LISA YVONNE LEFFERTS. Assessing Risks of Cholinesterase Inhibiting Pesticides. (Under the direction of ALVIS G. TURNER).

Cholinesterase-inhibiting (ChE-I) pesticides, which include both organophosphates (OP's) and carbamates, together constitute a very significant proportion of pesticides used in the U.S. and worldwide. ChE-I pesticides are known to disrupt nervous system functioning in animals and humans, and OP's are implicated in human poisonings more often than any other class of pesticides. The U.S. Environmental Protection Agency (EPA) uses ChE-I to characterize the risks of these pesticides since ChE-I is a sensitive predictor of exposure.

Assessing the risks of OP's and carbamates on the basis of ChE-I involves many uncertainties. The decisions and assumptions made to resolve these uncertainties are science policy decisions, and can have a significant impact on the final characterization of the risk. This report identifies the principal uncertainties throughout each of the four stages of risk assessment (as described by the National Research Council), discusses the nature and public health implications of these uncertainties, suggests an approach for describing and resolving uncertainties, and provides recommendations useful in developing a science policy for ChE-I pesticides. It is concluded that the EPA's use of an uncertainty factor as small as ten is not justified by the available scientific evidence.

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I. Introduction

Cholinesterase-inhibiting pesticides, which include organic phosphorus pesticides (OPs) and carbamates, together constitute over half of the total volume of insecticides used in the United States (Doull, 1980), and a very significant proportion of pesticides used worldwide. In 1976 annual production of OPs exceeded 10^6 Kg. OPs and carbamates have been rapidly replacing the persistent chlorinated hydrocarbon pesticides (e.g. DDT, endrin, kepone, toxaphene), most of which have been banned or severely restricted.

Both OPs and carbamate compounds share an ability to inhibit cholinesterases (ChEs), principally acetylcholinesterase (AChE) in nerve tissue. AChE is an enzyme critically important to central and peripheral nervous system functioning, which hydrolyzes acetylcholine (ACh), a neurotransmitter. OP pesticides had their origins in 1937, when Germany developed extremely potent OP compounds known as nerve gases (e.g., N-dimethyl phosphoramidocyanidate [tabun] and isopropyl methylphosphorofluoridate [sarin] as potential chemical warfare agents. OP insecticides are implicated in more human poisoning than any other class of pesticides (Doull, 1980).

Cholinesterase inhibition (ChE-I) is frequently considered the most sensitive health endpoint; i.e., other effects (e.g., reproductive, ocular) which may be caused by these compounds, generally occur at higher doses. As more sensitive methodologies are developed, neurobehavioral effects may be found to be an even more sensitive indication of exposure to ChE-I pesticides, but at

the present time no generally accepted means of assessing neurobehavioral changes resulting from exposures to toxic substances in human populations exists (Otto and Eckerman, 1985).

As the scientific data base on the risks associated with these compounds has been developed and refined over the years, so have the procedures for assessing and managing risk. The National Research Council's Risk Assessment in the Federal Government: Managing the Process (1983) describes the most recent approach (which EPA has adopted) for conducting and understanding risk assessments (EPA, 1986).

Risk assessment, the largely scientific process of characterizing the potential adverse health effects resulting from human exposure to environmental hazards, is distinguished from risk management, the largely political process which considers scientific factors from the risk assessment as well as economic, legal, technological, administrative and other factors to evaluate and select alternative regulatory actions. In this approach, a risk assessment would usually include the following four stages:

1) Hazard identification: What are the adverse health effects associated with exposures to the chemical under study?
2) Dose-Response Assessment: How is the probability of the occurrence of adverse health effects related to the magnitude and duration of exposure to the chemical?
3) Exposure Assessment: What is the extent of human exposure (past, present and future) to the chemical?

4) Risk Characterization: What are the risks to public health (a function of hazard and exposure) associated with the chemical? What are the uncertainties in assessing this risk?

Uncertainties are present in each of these procedures which require a decision or assumption to be made in order to proceed with the assessment. The points at which these decisions are made are referred to as components. The questions involved in reaching a decision at a component are science policy questions, so called because both science and policy considerations play a role in their resolution:

> The choices encountered in risk assessment rest, to varying degrees, on a mixture of scientific fact and concensus, on informed scientific judgments, and on policy determinations (the appropriate degree of conservatism). (National Research Council, 1983)

More specifically, this type of science policy is known as risk assessment policy, defined by the NRC as "policy related to and subservient to the scientific content of the process, in contrast with policy invoked to guide risk management decisions, which has political, social, and economic determinants."

The lack of a consistent, rational risk assessment policy for dealing with uncertainty in assessing risks of ChE-inhibiting pesticides has resulted in the use of different criteria to define "no observed effect levels" (NOELs) (both by researchers performing dose-response studies and by EPA reviewers of those studies), different uncertainty factors (UFs) being selected by different EPA offices, and conflicing estimates of acceptable daily intakes (ADIs) for the same pesticide. Although an intra-Agency group has decided on which ADI to use in the cases where

conflicting ADIs have been proposed for the same pesticide, there are many components in the risk assessment process which have been inconsistently evaluated for different pesticides. This problem has been recognized by various EPA scientists in regard to ChE-inhibiting pesticides but has not been resolved. (EPA internal memo, 1986).

It should be emphasized that the present state of affairs regarding the inconsistent treatment of uncertainties in the risk assessment of ChE-inhibiting pesticides is <u>not</u> due to either a) a failure to maintain a clear conceptual distinction between assessment of risks and consideration of risk management alternatives, as recommended by the NRC (1983), or b) faulty judgment on the part of Agency scientists. Excellent scientists can reasonably and rationally disagree over the human health implications associated with varying degrees of cholinesterase inhibition due to Ch-I pesticides. However, as the NRC (1983) points out:

> In establishing regulatory priorities, the same inference options should be chosen for all chemicals, because the main point of the analysis is to make useful risk comparisons so that agency resources will be used rationally. (NRC, 1983)

In addition, differential treatment of uncertainties is unfair to industry as well as the public potentially at risk. For example, the judgments and assumptions (all valid) made by a more conservative EPA risk assessor could ultimately lead to greater costs to the industry than if a different, less conservative scientist prepared the assessment (Fisher, 1980).

The objective of this review is to improve the risk assessment process for ChE-I pesticides by identifying and discussing

important components, particularly in the first two stages of the assessment, which can influence the outcome of the process. The results of this review could be a first step towards the development of a consistent and rational risk assessment policy for ChE-I pesticides. The NRC four stage model of the risk assessment procedure will be used to structure the analysis. Most examples were obtained from human and animal data on cholinesterase inhibition by malathion, parathion, and aldicarb. These particular pesticides were chosen because:

(a) they represent three main classes of ChE-I pesticides (phosphorodithioates, phosphorothionates, and carbamates, respectively)

(b) they have high risk potential due to their toxicity and/or extensive use

(c) there is ample health-related data, including studies in humans

(d) they have received public attention.

ChE-I was chosen as the health endpoint in this review since it is an early predictor of exposure and is often selected as the basis for characterizing risks. Data have been obtained from:

a) EPA risk assessment documents (e.g. registration standards, criteria documents)

b) EPA reviews of original studies (e.g., "Caswell file" reviews by scientists from the Office of Pesticide Programs (OPP))

c) open literature.

The major components to be analyzed in this review include the following:

Hazard Identification

- What degree of cholinesterase inhibition (ChE-I) is significant (i.e., regarded as an effect)?

Dose-Response Assessment

- What dose-response assessment methods should be used to extrapolate from experimental doses to exposure doses?
- What uncertainty factor should be employed for inter-species variation of ChE-I between animals and humans?
- What uncertainty factor should be employed for intra-species variation of ChE-I?

Exposure Assessment

- What special considerations should be included in exposure assessments for ChE-I pesticides?

Risk Characterization

- What are the uncertainties in estimating the extent of health effects for these compounds? How should they be estimated and presented to Agency decision makers?

Some of the uncertainties contributing to these components are included in Appendix V-1.

II. Hazard Identification

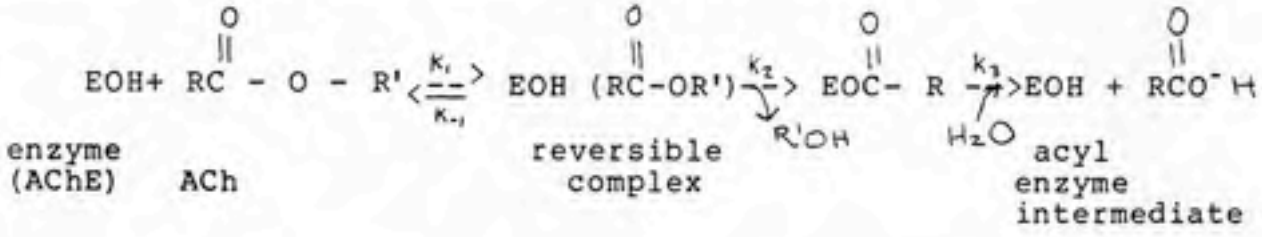
A. Cholinesterase Inhibition

Types of ChE

In mammals there are two types of enzymes which can react with ChE-I pesticides: acetylcholinesterase (AChE) (also called specific, "true" or "e" type cholinesterase, and acetylcholine acetyl hydrolase) and butyrylcholinesterase (BuChE) (also called nonspecific or pseudocholinesterase, "s" type cholinesterase, and acylcholine acyl hydrolase). These can be distinguished by location and substrate specificity. AChE is found in the central nervous system, motor end plates of skeletal muscle, and erythrocytes, whereas BuChE is found in smooth muscle, liver, adipocytes and plasma. BuChE has been found in almost all major body systems including the white matter of the brain, vascular system, respiratory system, digestive system, urogenital system, and also in certain endocrine and exocrine glands. AChE hydrolyzes acetylcholine (ACh) and acetyl beta methylcholine but very little benzylcholine, propionylcholine, or butyrlcholine, which BuChE readily hydrolyzes. BuChE can also hydrolyze ACh, but differs from AChE in that it is inhibited by ACh at concentrations greater than 100 micromoles (mM), whereas AChE is inhibited by ACh concentrations greater than 4 mM (in vitro). ChE-I pesticides differ in their ability to selectively inhibit these enzymes (i.e., some may inhibit only (or mainly) AChE, and others only BuChE).

AChE

Like all esterases, cholinesterases are hydrolases which split ester (specifically choline ester) bonds. AChE plays a key role in normal nervous system functioning by splitting the neurotransmitter acetylcholine (ACh). ACh, stored in synaptic vesicles, is released from the presynaptic cell into the synaptic cleft once an action potential reaches the nerve terminal. It then diffuses across the synaptic cleft and interacts with specific receptor sites in the postsynaptic membrane. This interaction triggers changes in ion conduction which lead to, for example, firing of a second neuron or a muscle contraction. ACh can have an inhibitory effect (e.g., slowing the heart rate) or excitatory effect (e.g., producing a skeletal muscle contraction). ACh interacts with muscarinic receptors (found primarily in smooth muscles, heart, and exocrine glands), nicotinic receptors (at autonomic ganglia and neuromuscular junctions) and receptors in the CNS. AChE is present in the synapse and destroys ACh very soon after it is released:

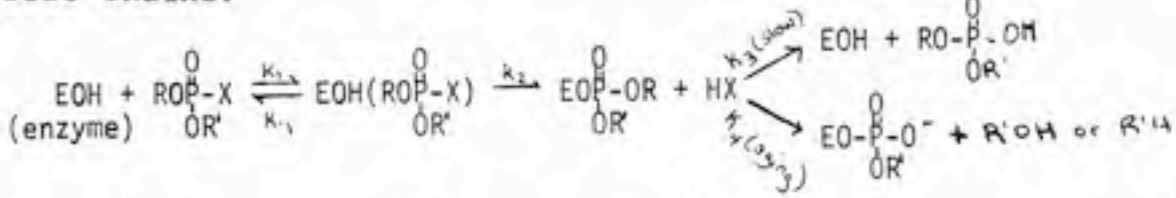


When a ChE-I pesticide is present, it reacts with ChE, preventing ACh from reacting with ChE. The only difference between a ChE-I pesticide (an inhibitor) and ACh (a substrate) in the reaction is the rate of the reaction (principally due to k_3). The acylated enzyme formed from ACh hydrolyzes very quickly,

whereas the phosphorylated or carbamylated enzyme formed from an organophosphate or carbamate hydrolyzes very slowly. This reaction (the restoration of the enzyme from its acylated (inhibited) state) is known as spontaneous reactivation or deacylation, or dephosphorylation in the case of OPs, and decarbamylation in the case of carbamates.

