in effect mechanically removing malathion and malaoxon from the skin surface-epidermis layer, then secondly moving into environmental settings conducive to dermal layer malathion and malaoxon evaporation over the remainder of the day. This swimming pool model dosage for malathion + malaoxon is about half the adjusted REL, yielding HI = 0.5. Therefore, by definition, this dose may be sufficiently close enough to HI = 1 to cause a specific ailment or disease related to malathion toxicity, and further analysis is warranted.

Chapter Six

Methods

Background: Historical studies have measured human dermal uptake of related aromatic chemicals from aqueous solutions, using the "disappearance method" in concert with the analysis of excreted metabolites. In all the studies that examined uptake from aqueous solutions using the disappearance method, both whole hands of each person was immersed for two hours in a one-liter beaker. For example, disappearance measurements of aqueous ethylbenzene at 125 ppm were compared to the absorbed amount computed by utilizing the urine metabolite mandelic acid. Chemical flux into skin was validated as the difference in chemical mass contained in the exposure solution at the beginning and end of the exposure period, divided by the corresponding dermal surface area (Dutkiewicz and Tyras, 1967).

Design: Prospective, controlled, and limited dermal exposure of hands and forearms of human volunteers to dilute aqueous solutions of 50-ppb (mcg/l) malathion in a laboratory setting.

Measurements: EPA-approved, commercial assays to 0.5-ppb (mcg/l) malathion will be utilized in order to measure the concentrations in water before and after hand and forearm exposure.

Subjects: Twenty volunteers

Test Solution: Twenty liter tanks were prepared with dilute aqueous solutions of 50-ppb (mcg/l) malathion. The solution was prepared by placing 1 mg of laboratory grade malathion (Supelco PS86, Malathion, Neat) into the distilled water bath.

A Standard Operating Procedure (SOP) was carefully developed, documented and verified to ensure volunteer subject safety. Several quality assurance based trial runs were undertaken prior to the first volunteer subject exposure period. During each trial run, each twenty liter tank prepared under the SOP was verified to contain a 50-ppb malathion concentration via an EPA-approved, commercial assay sensitive to 0.5 ppb. During the quality control evaluation period, the tank malathion concentrations were verified to be within ±30% (±15 ppb) of the nominal target concentration (50 ppb or 50 mcg/L).

The malathion exposure tank was stagnant, hand-stirred, and then mechanically stirred during three different stirring exposure phases of the protocol. The tanks were at room temperature (21°C) and swimming pool temperature (29°C or 90°F) during two different temperature exposure phases of the protocol as well. Volunteer exposure time was gradually increased during several different phases of the protocol from 30 minutes to 120 minutes. All upward adjustments in exposure level were carefully made in series to ensure maximum volunteer safety.

Method: Subjects place both hands and forearms (total body surface area: Fs = 0.11) into a dilute aqueous 50-ppb (mcg/l) malathion solution for up to two (2) hours. Malathion solution assays are then made via the "disappearance method" on the tank solution before and after the exposure period.

Malathion assays are completed by passing the tank exposure solution through Bakerbond C18 Speedisks (Part Number 8055-06). Five hundred milliliters of the exposure solution is drained through the Speedisk via full vacuum suction (about 25 mm Hg) applied to a Speedisk extraction station (for about five minutes). The Speedisks with extracted malathion solute were then shipped in a sealed container to Ecology and Environment, Inc., for completion of the EPA assay method.

Precise concentrations in the dilute aqueous malathion solution were determined before and after exposure using EPA SW-846 Method 8270C at Ecology and Environment, Inc (Lancaster, NY). This EPA-approved commercial assay method is sensitive to 0.5 ppb. The semi-volatile compound is introduced from a sample extract into the gas chromatograph by direct injection of 2 mcl of the extract. Sample extracts are prepared for analysis by transferring a measured aliquot of extract to an autosampler vial and adding the appropriate amount of internal standard solution. Chromatographic conditions are such that the compounds are separated by the gas chromatograph. The compounds are detected using a mass spectrometer from which both qualitative and quantitative information is obtained. Quantitation is by the internal standard technique using relative response factors.

Calculations of chemical flux into skin from the exposure solution are estimated according to Fick's Law as the difference in chemical mass contained in the exposure solution at the beginning and end of the exposure period, divided by the corresponding exposed dermal surface areas. In summary, a Mass Balance System was implemented upon the exposure solution, with the assumption that all lost malathion in solution was driven down the concentration gradient into the exposed human subject. $K_{p\text{-malathion}}$ (permeability constant across the skin) was then derived from the actual Fs=0.11 exposure model. $K_{p\text{-malathion}}$ was then theoretically applied to an Fs = 1.00 swimming pool model for both theoretical child and adult swimming pool models. Finally, hazard index (HI) for theoretical three-hour swimming pool exposures were calculated based upon experimentally derived Dd and $K_{p\text{-malathion}}$ values.

Table 15: Parameters for Kp = Dd*Mb / (Dc*De*Fs*As).

- Dd = skin dosage of malathion absorbed in mcg
- Dc = average concentration gradient of malathion from water to skin
- De = duration of contact with contaminated water
- Fs = fraction of skin in contact with exposure solution = 0.11;
- Fs_{adj} = adjustment for Maibach's relative skin absorption = 0.0775;
- Fs = fraction of skin in contact with water in swimming pool = 1.00;

Table 16: Parameters for Ds = Kp*Dc*De*Fs*As*Sys / Mb.

Adult:

- As = total skin surface area = 21 345 cm2;
- Mb = body mass = 85 kg;
- HI = Ds / (0.2 mg/kg/day)*(85 kg) = Ds / 17 mg;

Child:

- As = exposed skin surface area = 12 000 cm2;
- Mb = body mass = 34.5 kg;
- HI = Ds / (0.2 mg/kg/day)* (34.5 kg) = Ds / 7 mg;

Example Calculation: Utilize the theoretical exposure model Dd = Kp*Dc*De*Fs*As / Mb, and assume approximate $K_{p\text{-malathion}}$ = 0.001 L/cm2/hr. An 85 kg subject typically has hand area Fs=.025 (each) and forearm area Fs=.03 (each). Therefore, immersing both hands and forearms in the malathion solution yields a total exposure area Fs=.11, with Fs_{adj} = 0.0775 after adjustment for Maibach's relative skin absorption data. Therefore, this theoretical volunteer would dermally, but not systemically, absorb:

Dd = (.001 L/cm2/hr)(50 ppb or mcg/l)(3 hr)(.0775)(21 345 cm2) = 248 mcg.

The 20 liter immersion tank, then, would change from the original concentration of 50 ppb malathion to 37.6 ppb (mcg/l) or (1000 mcg - 248 mcg) / 20 liters. This change in malathion concentration over the time period of the volunteer subject exposure period is well within the sensitivity of the NIOSH/EPA laboratory assay procedure.

Chapter Seven

Volunteer Safety

Screening of Volunteer Subjects: Each female participant had a proven negative pregnancy test within 72 hours of study participation. Each volunteer subject was screened for a history of acute or chronic disease or disability, Gulf War Syndrome, or previous significant exposure to malathion, organophosphate or other pesticide. If the screening history is positive, the subject is eliminated from participation in the study protocol.

Medical Observation: Each subject is intermittently observed for acute cholinergic signs and symptoms by a licensed physician during the two (2) hour experimental protocol period as follows:

- Mild exposure symptoms include headache, giddiness, dizziness, anxiety, weakness, tremors of tongue and eyelids, and miosis.
- Moderate exposure symptoms include behavioral disturbances, sweating, nausea, vomiting, salivation, lacrimation, diarrhea, paresthesias, muscle tremors, and in-coordination.
- Severe exposure symptoms include fecal and urinary incontinence, pulmonary edema, heart block, respiratory failure, convulsions, coma and death.

Although highly unlikely at the malathion dosage selected for use in this experimental protocol, a volunteer exhibiting signs or symptoms of organophosphate exposure would be referred to a local emergency room for treatment at once. In addition, a resuscitation cart equipped for atropine and pralidoxime (2-PAM) administration was immediately available for use by a licensed physician should emergency reversal of

cholinergic events become necessary. Furthermore, volunteers exhibiting any cholinergic signs or symptoms would be reported as an adverse event to the Institutional Review Board at once.