In the case of OPs the k_3 step of dephosphorylation is so slow that these compounds are sometimes called "irreversible" inhibitors of ChE. With carbamates, k_3 is significantly slower than it is for ACh but faster than for OPs, while k_2 is slower than for OPs, so that once the carbamate is removed, the enzyme recovers (due to reversal of the reversible complex and decarbamylation (by k_3)). Hence, carbamates are considered "reversible" inhibitors of ChE. Acute toxicity and death from OPs and carbamates are thus really due to ACh poisoning as ACh accumulates. Symptoms of acute poisoning mimic the muscarinic, nicotinic, and CNS actions of ACh (Table II-1).

Phosphorylated ChE can be reactivated, not easily by water as with acetylated ChE, but by nucleophilic compounds which can displace the phosphate from the enzyme, such as pralidoxine (2-PAM), used as an antidote. This antidote is not effective for OP compounds which undergo "aging," or loss of one of the R-group side chains:



The negative charge on the aged phosphorylated AChE repels 2-PAM so that reactivation cannot occur. Some nerve gases age

rapidly, precluding the effective use of these antidotes. Atropine, another antidote for ChE-I pesticides, works by competitively inhibiting ACh at the receptor.

The role of AChE found in the synapses of nervous tissue is actually much more complicated than the preceding description implies, since AChE exists in a number of different varieties or molecular forms, each of which is thought to serve a different role, and might possibly be regulated separately (Brimijoin, 1983). However, it is typically the AChE found in erythrocytes that is measured to determine OP and carbamate toxicity, since it is impractical to measure synaptic AChE activity. The function of RBC AChE is not as well established as that of synaptic AChE. RBC AChE may protect synaptic AChE by reacting with some of an absorbed dose of AChE-I pesticide before it reaches the synapses, although this would vary between different ChE-I pesticides. Wills (1972) maintains that "there is no good evidence that that enzyme [RBC AChE] does anything more than control to a certain extent the permeability of the erythrocyte." Non-synaptic AChE is thought by some to maintain excitability and to initiate and propagate action potentials in nerve and muscle through regulation of electrolyte transport (Namba, 1971), although this has been disputed (Herz and Kaplan, 1973). From observations that RBC AChE levels increase after blood loss and are altered in several different types of anemia, it was inferred that RBC AChE activity is related to cell age, high levels being associated with rapid production of erythrocytes (Herz and Kaplan, 1973).

This is a potential source of variability in using measurements of AChE as indicators of exposure to ChE-I pesticides.

Not only are AChE levels high during the formation of RBCs, but Drews (1975) observed elevated ChE levels in the blastemal cells of developing organs in several species. Once the organ structure became established, ChE activity disappeared. ChE activity was always found in cells which, in the course of organ formation, moved actively. Drews hypothesized that ACh is involved in the short range regulation of movements in developing organs. Specificity tests were run on the embryonic ChE to determine its type, which was discovered to be AChE in the amphibian and chick embryo, as well as in human carcinoma, and nonspecific or BuChE in the rat embryo and juvenile rat mammary gland and uterine epithelium. Because ChE plays a role in embryonic development which is different from its known function in the adult, it is called "embryonic ChE." The presence of embryonic ChE may partially explain why the fetus is more sensitive to ChE-I pesticides than the adult. Differences in sensitivity due to age are discussed later in this section.

BuChE

Although BuChE has been detected in almost all major mammalian body systems, its biological function has not been established, and its natural substrates and inducers are unknown. It is faily well established that it is produced in the liver. Several hypotheses as to its function have been suggested (Kutty, 1980):

a) May be a precursor of AChE.

b) May be involved in myelin structure (BuChE has been found in Schwann cells of nerves and between folds of myelin in some central axons).

c) May control choline levels in plasma (used to synthesize ACh).

d) May act as a backup for AChE to destroy circulating ACh.

e) May be involved in structure and/or synthesis of beta-lipoproteins (is found complexed with beta-lipoprotein).

f) May removes toxic esters formed by fatty acid metabolism.

g) May be involved in assimilation of food (serum BuChE activity decreases after fasting, parallels level of food intake in undernourished children, and is elevated in obese and diabetic patients, and persons with hyperlipoproteinemia (abnormal lipid metabolism)).

Thus, although BuChE and AChE share many similarities (mechanism of action, molecular shape, etc.) it is not known how, if at all, they are related physiologically [Edwards and Brimijoin, 1982].

Hazard Identification Issues Re:ChE-I

Although there are still many questions to be answered, it should be remembered that AChE has been characterized as "one of the best studied of all enzymes" [Brimijoin, 1983]. Despite uncertainties regarding the roles of AChE in erythrocytes and

BuChE in plasma, substantial inhibition of these enzymes is associated with overt adverse effects in the whole organism following administration of OPs and carbamates and is currently used as a measure of toxicity as well as a measure of exposure to these pesticides (Table II-2).

If we assume ChE-I in RBCs and plasma to represent an adverse effect, the following questions should be considered:

a) How do AChE and BuChE levels respond to prolonged exposures to ChE-I pesticides (i.e., how does ChE-I relate to length of study?) What is the significance of such prolonged exposures?

b) What is the effect of age, sex, diet, race, health and other genetic and environmental factors on ChE levels? How does this affect susceptibitily to ChE-I pesticides?

c) How accurate and reliable are available methodologies for measuring ChE-I? How do they differ?

Each of these issues will be considered in turn.

Chronic Exposure to ChE-I Pesticides

The preceding description of ChE-I reflects the immediate biochemical events following exposure to a ChE-I pesticide. What happens when the exposure is continuous, for months or years?

It is known that animals repeatedly exposed to certain ChE-I pesticides develop tolerance to the (acute) toxicity of these pesticides (i.e., signs of acute poisoning disappear with continued administration). According to Chambers and Yarbrough (1982) this is due in some cases (e.g. disulfoton) to a decreased

number of muscarinic receptors in target tissues that are capable of binding the ACh which accumulates when AChE is being inhibited, and in some other cases (e.g. propoxur) due to enhanced enzymatic detoxication.

In spite of this, ChE levels may remain inhibited or decrease even more in long-term exposures. Figures III-1 - III-4 show the inhibition of ChE's in plasma and erythrocytes by parathion in dogs over two, four, and twelve months. In this experiment the percentage decrease (from control values) is the greatest at 12 months at every dose (except for RBC ChE of female dogs). In other words ChE was inhibited more after one year than after two or four months. Table II-3 compares ChE-I NOELs for the same species, pesticide, route of administration, and site of ChE (i.e., plasma or red blood cell (RBC) but differing study lengths, for seven pesticides (see Appendix III-3). In the majority of cases the NOELs are lower in longer (chronic) studies than in shorter (subchronic) studies. Possible differences in study designs (e.g., method used to determine ChE-I, comparisons to unexposed animals vs. pre-exposed animals, strain) and the small number of comparisons made (16) prevents any definitive conclusion, but the results suggest that ChE-I NOELs vary by duration of exposures such that chronic ChE-I NOELs are lower than subchronic NOELs. According to Bartholomew et al. (1985), a decrease (or absence) of acute toxic effects occurs despite continued inhibition of brain ChE activity and elevation of ACh concentrations.

Wills (1972) maintains that "In general, prolonged or repeated exposures to inhibitors of cholinesterases have emphasized the unreliability of estimations of the activities of plasma, or serum, and red blood cells for judging the severity of intoxication by these inhibitors." Levels of ChE-I fluctuate over the course of chronic and subchronic exposure. For example, Wills cites an example in which pigs fed 1.7 mg/kg/day of parathion had increased levels of plasma ChE at first, and then decreased levels until maximum inhibition (43%) occurred on about the 50th day. RBC AChE levels remained unaffected for 8 days of dosing, and then fell gradually until maximum inhibition (86%) occurred, also on day 50. Studies in which ChE activity is infrequently measured may not be able to determine maximum inhibition. In addition, there is some indication that the rate of ChE-I may affect toxicity (Wills, 1972). Jensen (1965) found that the lethal dose of paraoxon in guinea pigs increases at an increased rate of infusion.

Although tolerance appears to develop to the acute toxicity associated with ChE-I, what are the chronic toxic effects associated with ChE-I? (Recall that both acute and chronic effects may result from either acute or chronic exposures). Investigations to date have yielded conflicting claims regarding the existence of chronic neurobehavioral effects (Duffy et al., 1979; Ecobichon and Joy, 1982; Karczmar, 1984; Levin, 1976; Miller, 1982; National Research Council, 1985, Savage et al., 1982). These and other chronic effects (carcinogenic, nephrotic) linked to some ChE-I pesticides or their metabolites will not be considered here.

Intraspecies Variation of ChE

Not only is ChE activity affected by exposure to ChE-I pesticides, but it also varies among species (see section IIID), and is affected by age, sex, diet, genetic status, race, pregnancy, obesity, season, liver disease, myocardial infarction, and other health and environmental factors. Plasma ChE levels fluctuate more than RBC AChE levels.

o Genetic Factors

A small sub-group of the population (estimated by Williams (1985) to be 4.5%) possess genetically determined atypical plasma ChE. Atypical ChE was first discovered when the muscle relaxant succinylcholine, used in anaesthesia, was found to produce an unusually long period of paralysis and apnea (temporary suspension of breathing) in some patients. These individuals were found to have atypical or low serum ChE, which is responsible for the hydrolysis of succinylcholine, thus ending the drug's effects. Genetic studies since then have shown that most people with atypical BuChE are homozygous for a recessive gene (designated E_1^a E_1^a). The gene allele E_1^a directs the synthesis of a ChE which is unable to hydrolyze succinylcholine at pharmacological doses and which is also less sensitive to certain ChE-I's (e.g., dibucaine). The latter property is used to classify serum ChEs, by use of the dibucaine number (DN), a measure of the degree of inhibition (expressed as a percentage) of serum cholinesterase obtained with dibucaine under standardized conditions (Kalow, 1957). Whereas most people (with normal genotype E_1^u E_1^u) have a DN between 76 and 81, those who are heterozygotes (E_1^u E_1^a) have a DN between 55 and 69, have about seventy-eight

percent as much ChE as normal, and may display a mild increase in sensitivity to succinylcholine, and those who are homozygous for the recessive gene (E_1^a E_1^a) have a DN below 21 and have low BuChE activity (about twenty-five percent of normal) and sensitivity to succinylcholine [Ashby et al. (1970), Udsin (1970)].

Other genetic variants of plasma ChE have been identified, including a fluoride-resistant form (directed by E_1^f allele), a completely ineffective or absent "form" (directed by the "silent" allele E_1^s), and the J and K phenotypes (associated with reductions in measured enzyme activity of ca. 66% and 33%, respectively). The J and K phenotypes can only be identified with certainty when they occur with the atypical variant (i.e., E_1^a E_1^j and E_1^a E_1^k have distinctive inhibitor numbers). A variety of combinations are possible (e.g., E_1^u E_1^f, E_1^a E_1^f, E_1u E_1^s, E_1^f E_1^s, E_1^a E_1^s, E_1^j E_1^k, E_1^f E_1^f, E_1^s E_1^s, E_1^u E_1^k) (Evans and Wardell, 1984).

Genetic variants with an atypical number of electrophoretically-detectable isoenzymes have also been detected (Silver, 1974). Although most individuals possess bands C_1, C_2, C_3 and C_4, a fifth band (C_5) was identified in fourteen of three hundred randomly selected adults in England, and has been detected in other populations as well (e.g., Brazilian). Three additional bands are present in serum from certain Africans. The level of serum ChE is about 30% higher in subjects with C_5 than in C_5-negative subjects. (Silver, 1974).

Apart from increased sensitivity to succinylcholine, there are no reports of clinical abnormalities in people with rare genetic variants of serum ChE. Silver (1974) reports that in one

instance where other tissues (including brain) were investigated in a person with atypical serum enzyme, the tissue enzyme was similarly atypical. It is not apparent whether these people would be more susceptible to ChE-I pesticides, since their serum ChE is unable to hydrolyze accumulating cholines; less susceptible, since their serum ChE is less sensitive to certain ChE-I's (e.g. dibucaine, fluoride); or equally susceptible. Calabrese (1978) maintains that individuals with such pseudocholinesterase variants should be considered potentially at high risk to ChE-I pesticides. Calabrese also notes that the dibucaine variant has been found to be extremely sensitive to RO2-0683, and cautions that this is of "particular significance in light of the widespread use of carbamate insecticides." However, the OP's TEPP and DEP isofluorophosphate do not inhibit differentially among pseudocholinesterase variants and would not cause a higher risk to those individuals with atypical variants.

Another indication that genetic factors may affect susceptibility to ChE-I pesticides is provided through selective breeding experiments in animals by Overstreet et al. (1979). Male rats determined to be most resistant and most sensitive to DFP on the basis of drinking behavior, body weight, and core body temperature were bred with the most resistant and sensitive (respectively) female rats in an attempt to establish resistant and sensitive lineages. Although the former attempt failed, Overstreet et al. were successful in establishing a sensitive line of rats. However, the genetic differences in sensitivity were not found to be related to differences in brain or erythro-

cyte AChE or serum BuChE activity. The authors speculate that the genetic differences could be due to changes in sensitivity of AChE isoenzyme, changes in ACh synthesis or turnover, or changes in sensitivity of postsynaptic receptors for ACh. Subsequent studies (Overstreet et al., 1984) have shown that the latter two factors do indeed contribute to the enhanced sensitivity observed. Regardless, the data suggests that ChE-I may not always be an adequate measure of toxicity in genetically-susceptible individuals.

Besides genetic differences in ChEs, there is another esterase that is affected by organophosphates and carbamates, known as arylesterase, for which genetic variants exist. Arylesterases have not been as well studied as other esterases. Paraoxonase, an enzyme hydrolyzing paraoxon, the active metabolite of parathion, is an arylesterase which has been found to be polymorphically distributed in several populations (LaDu and Eckerson, 1984). Two alleles determine paraoxonase activity: A, a low activity allele, and B, a high activity allele. Heterozygotes (AB) also exhibit high activity. About one-half of the U.S. caucasian population is homozygous for the low activity allele (AA), which is speculated to place these individuals at higher risk of parathion poisoning than those with higher levels (Ortigoza-Ferado et al., 1984). Non-caucasian populations of African, Oriental, or American Indian subjects, for example, do not show the same distribution, but it is not known whether this is due to the presence of additional alleles or quite different gene frequencies (LaDu and Eckerson, 1984). Ortigoza-Ferado et al. state "It may be postulated that such differential suscepti-

bility would be particularly significant at low or intermediate levels of exposure to parathion since with marked exposure even high levels of paraoxonase would not be sufficient for protection against toxicity."

It appears, then, that both ChE and arylesterases (e.g., paraoxonase) affect the toxicity of ChE-I pesticides. A model describing the interaction of paraoxon with serum ChE and paraoxonase was developed by LaDu and Eckerson (1984). The level of paraoxonase was found to influence the degree of serum ChE-I in vitro. The authors recommend that the in vitro model system be applied to estimate what is likely to occur in vivo, and that epidemiological studies be undertaken to determine whether individual response to ChE-I pesticides shows the expected relationship to the type and level of paraoxonase.

o Sex Differences

Sex related differences in susceptibility to ChE-I pesticides are most likely to occur for those compounds which require metabolic activation to produce ChE-I (e.g., parathion). (Doull, 1980). Agarwal et al. (1982) found that the percentage of ChE-I by parathion was 2.6, 1.2, and 2.7 times greater in female than male rats measured in plasma, erythrocytes, and brain, respectively, following a single oral dose. Paraoxon treatment, however, resulted in comparable inhibition of plasma, erythrocyte and brain ChE in both sexes. Castration increased the susceptibility of male rats to a similar level as females. Pre-treatment with testosterone enabled these castrated males to recover from this increased sensitivity, whereas estradiol enhanced their

sensitivity slightly. Gonadectomy had little effect on ChE levels in the females. Pre-treatment with testosterone decreased the sensitivity of ovariectomized females. The authors concluded that testosterone plays an important role in determining parathion toxicity.

While it is well established that the female rat is more susceptible to the acute toxic effects of parathion (e.g., LD_{50} and ChE-I) than male rats, this sex difference is not as obvious for chronic or subchronic ChE-I. For example, in a 2 year feeding study in rats prepared by Daly (1984) for Monsanto (in Ghali, 1985), no consistent pattern is observed.

Sex differences exist in absorption, distribution, and excretion of ChE-I pesticides also. For example, Khaak et al. (1984) found that less parathion was lost by evaporation from the skin of male than female rats, males having a larger percentage of the dose in their carcasses. Similar amounts in both sexes were excreted in the urine and feces. Although the amount absorbed from the skin was about the same over a 120 hour period in male and female rats, males absorbed parathion from the skin much more rapidly. Females absorbed more in heart and liver tissue than males.

The plasma and red blood cells of human males have higher ChE activities than human females (Wills, 1972). Serum ChE is significantly decreased in women using oral contraceptive pills (Robertson, 1967). How this affects susceptibility in humans is not clear, although in the rat, the female is more susceptible and has higher ChE activities than the male.

- Age

Kacew and Reasor (1984) report that "it is clear that neonates are more susceptible than adults to AChE inhibitors." Although the hepatic cytochrome P450 system which catalyzes some OPs to their active metabolites (e.g., parathion to paraoxon) is poorly developed in neonates, neonates are still more sensitive, apparently due to differences in detoxification, excretion, or redistribution. For example, the level of the enzyme that degrades malaoxon (carboxylic ester hydrolase) is less than the level of the activating enzyme for the first thirty days of life of the rat. By thirty days of age malaoxon inactivation is equal to its rate of production (Kacew and Reasor, 1984).

Besides such differences in detoxification ability, the specific activity of brain AChE increases from a minimum in the one day old rat, either due to an increase in the amount of AChE or its catalytic activity (Kacew and Reasor, 1984).

In the human, adult levels of AChE are not reached until three to five months of age. Even though blood from neonates has a higher proportion of young cells (which have higher activity than more mature cells) than adults, AChE activity in newborn circulating erythrocytes is less (Herz and Kaplan, 1973). Thus, for a given concentration of ChE-I pesticide, more AChE is expected to be inhibited in newborns compared to adults (Kacew and Reasor, 1984). This has been found to be the case with beef cattle given ChE-I pesticides to control parasites, according to Kacew and Reasor. Chlorpyrifos is thirty-fold more toxic to calves than adult cattle. Brodeur and Dubois (1963) compared the LD_{50}'s of sixteen ChE-I pesticides given intraperitoneally for

twenty-three day old weanling and adult rats. The acute toxicity was from one to five times greater for weanlings than adults, except for OMPA, for which adults were five fold more sensitive. On average, weanlings were twice as sensitive as adults. Mendoza and Shields (1977) compared the LD_{50}'s of rat pups treated with malathion (99.3%) by gastric intubation and found that one-day old pups were three times more sensitive than six day old pups and nine times more sensitive than eighteen day old pups. Similarly, the I_{50} (concentration of malathion required to inhibit ChE by fifty percent at specified conditions) of one day old pups measured for brain AChE was one-third, one-fourth, and one-eighth the amount in six day, twelve day, and eighteen day old pups, respectively. Lu, Jessup and LaVallee (1965) compared oral LD_{50}'s for malathion (99.6%) in rats of different ages and observed that newborns were twenty-eight times more sensitve than adults and seven times more sensitive than pre-weaning rats. They also observed that dividing the dose over four days reduced the toxicity in adults but increased the toxicity in pre-weaning rats.

Parathion, malathion and aldicarb all pass through the placenta and are toxic to the fetus. Malathion and parathion have been shown to have teratogenic effects [Calabrese (1978), Fish (1966), Hoffman and Eastin (1981), Wyttenbach and Thompson (1985), etc.]. Cambon et al. (1979a) found that AChE in the brain and blood (but not liver) of rat fetuses was consistently more inhibited than that of the dams treated by gastric intubation with aldicarb on the eighteenth day of gestation. Carbofuran consistently inhibited brain AChE more in the dam than in

the fetus, while for pirimicarb, no consistent pattern was observed. Cambon et al. (1976b) hypothesize that the reason for the observed differences in sensitivity are due to differences in fixation of carbamate derivatives on the fetal versus maternal isoenzymes.

The sensitivity of the elderly to ChE-I pesticides is less clear than for the very young and unborn. Rider et al. (1957) found that plasma ChE showed a small but definite increase with age in both sexes. Other investigators [Calloway et al (1951), Gage (1969)] did not find age to be a factor influencing the magnitude of variability of ChE in adults. Ando et al. (1984) found that serum ChE activity increased according to age in females, while it decreased slightly according to age in males. The ChE activity was higher in males than in females under fifty years of age, whereas the reverse was found in persons over fifty-five years of age. They also detected seasonal variation (higher activity in winter than summer) in females but not males. There does not appear to be any conclusive evidence indicating that adults of any specific age may be more sensitive to ChE-I pesticides.

o Nutrition

Nutritional deficiencies have been found in some cases to increase the susceptibility of test animals to ChE-I pesticides. Parathion, malathion and banol all produced greater ChE-I in rats on low protein diets than on high protein diets (Casterline et al. (1969a, b, 1971 a, b), Vaishwanar and Mallik (1984)). Parathion-induced serum ChE-I was more dependent on dietary protein levels for subchronically-exposed rats (28 days) than for acutely exposed animals (single dose) (Casterline and Williams, 1971). Behavioral changes were noted more often in rats on low protein diets exposed to parathion or banol than in unexposed rats on low protein diets or exposed rats on high protein diets (Casterline, Brodie and Sobotka, 1971). These behavioral changes consisted of a higher proportion of "No escape" rats (i.e., rats failing to press a lever to either avoid or escape a negative stimulus [electric shock] after training in a standard operant conditioning chamber). None of the diet-pesticide groups tested were associated with significant changes in avoidance only behavior (lever pressing during conditioned stimuli [light and sound] preceding the unconditioned stimulus [shock from electric grids]). In this experiment, although behavioral changes were noted, the activites of ChE (and monoamine oxidase (MAO)) in the cerebellum and cerebrum were not significantly affected by the low casein diet and/or the presence of a ChE-I pesticide. Since brain ChE can not be assayed until the end of the experiment (at 9 weeks in the parathion experiment and 10 weeks in the banol experiment), it is possible that inhibition may have occurred earlier in the experiment, preceding adaptation to chronic

exposure. In fact, brain ChE-I was noted in a similar 28 day experiment by the same investigator (Casterline and Williams, 1971). It is noteworthy that behavioral effects can be observed following subchronic exposure to a ChE-I pesticide even when brain AChE levels are not inhibited. Subchronic exposure to parathion was found to decrease serum and liver triacetinesterase (AliE) activities in protein-deprived animals as well as ChE activities, thus reducing the detoxification ability in those animals and making them highly susceptible to poisoning, even at low doses (Casterline and Williams, 1971).