Hazard Index Model: Worst-case-scenario analysis assumes the subject volunteer absorbs all the malathion in the exposure tank, 1 mg, or 12 mcg/kg/day. Since the Reference Exposure Level is 200 mcg/kg/day, Hazard Index = 12/200 or 0.06. Therefore, health protection is achieved since the estimated dosage is an order of magnitude below the relevant REL and HI << 1.

Biological Exposure Indices: The ACGIH recommended BEI for organophosphorus cholinesterase inhibitors (e.g. malathion) is RBC Cholinesterase Activity. Significant exposure is widely recognized to have occurred if RBC Cholinesterase activity is decreased more than 30% from an individual's baseline condition. Continued depression may occur in dose-dependent fashion for up to 12 weeks post-exposure. RBC cholinesterase activity is the preferred measure as it is the same enzyme found within the nervous system.

In Moeller and Rider's NOAEL / LOAEL landmark malathion study, the defined objective was to determine the maximum amount of malathion which can be ingested daily for a prolonged period of time without depressing the pretest level of RBC Cholinesterase Activity. The subjects were healthy men in San Quentin State Prison. Malathion was orally administered at 8 mg/day for 32 days, 16 mg/day for 47 days, and 24 mg/day for 56 days. RBC Cholinesterase was analyzed at baseline then twice weekly. Neither 8 mg/day nor 16 mg/day affected RBC Cholinesterase Activity in a statistically significant manner. However, 24 mg/day for 56 days depressed RBC Cholinesterase Activity to about 75% of baseline.

Based upon Moeller and Rider's study, BEI monitoring with RBC Cholinesterase Activity during this protocol appears unwarranted. That is, in the worst case scenario, the volunteer subject would absorb all the malathion in the exposure tank: 1 mg. This dosage

is well below the known dosage to cause RBC Cholinesterase depression. Furthermore, volunteer subjects in this protocol have only a single exposure. In the Moeller and Rider study, volunteer subjects required 24 mg oral ingestion exposures on 56 consecutive days before RBC Cholinesterase was depressed.

Dermal Exposure Indices: Dermal absorption of neat malathion (24.6 mg), a 50% emulsifiable concentrate (25.7 mg), and a 1% (0.16 mg) and 10% (5.5 mg) aqueous mixture was examined in human volunteers. Each volunteer subject received a single application of the nominal dose of malathion applied to a 4.6 cm^2 area of the ventral forearm. The 1.0% aqueous mixture appeared to be more readily absorbed than the other formulations: 6.42% of the external concentration was absorbed per hour (Dary, 1994). Based upon this study analysis, <20% of the dilute malathion aqueous experimental solution will be absorbed after 2 hours time by the volunteers, or about 0.2 mg of Malathion. This dosage of malathion is well under the Reference Exposure Level (REL) and Hazard Index (HI) for Malathion

Theoretical Mathematical Exposure Model: Utilizing the exposure model described once again with assumed $K_{p\text{-}malathion}$ =0.001 L/cm2/hr, and the formula Ds = Kp*Dc*De*Fs_{adj}*As*Sys / Mb, the subject volunteers in the protocol will be exposed to about:

Ds = (.001 L/cm2/hr)(50 ppb)(3 hr)(.0775)(21 345 cm2)(0.10) / (85 kg);
= 25 mcg / 85 kg adult;
= 0.3 mcg/kg/day.
Reference Exposure Level (REL) = 200 mcg/kg/day;
Hazard Index (HI) = 0.3 / 200 = 0.0015.

Therefore, health protection is achieved since the estimated dosage is below the relevant REL and the HI is << 1.

Over-the-counter Exposure Model: Household over-the-counter malathion formulations (Spectracide®, manufactured for Spectrum Group, United Industries Corporation, St. Louis, EPA Reg. No. 10370-291-8845) are available in 50% xylene solution, or 500 mg/ml. The worst-case-scenario exposure of a subject volunteer in this protocol is equivalent to exposure to about 1/500 ml or 0.002 ml of the over-the-counter solution.

Carcinogen Status: At the time of the study protocol approval, malathion was not classified as a carcinogen by any certified organization including the ACGIH.

Chapter Eight

Results

Table 17: General Experimental Results.