In addition to protein (casein), varying dietary levels of calcium (Ca) and magnesium (Mg) also affected ChE-I by parathion and banol (Casterline and Williams, 1969a). Both high and low levels of these cations decreased serum and brain ChE-I by parathion and banol. Liver ChE-I was also decreased by parathion and banol, except by low magnesium which increased ChE-I by parathion. Serum and brain AliE by parathion and banol was unaffected or decreased by altered cation levels, while liver AliE-I by parathion and banol was significantly increased by high Mg or high Ca in the diet. These results are difficult to interpret, since they imply that low or high dietary concentrations of Mg or Ca might decrease susceptibility to parathion and banol since ChE-I by these compounds is reduced. However, the changes in ChE and AliE that occurred after pesticide administration did not influence the lethal action of the pesticides, except with the casein-free diet, where the mortality was increased.

Animals on food restricted diets, or deprived of water, were also shown to be more susceptible to subchronic (via diet) and acute exposures (via intraperitoneal injection) of parathion and paraoxon (Baetjer, 1983 and Villeneuve et al., 1978). Food restriction had a significantly greater effect than water deprivation on blood ChE-I by parathion, but not paraoxon.

In the subchronic study, food restriction increased plasma ChE-I elicited by parathion, but brain ChE was not inhibited by the doses of parathion used, either alone or with food restriction. Increased inhibition of plasma ChE in animals subjected to food restriction was not observed at the NOEL for the study.

o Pregnancy

During routine blood ChE monitoring at a pesticide industry, it was observed that a marked fall in plasma ChE occurred in pregnant women in their first trimester who had not been exposed to ChE-I pesticides (Howard et al., 1978). A more extensive survey by Evans and Wroe (1980) on 941 pregnant women distributed evenly throughout the 40 weeks of gestation revealed that a rapid fall occurred in the first trimester to a level which did not alter significantly during the remainder of pregnancy. Even lower values were observed in the 105 patients examined during the week following delivery. Three of the women surveyed possessed abnormal genotypes (i.e., E_1^a E_1^a, E_1^s E_1^s, and E_1^f E_1^f) and were excluded from the study. Nevertheless, some women were determined to be at risk to succinylcholine on the basis of ChE activity between 1.68 and 2 units/ml. The numbers of pregnant women at risk for each time period investigated

closely paralleled the changes in mean enzyme activity over time, with the highest risk of apnea and prolonged effects of succinylcholine occurring immediately after delivery.

How do low levels of plasma ChE occurring during pregnancy affect susceptibility to ChE-I pesticides? Recall that it was unclear whether individuals possessing non-normal genotypes and low levels of plasma ChE would be more susceptible to ChE-I pesticides due to the decreased ability of plasma ChE to hydrolyze cholines, or less susceptible since their plasma ChE was found to be less sensitive to fluoride, dibucaine, and some other ChE-I compounds. Weitman et al. (1983) found pregnant mice to be more susceptible to single doses of parathion and paraoxon than virgin female controls. In pregnant mice, signs of cholinergic stimulation (tremor, weakness, lacrimation, salivation) were more intense, brain and plasma ChE activites were lower, blood and brain concentrations of parathion and paraoxon were higher, and serum paraoxonase activities were lower, compared to controls. Whether pregnancy-induced alterations of hepatic function, ChE activity, serum protein binding, serum esterases or a combination of these are responsible for the enhanced susceptibility is unclear.

o Miscellaneous Health and Environmental Factors

The susceptibility to ChE-I pesticides can be affected by exposures to physical factors (e.g., cold), biological agents (e.g., viruses), and other toxic substances (e.g., pesticides, drugs).

Not only can the toxicity of ChE-I pesticides be altered in individuals with hypo- or hyperthermia, but ChE-I pesticides may

have hypothermic effects. Doull (1980) reports that hyperthermia increases the toxicity of parathion, while Chattopadhyay (1982) found that half the LD_{50} dose of parathion was lethal under cold temperature.

The percent whole blood ChE-I by DDVP was significantly less in cold-exposed rats than in rats at room temperature, but by parathion was significantly more at one-half the LD_{50} dose and unaltered at one-quarter of the LD_{50} dose. Chattopadhyay (1982) also noted hypothermic efects of OPs in rats under cold exposure. Body temperature decreased as the dose of OP increased, and the higher the ChE-I the lower was the body temperature of the animals under cold temperature.

Whole-body radiation produces a dose-dependent decrease in BuCh activity of the ilia in rats and mice, but there is no significant change in the acute toxicity of ChE-I pesticides in animals given lethal exposures of whole-body ionizing radiation (Doull, 1980).

Doses of parathion ordinarily considered sublethal were lethal to mice infected with the virus MCMV (murine cytomegalovirus), apparently due to a decrease in the ability of infected mice to detoxify parathion (Selgrade et al., 1984). MCMV is well established as a model for human cytomegalovirus, a ubiquitous herpes virus which infects a large portion of the population and remains with the host in a latent state for a lifetime.

The effects of human cytomegalovirus are usually subclinical or indistinct, but infections can be manifested congenitally, perinatally, in individuals who are immunosuppressed, and in some

other instances. Serum ChE was inhibited more by infected mice compared to uninfected mice.

Synergistic interactions among ChE-I pesticides are known to occur. Most of the studies of synergism among these compounds have tested for acute toxicity. According to Doull (1980), combinations of several OP pesticides fed at "recommended tolerance levels" failed to produce significant synergistic toxicity in chronic feeding studies.

Malathion is potentiated by many OPs since it is detoxified by carboxylesterases that are inhibited by other OPs. Potentiation of malathion is known to vary across species. For example, TOTP (triorthotolyl phosphate) given at a dose which alone did not significantly affect brain AChE potentiated the anticholinesterase action of malathion by 29-fold in mice, 17-fold in quail, 100-fold in frog, 11-fold in sunfish and 12-fold in bullheads (Cohen and Murphy, 1970). Isomalathion and other impurities in technical malathion potentiate malathion and are believed responsible for an epidemic poisoning in Pakistan in 1976 during a malaria eradication program (Aldridge et al., 1979). Potentiation of malathion by these impurities is significantly less in the mouse compared with the rat (Umetsu et al., 1977).

Potentiation can also occur between drugs and ChE-I pesticides. For example, chlorpromazine, a tranquilizer, has been shown to potentiate dichlorvos toxicity in rats, producing about twice as much ChE-I as dichlorvos alone. Drugs that deplete glutathione (e.g., acetaminophen) may potentiate the toxicity of some OPs which are detoxified by glutathione transferases (Marquis, 1986).

In contrast, organochlorine (OC) insecticides protect against the acute toxicity of several OP insecticides. This is probably because OC's stimulate detoxification of OPs by liver microsomes and increase noncatalytic binding sites for OPs (Doull, 1980). Lead was observed not to potentiate parathion toxicity (Phillips et al., 1973). However, both lead and cadmium decreased AChE in rat brain following chronic exposure in the animals' drinking water (Marquis, 1986). Marquis (1986) summarized her review of heavy metal interaction with pesticides: "Clearly, the CNS is rendered more susceptible to the hazards of ChE inhibition in animals chronically intoxicated by heavy metals."

ChE levels are known to vary with certain disease conditions (Silver, 1974). A decrease in serum ChE levels has been associated with some forms of anemia, liver disease, carcinoma, epilepsy, eczema, rheumatic fever, typhus, tetanus, kwashiorkor, and tuberculosis, while an increase in serum ChE levels has been associated with diabetes, asthma, obesity, kidney disease, hyperthyroidism and hyperlipoproteinemia (abnormal lipid metabolism). Patients with mental abnormalities, including psychopathic patients, had raised levels of serum ChE more often than control subjects. A decrease in the ChE's of both serum and erythrocytes has been noted in cases of renal ischaemia. Spastic children have higher activities towards ACh in serum than either mongoloid or moronic children, who have activities within normal limits. ChE levels in erythrocytes are below normal in schizophrenics. According to a study reported by Silver, the stress associated

with sitting university examinations can cause an increase in ChE levels in whole blood from healthy students. Alcohol and nicotine have been shown to depress brain AChE levels. It is not clear whether or to what extent individuals with these diverse conditions may have altered susceptibility to ChE-I pesticides. Individuals with low ChE activities may be at high risk.

Methodology

Several reviews of the methods for determining ChE activity are available [Silver (1974), Wills (1972), Augustinsson (1971)]. These methods vary in their accuracy, complexity, efficiency, and units measured. The conditions to which the enzyme is subjected differ according to the method used, and thus the results obtained by different methods are not directly comparable (Silver, 1974). Temperature, pH, substrate, buffer, and contaminants (e.g. salts, detergents) can affect the measurement of ChE activity. Activities may be reported as the change in pH in a weak buffer solution due to the release of acetic acid from ACh (e.g., Michel method); the amount of CO_2 evolved from bicarbonate by acetic acid released from ACh (e.g. Warburg manometric method); the volume of sodium hydroxide needed to hold the pH of the reaction mixture constant (e.g. Hall and Lucas continuous titration or pH stat method); or the amount of substrate (e.g., ACh,. BzCh (benzoylcholine), BuCh) hydrolyzed. Reporting ChE activity in terms of micromoles of substrate hydrolyzed per ml per minute makes comparisons more valuable (Cornish, 1971). Analysis of ChE following inhibition by carbamates requires special methods,

since the complexes formed with ChEs by all but a few of the carbamates are readily dissociable by dilution (Wills, 1972).

As previously mentioned, it is important that ChE activity be assayed frequently to detect peak inhibition during chronic studies of ChE-I by pesticides. Different pesticides reach peak ChE-I at different times in different species. Also, a lack of ChE-I should not be considered as a true absence of effect when ChE is measured at only one site (e.g., plasma). For example Cornish (1971) cites a study with guthion in which brain ChE activity was inhibited by 60% while serum ChE was unaffected. Measuring only serum ChE would have resulted in a serious underestimation of the risk posed by guthion. Brain and blood contain both AChE and BuChE, so it is recommended that activities be determined separately. Reporting activities in terms of percent of mean control activity, while permitting comparisons between levels, does not provide information on the pre-exposure condition of the animal nor the variability involved. Even in studies using laboratory animals, where one would expect little variation, considerable variation may be present (e.g., see figures III 1-4). Gage (1967) has pointed out the limitations of comparing individual ChE activities to a population average since the coefficients of variation in control studies range from 10 to 25 percent. A much better control is a series of pre-exposure levels on the individual or animal to be studied. Reporting the variability encountered in a study provides more flexibility in the choice of dose-response assessment methods (see Section III of this report).

Table II-1: Effects of ChE-I Pesticides Linked to ACh Accumulation at Various Receptor Sites

Receptor		Effect
1.	Muscarinic (smooth muscles, heart, exocrine glands)	a) bronchoconstriction and increased bronchial secretions, resulting in wheezing and chest tightness.
		b) increased salivation, lacrimation, and sweating
		c) increased GI tone and peristalsis, resulting in nausea, vomiting, abdominal cramps, diarrhea, tenesmus, and involuntary defecation
		d) bradycardia
		e) smooth muscle contraction in bladder, resulting in involuntary urination
		f) contraction of pupils
2.	Nicotinic neuromuscular junction	a) easy fatigue, weakness, involuntary twitching, cramps, fasiculation, and respiratory muscular weakness leading to dyspnea and cyanosis
	autonomic ganglia	b) pallor, elevated blood pressure, hyperglycemia, and other effects which can mask

Receptor	Effect
	muscarinic effects (e.g., tachycardia)
3. Central nervous system	a) behavioral effects: tension, anxiety, restlessness, insomnia, headache, emotional instability and neurosis, excessive dreaming and nightmares, apathy, confusion
	b) neurological effects: slurred speech, tremor, generalized weakness, ataxia, convulsions, depression of respiratory and circulatory centers, coma

[Adapted from Doull, 1980]

Table II-2: Incidence of Symptoms from Poisoning by ChE-I Pesticides Associated with ChE-I in Red Blood Cells *

Incidence of Symptoms Strongly Correlated ($r \geq 0.90$) with Amount of ChE-I	Incidence of Symptoms Associated with ChE-I, but not Strongly Correlated with Amount of ChE-I
Eye	Nose
Miosis	Rhinorrhea
Lacrimation, Pain	
Dimness of Vision	Respiratory
Impaired Accomodation	Constriction of Chest
Pain on Accomodation	Cough
Injection Conjunctiva	Wheezing and Rales
	Dyspnea
Central Nervous System	
Dreams, Poor Sleep	Central Nervous System
Increased Perspiration	Headache (r=.89)
Dizziness	Fatigability
Paresthesia and Cold	Nervous and Irritable, Mood Changes (r=.82)
Gastrointestinal	Tremor and Twitch, Fasiculation (r=.74)
Anorexia and Nausea	

* Derived from 449 cases of anticholinesterase poisoning. Amount of RBC ChE-I determined using an average of pre-exposure values as the control (adapted from Holmes and Gaon (1956).

Table II-3: ChE-I NOEL (mg/kg/d) and Length of Feeding Study

Pesticide, Species	Length of Study						Trend
	33-34 d.	56 d.	90 d.	6 mo.	1 yr.	2 yr.	
Parathion, rat (SD)							
plasma			<.125			.025	(↓)?
Parathion, dog (beagle)							
plasma			<.3		<.01		(↓)?
RBC			.3(male)		<.01		↓
Malathion, rat							
plasma	50					5.0	↓
RBC	5					5.0	=
Chlorthiophos, rat							
plasma			.025			.02	↓(=)
RBC			.1			.08	↓
Chlorthiophos, dog (beagle)							
plasma			<.025		.05		↑
RBC			.025		.025		=
Ethion, dog							
plasma			.025			.15	↑
Chlorpyrifos, rat							
plasma			<.3	.15		.1	↓(?)
RBC			<.3	.15		.1	↓(?)
Dimethoate, rat							
plasma	.7[b]		.16			.05	↓
RBC	.7[b]		.16			.05	↓
Aldicarb sulfone, rat							
plasma		2.4	1.2	.6			↓
RBC		2.4	1.2	.6			↓

SD: Sprague-Dawley strain

* Studies from sample, described in Appendix III-3

[a] Trend as increase study length: ↓ indicates NOEL decreases as study lengh increases; ↑ indicates NOEL increases as study length increases; = indicates no change

[b] Dosing by intraperitoneal injection

III. Dose-Response Assessment

A. Approaches to Dose-Response Assessment

There are four main appproaches to using experimental dose-response data to obtain estimates of a response at low, policy-relevant doses:

1) the NOEL uncertainty factor approach
2) the "benchmark dose" uncertainty factor approach proposed by Crump (1984))
3) mathematical modeling of the entire dose-response curve
4) linear extrapolation.

NOEL-Uncertainty Factor Approach

In this approach, an acceptable daily intake (ADI) is estimated by

$$ADI = \frac{NOEL}{\text{uncertainty factor}}$$

where the NOEL is the "no observed effect level," defined by EPA as

> The level (quantity) of a substance administered to a group of experimental animals which demonstrates the absence of adverse effects observed or measured at higher dose levels. The NOEL produces no biologically significant difference between the group of chemically exposed animals and an unexposed control group of animals maintained under identical conditions. [U.S. EPA, 1985] (Emphasis added from earlier version.*)

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* Underlined terms were omitted in the definition written the decade before by three OPP scientists in "United States Pesticide Tolerance System" as reported in U.S. House of Representatives (1983) Regulation of Pesticides: Appendix to hearings before the Subcommittee on Department Operations, Research and Foreign Agriculture of the Committee on Agriculture, 98th Congress First Session (serial No. 98-22 vol. III).

An ADI is defined as the amount of toxicant in milligrams per kilogram bodyweight per day (or in milligrams per day for a 70 kg person) which is not anticipated to result in any adverse effects after chronic exposure to the general population of humans, including sensitive subgroups [U.S. EPA, 1980]. An uncertainty factor is a number intended to account for the uncertainties in using a response at a single dose (the NOEL) from an experimental study to estimate a level of risk for the diverse human population.

Traditionally, the uncertainty factor is a multiple of 10. The appropriateness of selected values for uncertainty factors for ChE-inhibiting pesticides has been an area of debate within the Agency, reflecting the larger debate over uncertainty factors in general. The determination of a NOEL for these compounds is also controversial.

EPA uses the NOEL-UF approach for ChE-I pesticides and other substances (referred to by EPA as "systemic toxicants") believed to exhibit a threshold (EPA, 1986). This approach is not considered appropriate for agents that do not exhibit a threshold, which is usually the assumption for carcinogens. The NOEL, although actually a subthreshold dose level, is used to estimate the threshold dose, which in reality lies between the true NOEL and "lowest observed effect level," or LOEL. The measurement of the actual or true NOEL, LOEL, or threshold dose is a "trans-scientific" problem [see Section V] since these values can not be measured due to practical limitations on the size and sensitivity of experiments. The term threshold is generally applied to individuals, although in establishing an

ADI, a population threshold is implied. A population threshold is theoretically the threshold of the most sensitive individual in a population [EPA, 1986].

In keeping with the approach recommended by the NRC (1983), EPA conceptually separates risk assessment from risk management, and recommends that the term "reference dose" (RfD) be used in lieu of "ADI" [EPA (1986)]. It is argued that the use of the value-laden word "acceptable" is inappropriate in the largely scientific process of risk assessment. Other non-scientific factors also determine what level may be considered "acceptable." Use of the term ADI implies that doses higher than the ADI are "unacceptable" and that all doses less than the ADI are "acceptable" or "safe." In reality, there are many uncertainties in estimating an ADI which do not permit such a strict interpretation. The concept of a "reference dose" (RfD) is presented as a dose to be used as a reference point for gauging the potential effects of other doses, as a way of circumventing these connotations. For similar reasons, the term "uncertainty factor" is preferred over "safety factor." The RfD is estimated in the same way as an ADI. The more the RfD is exceeded (both in frequency and in magnitude), the more likely it is that adverse effects may be observed in a human population. Likewise, the more the RfD is avoided (in frequency and in magnitude) the less likely it is that adverse effects may be observed in a human population.

"Benchmark Dose" -- Uncertainty Factor Approach

In response to criticisms of the NOEL-UF approach (e.g., problems in measuring a NOEL), Crump (1984) suggested a new

method for determining ADIs which uses a "benchmark dose" (BD) in lieu of a NOEL. He defines the BD as the statistical lower confidence limit of a dose producing some predetermined increase in response rate such as 0.01 or 0.1 percent. Although the BD is calculated using a mathematical model, this approach is different from the mathematical modeling approach described below since it does not attempt to model the response at low doses. In fact, the particular model used is not very important since the method does not involve extrapolation much below the experimental range. This approach can be applied either to data where responses are quantal ("all or none") or (as is the case with ChE-I) to continuous ("graded") responses. It also requires the use of uncertainty factors.

Mathematical Modeling Approach

This approach is used by EPA to assess the risks of substances believed to pose a risk at any dose, no matter how small (e.g., carcinogens). For these non-threshold toxicants, a mathematical model is fitted to animal dose-response data and used to predict risks at lower doses which correspond to those experienced by humans. The choice of a model, although partially based on biological plausibility, is a matter of policy (degree of conservatism), since the choice of model can result in risk estimates which may differ by orders of magnitude. There is no inherent property of threshold toxicants which renders them unsuitable for dose-response modeling; the choice of the NOEL-UF approach for these compounds appears to be largely based on tradition.

Two types of models are often used: (1) tolerance-distribution models and (2) stochastic or probabilistic models. Tolerance-distribution models assume that every person in the population has their own tolerance to the toxicant. These models (which include the probit, log-probit, logistic, and Weibull) can be used for "non-threshold" toxicants by assuming that the population tolerance (the minimum of the individual tolerances) is zero. A number of factors (e.g., gender, race, diet) appear to affect individual susceptibility to ChE-I pesticides, so that the assumption of a distribution of tolerances appears to be well founded for these (and many other) compounds. In contrast, the stochastic models (which include the one-hit, multi-stage and multi-hit) assume that a response happens as a consequence of a random occurrence of one or more biological events, and that each individual in the population has an equal probability of responding. The one-hit, multi-stage, and multi-hit models were derived from theories on the mechanism of carcinogenesis, and are not applicable to ChE-I pesticides. However, it is possible that other stochastic models (based on our knowledge of the mechanism for ChE-inhibition) could be developed. The merits of one such model (Wilkinson, 1983) will be discussed.

Linear Extrapolation (Interpolation) Approach

Partially in response to the criticism that the choice of a particular mathematical model could not be scientifically justified, the U.S. Food and Drug Administration adopted a procedure recommended by Gaylor and Kodell (1980) for dose-response assessment of carcinogens which does not depend on any mathematical

model for extrapolation. This procedure assumes that the dose-response curve has a sigmoidal shape (a fundamental toxicological premise supported empirically), and rather than providing an estimate of risk in the low dose region, it places an upper limit on the potential risk at low doses. This procedure consists of four steps:

1) Approximate the dose-response relationship in the experimental data range using any appropriate mathematical model which adequately fits the data.

2) Obtain the upper confidence limits on the response above background (control) levels in the experimental dosage range.

3) Connect a straight line from the origin to the point representing the upper confidence limit at the lowest experimental dosage.

4) Obtain upper limits of risk for low dosage or dosages corresponding to upper limits of small risk from the interpolation line obtained in step 3.

Note that although the interpolation line goes through the origin, this does not imply that it assumes a no threshold response, since it provides an upper limit, not an estimate, of the actual risk in the low dose region. The procedure is actually interpolative since it is estimating a value between two known data points. Several variations of this basic procedure have also been suggested (Krewski et al. 1984).