- Demographics: All volunteer subjects were between ages 25-50 yo, weight 70 – 100 kg, and 2/20 (10%) were female.
- Subject eleven was removed from further data analysis due to the null concentration of malathion during the exposure period.
- Eight of nineteen subjects (42%) absorbed no malathion from solution, thus receiving no malathion dosage during exposure.
- Eleven of nineteen subjects (58%) absorbed malathion from solution, thus receiving a malathion dosage during exposure.
- Begin versus end malathion concentrations were highly variable from volunteer to volunteer, but not significantly different ($p = 0.185$).
- Volunteer Safety: No volunteer experienced signs or symptoms of organophosphate exposure or toxicity at any time.
- Experimental $Kp_{malathion}$ derived from human volunteer exposures ranged from 0 - 0.0051 $L/cm^2/hr$ (ave=0.0005, SD=0.0011, UCL=0.0028).
- Hazard Index (HI) for adult swimmers immersed for three hours in Malathion=30 ppb+Malaoxon=0 ppb (utilizing experimentally-derived $Kp_{malathion}$) ranged from 0 – 0.06 (ave=0.01, SD=0.01, UCL=0.03).
- Hazard Index (HI) for child swimmers immersed for three hours in Malathion=30 ppb+Malaoxon=0 ppb (utilizing experimentally-derived $Kp_{malathion}$) ranged from 0 – 0.08 (ave=0.01, SD=0.02, UCL=0.04).

- Hazard Index (HI) for adult swimmers immersed for three hours in Malathion=30 ppb+Malaoxon=30 ppb (utilizing experimentally-derived $Kp_{malathion}$) ranged from 0 – 2.0 (ave=0.21, SD=0.45, UCL=1.11).
- Hazard Index (HI) for child swimmers immersed for three hours in Malathion=30 ppb+Malaoxon=30 ppb (utilizing experimentally-derived $Kp_{malathion}$) ranged from 0 – 2.8 (ave=0.29, SD=0.62, UCL=1.53).