B. Selecting the Best Approach

A key consideration in the choice of a dose-response assessment method is the way in which uncertainties necessarily involved in low dose extrapolation are handled. Since the goal is a consistent and rational risk assessment policy, the approach should be justifiable on scientific and policy grounds. With this in mind, each of the possible approaches can be evaluated using existing data on ChE-I pesticides.

1. NOEL-UF Approach

Although the NOEL-UF approach has been the method used for threshold toxicants for many years by many agencies, using this approach for ChE-I pesticides has some limitations, including:

1) the uncertainty regarding the meaning and estimation of a NOEL for ChE-I
2) the uncertainty regarding the appropriate UF to use when calculating an ADI for ChE-I pesticides
3) the effect of study design on establishment of a NOEL (e.g., sample size and choice of experimental doses)
4) neglect of available information on the dose-response relationship
5) it is not a cost effective way of using available data.

As discussed in the Hazard Identification section of this report, there are several uncertainties in regard to establishing a NOEL for ChE-I:

1) What is the biological significance of ChE-I? How does ChE-I relate to impairment of normal physiological functioning?

2) Is it possible to establish a biological threshold for ChE-I that is indicative of a NOEL? If so, how should it be measured?

There are several science policy options for handling this uncertainty:

1) Establish a level of ChE-I which will be considered an effect for regulatory purposes, stipulating the parameters under which it will be applicable. These parameters may include:

   a) whether post-exposure values of ChE are to be compared to control values or to pre-exposure values to determine the amount of inhibition (e.g., in a species with a large degree of intra-species variation in ChE values, such as humans, comparison to pre-exposure levels may be much more meaningful.

   b) number of pre-exposure baseline determinations (e.g., a fall in activity could be regarded as significant ($p < .05$) if it exceeds a value given by the expression $[1.65\ s\sqrt{n+1}]/n$ (Gage, 1967*) where s is the standard deviation around the individual average and n is the number of determinations on which the pre-exposure average is based

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* The expression given in Gage (1967), cited from Callaway (1951) is $1.65s\sqrt{(n+1)n}$ which appears to be in error since increasing pre-exposure baseline determinations (n) decreases the degree of inhibition needed to show statistical significance (thus divide by n, not multiply).

c) site and type of ChE measured (e.g., a smaller amount of brain AChE-I may be more significant than AChE-I in erythrocytes)

d) length of study/age of animal (e.g., a comparison of pre-exposure ChE levels when an animal is young with post-exposure ChE levels near the end of its life span may be confounded by age)

e) species or strain of experimental subject (e.g., depression of RBC ChE by 25% may be of greater biological significance in rabbits than dogs)

f) measurement method of ChE-I

g) type of pesticide (e.g., OP or carbamate)

h) rate of ChE-I

i) severity of other effects seen at higher doses.

2) Determine a statistical level(s) for which differences in ChE measurements will be considered significant for regulatory purposes (e.g., $p < 0.5$), stipulating the conditions under which it will be applicable (see a-i, above).

3) Specify the conditions for which ChE-I per se may not be an adequate toxicological endpoint (e.g., carbamates for which the inhibition of ChE is rapidly reversible).

4) Use a dose-response assessment approach that does not require the establishment of a NOEL (e.g., for all ChE-I, or only for some, such as carbamates).

5) Incorporate this uncertainty as a component of the UF.

The second criticism concerns the choice of an UF. What factors should be considered in establishing the UF? How should they be quantified? Various federal governmenal agencies and scientific organizations have recommended different UFs, as summarized in Table III-1. However, the actual UF employed does not always adhere to these recommendations. For example, a variety of UFs have been used in calculating ADIs (RfDs) for ChE-I pesticides. These may be justifiable in light of the recently articulated "modifying factor" (MF). To assure consistency in the selection of a MF, some sort of guidance will be required.

There have been various attempts to justify the selection of UF values, with little success. Dourson and Stara (1983) of EPA have characterized the uncertainty represented by a 100X UF as being basically of two types: interspecies variability and intraspecies variability. Most of the justifications for a 100X UF given in Table III-1 fall into one of these two categories, with the exception of uncertainties arising from possible synergistic action with other contaminants and small sample sizes. A 10X UF is generally ascribed to interspecies and intraspecies variability. Section IIIC documents the evidence to support an UF for these two types of uncertainty in the dose-response assessment of ChE-I pesticides, in an attempt to resolve some of the controversy over the most appropriate UF to choose.

The third criticism of the NOEL-UF approach is its dependence on study design. EPA's use of this approach discourages a registrant from planning more sensitive studies using a large number of animals. A larger study has a higher probability of showing a statistically significant result at a given dose level

and thus will more likely result in a smaller ADI. Gaylor (1983) illustrates this using results from two hypothetical experiments:

	Proportion of diseased animals (%)	
Dose Units	Experiment A	Experiment B
0	0/20 (0)	0/60 (0)
1	1/20 (5)	3/60 (5)
2	2/20 (10)	6/60* (10)
3	10/20* (50)	30/60* (50)

* Statistically different from control ($p < .05$)

Although the results of both experiments are proportionally identical, the NOEL in experiment A is 2 and in B is 1 dose unit. This is because the results at 2 dose units are statistically significant only at the $p < 0.244$ level in Experiment A, whereas in B they are significant at the 0.05 level. Dividing by an UF of 100 yields an ADI (RfD) for Experiment A of 0.02 dose units and 0.01 dose units for B. We would have expected Experiment B to produce a larger ADI than A since there is less random variation, but that is not the case if this approach is used.

Another study-design related concern in using the NOEL-UF approach is the selection of experimental doses. The highest true NOEL may be anywhere between the NOEL determined in the study and the LOEL. Thus, the NOEL is either an unreliable estimator of the threshold dose (assuming a threshold exists). Or, it may not be possible to determine a true NOEL if it is above or below the experimental dose range. Proponents of the NOEL-UF approach might counter these criticisms by pointing out that Good Laboratory Practice Standards and other EPA guidelines specify the minimum numbers of animals/dose level to ensure an

adequate study design. Although this ensures that "unacceptably" small (i.e. substandard) studies will not be used, studies using larger than the minimally acceptable sample size to demonstrate greater evidence of safety are discouraged.

One partial solution to this problem (of discouraging large sample sizes) would be a policy guiding the use of modifying factors (MFs). Sample size is, in fact, cited as a justification for employing a MF other than the default value of one in a draft EPA concept paper introducing the term MF (EPA, 1986). However, without a risk assessment policy in place to offer guidance, different scientists will likely select different MFs in a given case, since the value is largely arbitrary. Such inconsistency is scientifically unjustifiable and would undoubtably leave the Agency vulnerable to charges that personal values had entered into the risk assessment. In contrast, a well-established policy regarding the use of MFs could encourage larger, more accurate studies.

The fourth major criticism of the NOEL-UF approach is that it ignores dose-response information, since the NOEL is limited to the dose levels tested. Two alternatives are possible: (a) utilize a different approach, such as the BD concept, or (b) develop a risk assessment policy based on the dose-response curve to guide the choice of MFs. Since uncertainty in the estimation of a NOEL can result in a much greater underestimation of risk when the dose-response curve is steeply rather than shallowly sloped, larger MFs could be applied.

Lastly, the NOEL-UF approach does not use available data cost-effectively. In three out of five studies attempting to determine subchronic or chronic NOELs for ChE-I cited in an EPA risk assessment document for parathion (Ghali, 1985), NOELs for ChE-I could not be determined since effects were observed at all doses tested. Under the current approach, the only information gleaned from these studies (which can cost between $500,000 and $1 million) is that the NOEL is less than the lowest dose tested. If another approach was used, this same data could be utilized to estimate that dose producing some small additional level of risk.

2) Benchmark Dose (BD)-UF Approach

The BD-UF approach suggested by Crump (1984) was developed in an attempt to respond to the criticisms lodged against the traditional NOEL-UF approach. Specifically, it addresses the following criticisms of the traditional approach:

- the effect of the study method in establishing a NOEL: larger experiments tend to produce larger BDs (in contrast with NOELs) and BDs are not limited by having to be one of the doses tested.
- the disregard of existing information on the dose-response relationship: BDs reflect the dose-response pattern to a much greater degree than NOELs, since estimation of the BD involves fitting a model to the dose response data.
- the inability to make cost-effective use of data: any properly conducted and reported study can be used to calculate a BD, unlike a NOEL.

- the uncertainty regarding the meaning and estimation of a NOEL: although it is true that it is not necessary to define a NOEL to determine an RfD using this approach, the biological significance associated with the dose used to determine the RfD remains unresolved.

For continuous data (such as ChE-I) the BD is defined as the dose which corresponds to a specified amount of absolute change in the mean value relative to the mean value in the absence of the dose (the "extra response"):

$$BD = \frac{m(d) - m(o)}{m(o)}$$

where d = dose and m(d) = mean response at dose d. Since ChE-I is a continuous response showing a smooth dose-response trend it is difficult to pinpoint a threshold for a given subject and even more difficult for a large human population. If it is assumed that exposure to ChE-I pesticides is capable of producing some, albeit immeasurable, inhibition of ChE, a policy decision could be made to establish what portion of the extra response (%) would constitute the BD.

Crump suggests three models for continuous data: (1) the continuous linear regression (CLR) model

$$m(d) = c + q_1(d - d_0) \quad \text{for } d \geq d_0$$
$$= c \quad \text{for } d < d_0$$

where $d_0 \geq 0$ and c and q_1 are unrestricted, (2) the continuous polynomial regression (CPR) model

$$m(d) = c + q_1 (d - d_0) + \ldots + q_k(d - d_0)^k \quad \text{for } d \geq d_0$$
$$= c \quad \text{for } d < d_0$$

where $d_0 \geq 0$ and q_i's are either all positive (increasing dose response) or all negative (decreasing dose response) and (3) the continuous power (CP) model

$$m(d) = c + q_1(d - d_0)k.$$

The assumption is made that the responses of subjects in a dose group are normally distributed with mean $m(d_i)$ and variance σ_i^2. With this information a maximum likelihood estimate can be computed. Unfortunately, ChE-I data is often reported as mean ChE/dose group, without reporting the variance. Since the animals used in a given experiment are genetically homogeneous, one could assume a relatively small variance. However, as Figures III 1-4 show, this may not be a valid assumption. When investigators do not report variance, these methods can not be used.

As with the NOEL-UF approach, there is also the problem in selecting the apropriate UF. Gaylor (1983) suggests an alternative to the use of UFs (Table III-1) in which the size of the safety factor is chosen so that a specified low level of disease risk will not be exceeded. Although Gaylor uses quantal data, continuous data may also be used. Once the BD is estimated for a predetermined extra response (say, 10% above controls), then (a) select (via a policy decision) the level of extra response which is of interest for the study ("acceptable") (e.g., 1 in 1,000 animals, or R = 0.001), (b) determine the upper confidence limit (e.g., 95%) on the percentage of extra response associated with the BD (call this value U), (c) calculate UF = U/R, (d) calculate RfD = BD/UF. This method of determining UFs can also be used

with the NOEL-UF approach. Appendix III-1 illustrates this method using actual data for ChE-I pesticides.

3) Mathematical Modeling of Dose-Response Curve

It seems paradoxical that mathematical models have been developed to describe carcinogenesis, which is so little understood, and not ChE-I, for which detailed knowledge of the mechanism of toxic action is known and which is an effect caused by the majority of insecticides in use in the U.S. (see Introduction). For example, Awad (1984) has described the molecular mechanism and rate equations for ChE-I by malathion. Although it is beyond the scope of this paper, it would certainly be worthwhile to take advantage of the available literature and explore the use of kinetic models, as well as tolerance distribution models already developed (such as the probit) for dose-response assessment of ChE-I pesticides.

Wilkinson (1983) has in fact developed a kinetic model for aldicarb, using available human data. Data on whole blood ChE-I over time (t) for each dose were fitted to an exponential equation:

$$Y = A\,(e^{-k_r t} - e^{-k_i t})$$

where k_i is the rate of ChE-I

k_r is the rate of ChE recovery

and A is the Y intercept (of the recovery portion of the curve).

When using the available human data, this model (1) bypasses the need for interspecies extrapolation, (2) the dose aproximates the exposure condition of interest (i.e., exposure via aldicarb-

contaminated water) and (3) dosing conditions represent the worst-case situation (i.e., single bolus). The principal disadvantages of the data used were the small sample size (only 12 subjects), whole blood ChE was measured rather than ChE in RBC and plasma, and the study was never published. However, our principal interest is in the merits of this method of dose-response assessment, not the data used in Wilkinson's example. It addresses several of the criticisms of the NOEL-UF approach:

1) It does not require the definition of a "NOEL" or threshold level. It assumes that any exposure to aldicarb is capable of producing some ChE-I, even if not measurable. ChE-I is thus used as a measure of exposure, not effect.

2) Mathematical modeling does not generally employ UFs, thus bypassing this controversy.

3) The limitations of the study design are not as pronounced as with the NOEL-UF approach, although still may be present. Wilkinson averages the values of k_i, k_r, and A obtained for each of the doses used to obtain the general form of the equation, which is then used to predict the effects at other doses. The general form of the equation tends to predict somewhat higher values of ChE-I (except immediately after dosing) than do the dose-specific equations. Using a larger number of doses would reduce the overestimation predicted by the general form of the equation, thus rewarding studies with a larger number of doses. Uncertainty resulting from small sample size, on the other hand, does not

appear to be considered. Other mathematical modeling approaches (e.g., probit) do consider sample size.

4) Mathematical modeling does utilize information from the dose-response relationship.

5) Wilkinson's modeling approach can be used for studies in which NOELs can not be established, as long as data on ChE-I over time are provided. This approach could obviously not be used for many studies, but is a cost-effective way of using single dose human studies which otherwise might not be utilized to determine a RfD. The level of ChE-I from repeated exposures over long periods of time can be estimated if the following assumptions are made: (a) subsequent exposures occur before the effects of previous exposures are eliminated, (b) the magnitude of all exposures is similar, (c) the intervals between exposures are equal, and (d) the kinetic parameters remain constant throughout exposure.

Another advantage to the mathematical modeling approach is that the effects of these and other assumptions can be explored. Assumptions that have a large effect on estimates of doses or responses can be explored in more detail.

In conclusion, the mathematical modeling approach is an alternative to the NOEL-UF approach which merits more attention, through validating existing models (such as the kinetic model for aldicarb by Wilkinson et al.) and through research into other

models, particularly for data which is applicable to the human experience and could otherwise not be used to determine a RfD.

4) Linear Extrapolation

While linear extrapolation may often be thought of as a type of mathematical model, it can also be interpreted as a variation on the NOEL-UF approach. Rather than connecting a straight line from the origin to the point on the upper confidence limit at the lowest experimental dosage (step 3 of procedure listed previously) to obtain the upper limits of the response at a low dose, an equivalent procedure is to divide the upper confidence limit (U) on the response produced by the NOEL by a (policy predetermined) extra response of interest R (say, R = 1% ChE inhibition) to obtain an UF = U/R (Gaylor, 1983).

The RFD is then obtained as usual by dividing the NOEL by this UF. This approach can also be applied to an ED (e.g. ED_{90} representing 10% ChE-I). This is illustrated in Appendix III-1 using data for parathion.

The main advantages to linear extrapolation are that:

1) an upper limit on the level of risk in the experimental population can be obtained, since as the dose is decreased, the risk response decreases proportionately more rapidly (zero is the lower bound on the risk),
2) the influence of sample size is controlled through the use of confidence limits (so that smaller experiments are not rewarded by higher allowable doses),
3) the problem of selecting or developing an appropriate mathematical model is circumvented.

Applying this approach to an ED (dose for which the BD is an upper limit) also incorporates many of the advantages attributed to the BD-UF approach (e.g., a threshold dose need not be demonstrated).

Summary

In conclusion, there are situations in which each of the four approaches to dose-response assessment of ChE-I appears to be advantageous. When information on kinetics and resources to develop, refine and validate the method are available, a mathematical modeling approach is desirable. When ChE-I data is expressed as mean ChE activity $\pm$ standard error, the BD-UF or linear extrapolation approach are desirable.

Finally, the NOEL-UF approach can be used in the remaining situations, if the recommendations outlined for developing a clear, sound science policy are followed. Policy that allows some flexibility in the choice of dose-response assessment method will result in a more productive use of available data.

C. Selection of Uncertainty Factor

1. Animal to Human Extrapolation

In an attempt to quantitatively estimate the uncertainty in extrapolating experimental results from animals to humans, human NOELs for ChE-I were compared to other animal NOELs for nine pesticides (Appendix III-3). Unfortunately, differences in study design and inadequate reporting of human data in several cases made valid comparisons difficult. Much of the human data were collected on prisoners or "volunteers" (usually male) and thus did not reflect the human population at large. Individual body weights of human subjects were not always reported. The experiments on humans were typically of shorter duration than the animal experiments to which they were compared, making such comparisons questionable. In two instances human and animal studies of ChE-I were of comparable duration:

Malathion:

$$\frac{\text{Rat (RBC) ChE-I NOEL (33 day)}}{\text{Human (RBC) ChE-I NOEL(47 day)}} = \frac{5.0 \text{ mg/kg}}{0.23 \text{ mg/kg}} = 21.7$$

Dimethoate:

$$\frac{\text{Rat (RBC, plasma, and brain) ChE-I NOEL(34 day)}}{\text{Human ("whole blood" and RBC) ChE-I NOEL(39 day)}} = \frac{0.7 \text{ mg/kg}}{0.2 \text{ mg/kg}} = 3.5$$

This implies that healthy adult men are 22 times more sensitive to ChE-I by malathion than rats, and adults (presumably healthy men and women) are 3.5 times more sensitive to ChE-I by dimethoate than male rats. However, this should be interpreted cautiously since (for malathion):

- o Only 5 men (prisoners) were tested with malathion
- o Body weights of men were not recorded (assumed 70 kg)
- o Dosing procedure was not clear (e.g., not indicated whether dosed on full or empty stomach), although both rats and humans were dosed orally
- o Dosing schedule was unusual (i.e., administered 8 mg to each man every day for 32 days, gave no treatment for 3 weeks, then administered 16 mg/d for 47 days)
- o Age of rats and men not reported in available reviews (assumed adult age)

and for dimethoate:

- o Only nine humans (gender not reported) were tested at this dose level
- o Route of administration differed (intraperitoneal injections for rat and oral aqueous solution for humans).
- o Age of rats and humans not reported (assumed adult age)

However, the human study of dimethoate was rated as "supplementary upgraded to minimum" by EPA's Office of Pesticide Programs and "High Confidence" by EPA's ADI work group. The human study of malathion is the basis for the ADI set by the World Health Organization (WHO, 1982).

Table III-2 compares NOELs for studies of comparable length among different species. Studies where the NOEL was lower than the lowest dose tested or higher than the highest dose tested resulted in minimum or maximum ChE-I ratios (e.g., (Rat NOEL)/(Dog NOEL) $\leq$ 1.9, implying that the dog is no more than 1.9 times

more sensitive to ChE-I by chlorthiophos than the rat). Inter-species differences ranged from no difference (ratio = 1) to greater than 25 fold difference ($\bar{x}$=5*). Comparisons made on a per unit surface area basis rather than a weight basis did not consistently reduce the variability observed.

Comparisons of sensitivity to ChE-I pesticides among non-human species is useful in estimating the uncertainty in animal-to-human extrapolation because (a) if different species of test animals differ from each other in sensitivity, it is likely that humans will differ from test animals and (b) directly comparing the sensitivity of humans to test animals is difficult due to scanty reliable human data and ethical prohibitions against collecting additional human data.

Due to the small number of comparisons which were made and differences between studies (e.g., in how, when, and for how many animals ChE levels were measured), no firm conclusions can be drawn from the data in Table III-2. However,

a) Dogs are often (but not always) more sensitive than rats to ChE-I.

b) ChE-I measured at a particular site (i.e. plasma, erythrocytes, or brain) did not vary more (across species) than at other sites.

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* Mean calculated after inverting ratios less than one, and assuming minimums and maximums represent the actual ratio (note that the number of minimum ratios balances the number of maximum ratios).

c) ChE-I measured after chronic exposure did not vary more across species than ChE-I measured after subchronic exposure to the pesticides analyzed.

d) Humans were more sensitive to ChE-I than rats in the two instances where comparisons could be made.

Edson (1964) conducted several studies comparing ChE-I NOELs in the rat, pig, and human. This reduced the variability expected when studies conducted in different laboratories are compared. These results are shown in Table III-3. One problem in estimating NOELs from these studies is that only a qualitative description of some of the results is provided. For example, when Edson reports that "Red cell ChE was slightly reduced at 0.025 ppm," it is not apparent whether this dose is the NOEL or LOEL. The difficulties are compounded by inconsistencies in the article summarizing the data. For example, he reports 0.05 ppm as the NOEL for rats exposed to parathion, yet concludes that the NOEL is 0.02 mg/kg/d. According to the diet conversions used by the ADI work group (from Layman's tables), the percent of body weight consumed as food, F, is 0.05 for rats, and F times X ppm of pesticide in feed is 0.05 x 0.05 = 0.0025 mg/kg.

The ratio of ChE-I measured in red blood cells ranged from 1 to 48 and from 1 to 480 (and possibly as high as 960, depending on the interpretation of Edson's data) in plasma. These results should also be interpreted cautiously (due to small sample sizes, variable dosing schedules, poor reporting of data, etc); however much of the uncertainty in the data which would result from different study designs, methods, individual techniques, and scientific judgment were probably minimized.

Undoubtably the major factor contributing to the interspecies variation in sensitivity to ChE-I pesticides is differences in metabolism, although differences in absorption, plasma protein binding, and other factors also play a role. Table III-4 provides some examples of these differences (from Hollingworth (1971) and Calabrese (1983) reviews of the literature on comparative metabolism and sensitivity to OPs and carbamates.) Wills (1972) provides estimates of the "normal" activities of red blood cell and plasma cholinesterases in 15 species. Humans have the highest activity of plasma ChE of the 15 species compared, about 15 times the activity of rabbits (the largest difference) and twice that of dogs. Estimates of plasma cholinesterase in rats varied greatly (22 fold for males, even when measured by the same method), probably due to strain differences. Humans, rabbits and pigs had higher ChE activity in RBCs than in plasma, although the opposite was true in the dog. Exactly how differences in ChE activities may translate to differences in susceptibility to ChE-I pesticides is unclear, although it is interesting to note that the species with high ChE activities (human, monkey, dog) generally appear to be more susceptible (Tables III-2 and III-3) than those with lower ChE activities (rat, mouse). According to Wills, the ChE in the blood of various species seems to fall in the following order of decreasing concentration: human, horse and monkey, cattle, turkey, dog, rat, duck, cat, goose, mouse, and rabbit. Only in 3 of the 24 comparisons shown in Table III-2 was the correlation between higher ChE activity and greater sensitivy to ChE-I pesticides not as predicted. These three were

comparisons between the rat and dog. Some of the estimates given by Wills for the plasma ChE activities of the rat were lower and some higher than those for the dog.