Table 18: Dermal Absorption by Volunteer - I

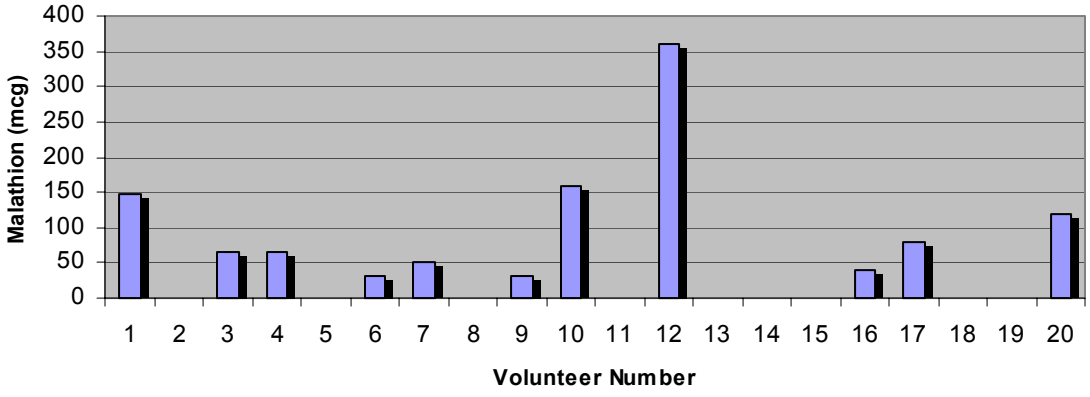

Table 19: Dermal Absorption by Volunteer - II

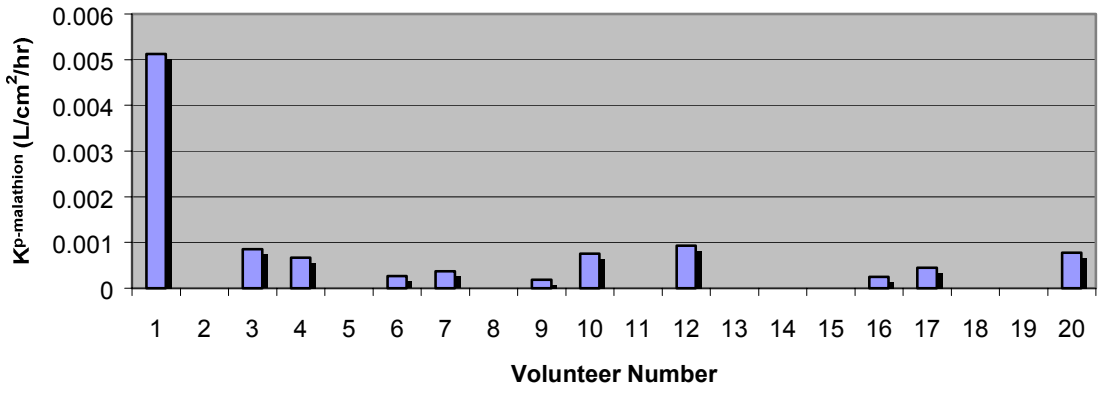

Table 20: Dermal Absorption by Dosage

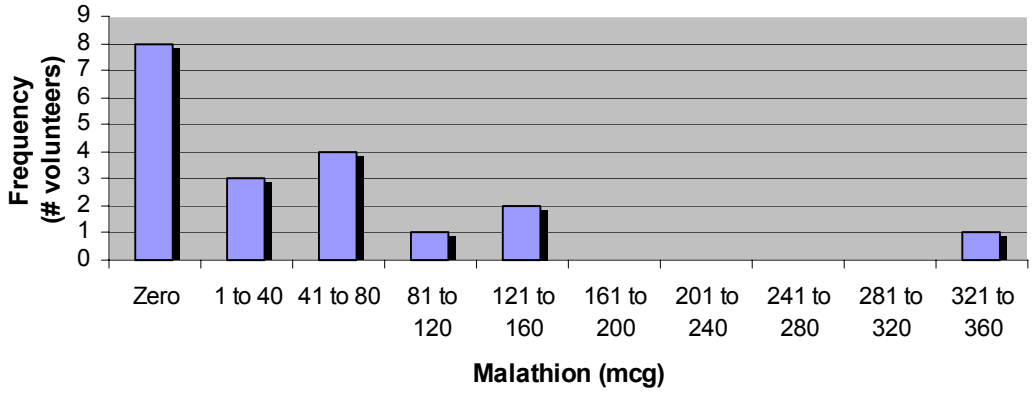

Table 21: Dermal Absorption by $K_{p\text{-malathion}}$

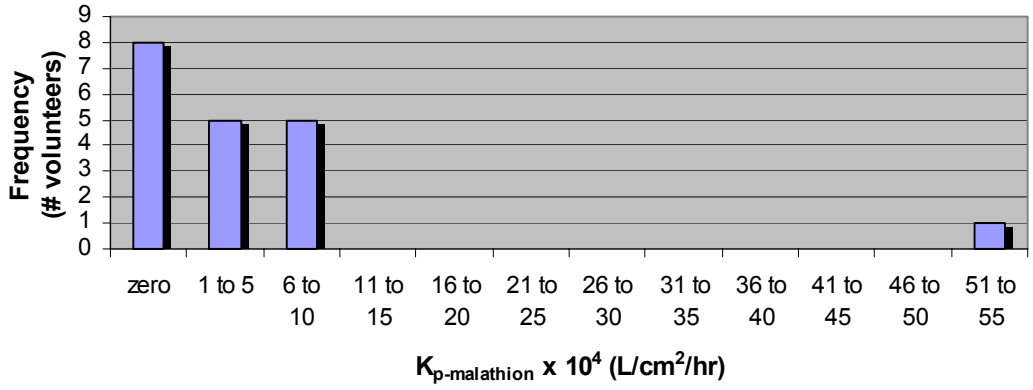

Table 22: Hand / Forearm Exposure in Aquarium;
Nominal Contamination: Malathion=50 ppb + Malaoxon=0 ppb;
Disappearance Method: Solution Concentrations.

subject	pre-malathion (mcg/l)	post-malathion (mcg/l)	stir	temp (°C)	time (min)
1	38.6	31.2	no	21	30
2	51.6	52.6	no	21	60
3	46.8	43.6	no	21	60
4	40.4	37.2	no	21	90
5	42.4	44.0	no	21	90
6	50.0	48.4	no	21	90
7	39.2	36.6	no	21	135
8	32.4	34.8	no	21	135
9	47.2	45.6	no	21	135
10	68.0	60.0	yes	21	120
11	0.0	0.0	yes	21	120
12	126.0	108.0	yes	21	120
13	46.0	46.0	yes	21	120
14	46.0	46.0	yes	21	120
15	44.0	46.0	yes	21	120
16	50.0	48.0	yes	29	120
17	56.0	52.0	yes	29	120
18	34.0	48.0	yes	29	120
19	50.0	50.0	yes	29	120
20	50.0	44.0	yes	29	120

Table 23: Hand / Forearm Exposure in Aquarium;
Nominal Contamination: Malathion=50 ppb + Malaoxon=0 ppb;
Disappearance Method: Experimental determination of $Kp_{malathion}$.

subject	Skin Dosage (Dd in mcg)	$K_{p\text{-}malathion}$ (L/cm2/hr)
1	148	.00513
2	0	0
3	64	.00086
4	64	.00067
5	0	0
6	32	.00026
7	52	.00037
8	0	0
9	32	.00019
10	160	.00076
11	0	0
12	360	.00093
13	0	0
14	0	0
15	0	0
16	40	.00025
17	80	.00045
18	0	0
19	0	0
20	120	.00077

Table 24: Swimming Pool Model using experimental $Kp_{malathion}$; Contamination: Malathion=30 ppb + Malaoxon=0 ppb; Adult Swimmer immersed for three hours.

subject	Skin Dosage (Dd in mg)	Systemic Dosage (Ds in mg)	Hazard Index
1	9.85	1.0	0.06
2	0	0	0
3	1.64	0.2	0.01
4	1.28	0.1	0.01
5	0	0	0
6	0.50	0.05	0.003
7	0.71	0.1	0.004
8	0	0	0
9	0.36	0.04	0.002
10	1.45	0.1	0.01
11	0	0	0
12	1.79	0.2	0.01
13	0	0	0
14	0	0	0
15	0	0	0
16	0.47	0.05	0.003
17	0.86	0.1	0.005
18	0	0	0
19	0	0	0
20	1.48	0.1	0.01

Table 25: Theoretical Swimming Pool Model using experimental $Kp_{malathion}$; Contamination: Malathion=30 ppb + Malaoxon=0 ppb; Child Swimmer immersed for three hours.

subject	Skin Dosage (Dd in mg)	Systemic Dosage (Ds in mg)	Hazard Index
1	5.54	0.5	0.08
2	0	0	0
3	0.92	0.1	0.01
4	0.72	0.1	0.01
5	0	0	0
6	0.28	0.03	0.004
7	0.40	0.04	0.006
8	0	0	0
9	0.20	0.02	0.003
10	0.82	0.08	0.01
11	0	0	0
12	1.00	0.1	0.01
13	0	0	0
14	0	0	0
15	0	0	0
16	0.27	0.03	0.004
17	0.48	0.05	0.007
18	0	0	0
19	0	0	0
20	0.83	0.08	0.01

Table 26: Theoretical Swimming Pool Model using experimental $Kp_{malathion}$; Contamination: Malathion=30 ppb + Malaoxon=30 ppb; Adult Swimmer immersed for three hours.

subject	Skin Dosage (Dd in mg)	Systemic Dosage (Ds in mg)	Hazard Index
1	345	34.5	2.0
2	0	0	0
3	57	5.7	0.3
4	45	4.5	0.3
5	0	0	0
6	18	1.8	0.1
7	25	2.5	0.2
8	0	0	0
9	12	1.2	0.1
10	51	5.1	0.3
11	0	0	0
12	63	6.3	0.4
13	0	0	0
14	0	0	0
15	0	0	0
16	17	1.7	0.1
17	30	3.0	0.2
18	0	0	0
19	0	0	0
20	52	5.2	0.3

Table 27: Theoretical Swimming Pool Model using experimental $Kp_{malathion}$; Contamination: Malathion=30 ppb + Malaoxon=30 ppb; Child Swimmer immersed for three hours.

subject	Skin Dosage (Dd in mg)	Systemic Dosage (Ds in mg)	Hazard Index
1	194	19	2.8
2	0	0	0
3	32	3.2	0.5
4	25	2.5	0.4
5	0	0	0
6	10	1.0	0.1
7	14	1.4	0.2
8	0	0	0
9	7	0.7	0.1
10	29	2.9	0.4
11	0	0	0
12	35	3.5	0.5
13	0	0	0
14	0	0	0
15	0	0	0
16	9	0.9	0.1
17	17	1.7	0.2
18	0	0	0
19	0	0	0
20	29	2.9	0.4

Chapter Nine

Discussion

It is not uncommon in dermal absorption studies for wide Kp variation within individual experimental results. Absorption constants and as a result dosage received can vary by several orders of magnitude between human subjects. For example, during this protocol, eight of nineteen subjects absorbed nil malathion, or at least so little that no absorption could be detected by the disappearance technique. In contrast, the absorption constant derived from the first volunteer was an order of magnitude greater than the following nineteen subjects, this despite the most conservative set of experimental conditions. This wide variation in results is similar to other investigators experience in similar published dermal absorption study settings.

As compared to malathion and malaoxon, toluene and chloroform are smaller in molecular weight but with similar oil-water partition coefficients. Thus, according to Bogen's equation, toluene and chloroform should have considerably larger permeability constants (Kp). However, the experimentally derived $Kp_{malathion}$= .0005 L/cm2/hr obtained in this study is in fact similar to those previously obtained by other investigators for $Kp_{toluene}$=.0008 L/cm2/hr and $Kp_{choloroform}$= 0.0001 L/cm2/hr.

It is interesting to note that many factors in the swimming pool exposure model may enhance the typical $Kp_{malathion,}$. For example, water, ambient, and skin temperatures may well be greater in swim exercise. These increased temperatures enhance skin absorption. Also, during swimming or bathing, greater hydration of skin surfaces takes place, again promoting Kp. For lipid-soluble malathion, lowered viscosity of tissue lipids at higher temperatures reduces activation energies needed for diffusion. In addition, sunburned or

wounded skin, as well as hair follicles and sweat glands, accelerate dermal absorption. Finally, skin sites in children appear to be more permeable than skin sites in adults. Thus, the experimental $K_{p_{malathion}}$ derived from this study is likely a conservative value.

Utilizing the experimentally-derived $K_{p_{malathion}}$, the Hazard Index (HI) for adult and child swimmers immersed for three hours in a malathion only contaminated pool ranged from 0 – 0.08. These data indicate that exposure to dilute aqueous malathion solutions following usual aerial bait applications is not appreciably absorbed, and therefore, not a public health hazard.

Importantly, a potential problem arises in the analysis from the statement 'usual aerial bait application'. An atypical problem scenario exists with aerial bait application in that malathion is easily oxidized to the more potent malaoxon by chlorine within swimming pools. This fact is evidenced by the fact that both California and Florida environmental agencies and public health authorities found considerable malaoxon contamination of public and private swimming pools in the days and weeks following aerial bait application.

In vivo, parent malathion must be metabolically activated to malaoxon by oxidative desulfuration to be an effective AChE inhibitor. This activation step is carried out by both mammals and insects, but insects are relatively deficient in the carboxylesterases used by mammals for detoxification. Thus, insects have higher toxicity to malathion generally speaking. However, humans may have similar malathion toxicity profiles to insects if and when liver carboxylesterase is inactivated. One compound well known to inactivate liver carboxylesterase is malaoxon, which may do so if absorbed ex vivo from contaminated swimming pools.

Since malaoxon is 68 times as toxic as malathion, human volunteer exposure to same, with or without concurrent exposure to malathion, is generally deemed too dangerous for human experimentation. Therefore, in order to analyze the potential toxicity of external malaoxon contamination and exposure in the swimming pool model, it is necessary to

estimate $Kp_{malaoxon}$. Fortunately, Bogen's empirical formula for Kp values based upon molecular weight and oil-water partition coefficient values can be utilized for same. In this case, owing to the more polar qualities of malaoxon, $Kp_{malaoxon}$ is estimated to be one-half that of $Kp_{malathion}$.

The Hazard Index for swimmers immersed for three hours in a malathion plus malaoxon contaminated pool was modeled utilizing the experimentally-derived $Kp_{malathion}$, and Bogen's technique to estimate $Kp_{malaoxon}$. This methodology yielded an average HI=0.21 for adult swimmers, with HI for child swimmers similarly averaging 0.29. Although HI>1 (albeit slightly) at the UCL (two standard deviations) for the volunteer group modeled in this malathion plus malaoxon exposure model, even these data indicate there is no appreciable public health hazard.

In order to lessen uncertainty in the hazard index estimate for malathion plus malaoxon exposure scenarios, further animal or human experimental dermal absorption data is clearly indicated in order to determine $Kp_{malaoxon}$ with more certainty. Since human volunteers should not be utilized in experiments with this toxic compound, the next best available alternative is hairless guinea pig skin. Bogen and others have determined that the hairless guinea pig is a superb model for human dermal skin absorption, and a similar disappearance method to that used in this protocol can be effectively used with this guinea pig animal model. Thus, potentially synergistic contaminant toxicity profiles for malathion plus malaoxon may be more precisely determined in the future (Bogen, 1992).

Additional routes of entry for malathion and its co-products to enter into a swimmers body exist but have not yet been integrated within the theoretical model described in this paper. EPA estimates that children who swim for 3 hours daily take in and squirt out of their mouths 16 liters of pool water and ingest 1% of that amount. The residence time for each 5 ml aliquot in the mouth will be about 1.75 seconds, and during this time period about 1% of the malathion will be absorbed through the buccal and sublingual mucous membranes. Additional absorption is possible again through the orbital, nasal, aural, and

inhalation routes. These unaccounted for routes of ingestion, absorption, and inhalation in the swimmer may increase total dosage exposure scenarios by up to 50%.

The most neglected aspect of pesticide toxicity investigation in mammals remains zeroing in on the toxicity of breakdown products of the parent compound. In fact, the toxicity profile of mixtures of parent and breakdown products may be synergistic in nature. Measurements of skin absorption rely on data obtained from single compound solutions. This technique may underestimate absorption in the more common scenario of multiple exposures to solvent mixtures in the contaminated water. Studies show that combinations of compounds have greater effect on the stratum corneum, and are absorbed more readily. In addition, potent impurities such as malaoxon, isomalathion, O,O,S-trimethyl phosphorothioate and diethyl fumarate may contribute synergistically to the toxicity of technical malathion formulations both before and after usual aerial bait application (Aldridge, 1979). For example, a difference of a day or two in bait application on two adjacent areas could result in a condition in which a considerable quantity of the breakdown product along with a substantial quantity of the parent compound could be washed by rainfall into the common water source. In a single body of water, daily pH fluctuations could conceivably be responsible for producing synergistic conditions as well. The rapid production of diethyl fumarate could occur during the period when pH is high, its rate of production being reduced after the active photosynthetic period, leaving enough malathion to produce synergistic toxic effects (Bender, 1969).

These data suggest that the toxic exposure profile of the population swimming in the spray area may be significantly different than the profile measured in the original bait spray tank. As a minimal standard, prior to spray application, analysis should be considered for potent and toxic contaminants (e.g. isomalathion, malaoxon, diethyl fumarate) within the technical malathion formula. In addition, after spray application, similar analysis should be considered for swimming pools within these same designated spray areas. In fact, partially as a result of this type of analysis, California ultimately cut its target aerial spray malathion concentrations from 2.4 to 1.2 ounces per acre, then subsequently abandoned malathion completely in favor of biological controls in 1994.

Chapter Ten

Conclusion

Malathion skin absorption measured among 58% of the human volunteers by the disappearance method allowed for experimental determination of $Kp_{malathion}$=.0005 L/cm2/hr. The dosage in human volunteers from this controlled exposure to a contaminated aquarium solution was several orders of magnitude less than the minimal dose necessary to cause measurable change in red blood cell acetylcholinesterase (RBC-AChE). Following experimental determination of $Kp_{malathion}$, a mathematical model was evaluated for swimmers using dilute aqueous malathion contamination concentrations typically detected after bait application. Extrapolation of $Kp_{malathion}$ into swimming pool exposures (both adults and children, with and without malaoxon) resulted in dosages an order of magnitude below that needed to cause a detectable change in RBC-AChE. These data indicate that exposure to aqueous malathion following usual aerial bait applications is not appreciably absorbed, and therefore, not a public health hazard.

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