In addition to differences in ChE activities across species, other differences in metabolism also contribute to interspecies variability in response to ChE-I pesticides. Serum paraoxonase activities are higher in rabbits than in rats, for example (La Du and Eckerson, 1984).

2. Variation in Human Sensitivity

Section IIB outlined the major factors accounting for (or potentially contributing to) differences in sensitivity among people to ChE-I pesticides. Factors which generally seem to be (or are likely to be) associated with increased sensitivity include young age (including pre-natal), genetically determined ChE and arylesterase (e.g., paraoxonase) with low activity, pregnancy, food or water deprivation, malnutrition, stress due to temperature or viral infection, and exposure to certain other substances (e.g., other ChE-I's, alcohol, nicotine, chlorpromazine). Certain disease conditions may also contribute to enhanced sensitivity to ChE-I pesticides (e.g., anemia, liver disease, carcinoma, epilepsy, renal ischaemia, schizophrenia, eczema). Since ethical prohibitions prevent the deliberate exposures of potentially high risk individuals to pesticides under controlled experimental conditions, it is difficult to quantitatively determine with certainty the variation in human sensitivity to ChE-I pesticides. The contribution of some of the above mentioned factors to increased sensitivity in animals sometimes

exceeds the customary ten-fold factor for intraspecies variation. For example (see Section IIB) chlorpyrifos (used to control parasites in cattle, and widely used for indoor pest control) is thirty times more toxic to calves than adult cattle, and malathion (also widely used in the home or by homeowners) is twenty-eight times more acutely toxic to newborn rats than adults.

3. Recommendations

The evidence adduced in Sections IIB and IIID indicates that an uncertainty factor as small as ten is clearly inadequate to account for the inter- and intraspecies variability in response to ChE-I pesticides. Ten is the UF usually applied to NOEL's based on Che-I, even when derived from an animal study (Federal Register, 1981).

In keeping with the recommendations of NAS, WHO, FDA, and EPA (for non-carcinogenic compounds other than ChE-I pesticides), it is recommended that an uncertainty factor of one hundred be used when employing the NOEL-UF or BD-UF dose-response assessment methods. In cases where available data demonstrates that the inter- and intra-species variation is significantly different from one hundred, a modifying factor (MF) might be used to account for this difference. Documentation to justify the selection of a MF other than one should be included in the risk assessment.

a) May be a precursor of AChE.

b) May be involved in myelin structure (BuChE has been found in Schwann cells of nerves and between folds of myelin in some central axons).

c) May control choline levels in plasma (used to synthesize ACh).

d) May act as a backup for AChE to destroy circulating ACh.

e) May be involved in structure and/or synthesis of beta-lipoproteins (is found complexed with beta-lipoprotein).

f) May removes toxic esters formed by fatty acid metabolism.

g) May be involved in assimilation of food (serum BuChE activity decreases after fasting, parallels level of food intake in undernourished children, and is elevated in obese and diabetic patients, and persons with hyperlipoproteinemia (abnormal lipid metabolism)).

Thus, although BuChE and AChE share many similarities (mechanism of action, molecular shape, etc.) it is not known how, if at all, they are related physiologically [Edwards and Brimijoin, 1982].

Hazard Identification Issues Re:ChE-I

Although there are still many questions to be answered, it should be remembered that AChE has been characterized as "one of the best studied of all enzymes" [Brimijoin, 1983]. Despite uncertainties regarding the roles of AChE in erythrocytes and

number of muscarinic receptors in target tissues that are capable of binding the ACh which accumulates when AChE is being inhibited, and in some other cases (e.g. propoxur) due to enhanced enzymatic detoxication.

In spite of this, ChE levels may remain inhibited or decrease even more in long-term exposures. Figures III-1 - III-4 show the inhibition of ChE's in plasma and erythrocytes by parathion in dogs over two, four, and twelve months. In this experiment the percentage decrease (from control values) is the greatest at 12 months at every dose (except for RBC ChE of female dogs). In other words ChE was inhibited more after one year than after two or four months. Table II-3 compares ChE-I NOELs for the same species, pesticide, route of administration, and site of ChE (i.e., plasma or red blood cell (RBC) but differing study lengths, for seven pesticides (see Appendix III-3). In the majority of cases the NOELs are lower in longer (chronic) studies than in shorter (subchronic) studies. Possible differences in study designs (e.g., method used to determine ChE-I, comparisons to unexposed animals vs. pre-exposed animals, strain) and the small number of comparisons made (16) prevents any definitive conclusion, but the results suggest that ChE-I NOELs vary by duration of exposures such that chronic ChE-I NOELs are lower than subchronic NOELs. According to Bartholomew et al. (1985), a decrease (or absence) of acute toxic effects occurs despite continued inhibition of brain ChE activity and elevation of ACh concentrations.

Wills (1972) maintains that "In general, prolonged or repeated exposures to inhibitors of cholinesterases have emphasized the unreliability of estimations of the activities of plasma, or serum, and red blood cells for judging the severity of intoxication by these inhibitors." Levels of ChE-I fluctuate over the course of chronic and subchronic exposure. For example, Wills cites an example in which pigs fed 1.7 mg/kg/day of parathion had increased levels of plasma ChE at first, and then decreased levels until maximum inhibition (43%) occurred on about the 50th day. RBC AChE levels remained unaffected for 8 days of dosing, and then fell gradually until maximum inhibition (86%) occurred, also on day 50. Studies in which ChE activity is infrequently measured may not be able to determine maximum inhibition. In addition, there is some indication that the rate of ChE-I may affect toxicity (Wills, 1972). Jensen (1965) found that the lethal dose of paraoxon in guinea pigs increases at an increased rate of infusion.

Although tolerance appears to develop to the acute toxicity associated with ChE-I, what are the chronic toxic effects associated with ChE-I? (Recall that both acute and chronic effects may result from either acute or chronic exposures). Investigations to date have yielded conflicting claims regarding the existence of chronic neurobehavioral effects (Duffy et al., 1979; Ecobichon and Joy, 1982; Karczmar, 1984; Levin, 1976; Miller, 1982; National Research Council, 1985, Savage et al., 1982). These and other chronic effects (carcinogenic, nephrotic) linked to some ChE-I pesticides or their metabolites will not be considered here.

Intraspecies Variation of ChE

Not only is ChE activity affected by exposure to ChE-I pesticides, but it also varies among species (see section IIID), and is affected by age, sex, diet, genetic status, race, pregnancy, obesity, season, liver disease, myocardial infarction, and other health and environmental factors. Plasma ChE levels fluctuate more than RBC AChE levels.

o Genetic Factors

A small sub-group of the population (estimated by Williams (1985) to be 4.5%) possess genetically determined atypical plasma ChE. Atypical ChE was first discovered when the muscle relaxant succinylcholine, used in anaesthesia, was found to produce an unusually long period of paralysis and apnea (temporary suspension of breathing) in some patients. These individuals were found to have atypical or low serum ChE, which is responsible for the hydrolysis of succinylcholine, thus ending the drug's effects. Genetic studies since then have shown that most people with atypical BuChE are homozygous for a recessive gene (designated E_1^a E_1^a). The gene allele E_1^a directs the synthesis of a ChE which is unable to hydrolyze succinylcholine at pharmacological doses and which is also less sensitive to certain ChE-I's (e.g., dibucaine). The latter property is used to classify serum ChEs, by use of the dibucaine number (DN), a measure of the degree of inhibition (expressed as a percentage) of serum cholinesterase obtained with dibucaine under standardized conditions (Kalow, 1957). Whereas most people (with normal genotype E_1^u E_1^u) have a DN between 76 and 81, those who are heterozygotes (E_1^u E_1^a) have a DN between 55 and 69, have about seventy-eight

instance where other tissues (including brain) were investigated in a person with atypical serum enzyme, the tissue enzyme was similarly atypical. It is not apparent whether these people would be more susceptible to ChE-I pesticides, since their serum ChE is unable to hydrolyze accumulating cholines; less susceptible, since their serum ChE is less sensitive to certain ChE-I's (e.g. dibucaine, fluoride); or equally susceptible. Calabrese (1978) maintains that individuals with such pseudocholinesterase variants should be considered potentially at high risk to ChE-I pesticides. Calabrese also notes that the dibucaine variant has been found to be extremely sensitive to RO2-0683, and cautions that this is of "particular significance in light of the widespread use of carbamate insecticides." However, the OP's TEPP and DEP isofluorophosphate do not inhibit differentially among pseudocholinesterase variants and would not cause a higher risk to those individuals with atypical variants.

Another indication that genetic factors may affect susceptibility to ChE-I pesticides is provided through selective breeding experiments in animals by Overstreet et al. (1979). Male rats determined to be most resistant and most sensitive to DFP on the basis of drinking behavior, body weight, and core body temperature were bred with the most resistant and sensitive (respectively) female rats in an attempt to establish resistant and sensitive lineages. Although the former attempt failed, Overstreet et al. were successful in establishing a sensitive line of rats. However, the genetic differences in sensitivity were not found to be related to differences in brain or erythro-

cyte AChE or serum BuChE activity. The authors speculate that the genetic differences could be due to changes in sensitivity of AChE isoenzyme, changes in ACh synthesis or turnover, or changes in sensitivity of postsynaptic receptors for ACh. Subsequent studies (Overstreet et al., 1984) have shown that the latter two factors do indeed contribute to the enhanced sensitivity observed. Regardless, the data suggests that ChE-I may not always be an adequate measure of toxicity in genetically-susceptible individuals.

Besides genetic differences in ChEs, there is another esterase that is affected by organophosphates and carbamates, known as arylesterase, for which genetic variants exist. Arylesterases have not been as well studied as other esterases. Paraoxonase, an enzyme hydrolyzing paraoxon, the active metabolite of parathion, is an arylesterase which has been found to be polymorphically distributed in several populations (LaDu and Eckerson, 1984). Two alleles determine paraoxonase activity: A, a low activity allele, and B, a high activity allele. Heterozygotes (AB) also exhibit high activity. About one-half of the U.S. caucasian population is homozygous for the low activity allele (AA), which is speculated to place these individuals at higher risk of parathion poisoning than those with higher levels (Ortigoza-Ferado et al., 1984). Non-caucasian populations of African, Oriental, or American Indian subjects, for example, do not show the same distribution, but it is not known whether this is due to the presence of additional alleles or quite different gene frequencies (LaDu and Eckerson, 1984). Ortigoza-Ferado et al. state "It may be postulated that such differential suscepti-

bility would be particularly significant at low or intermediate levels of exposure to parathion since with marked exposure even high levels of paraoxonase would not be sufficient for protection against toxicity."

It appears, then, that both ChE and arylesterases (e.g., paraoxonase) affect the toxicity of ChE-I pesticides. A model describing the interaction of paraoxon with serum ChE and paraoxonase was developed by LaDu and Eckerson (1984). The level of paraoxonase was found to influence the degree of serum ChE-I in vitro. The authors recommend that the in vitro model system be applied to estimate what is likely to occur in vivo, and that epidemiological studies be undertaken to determine whether individual response to ChE-I pesticides shows the expected relationship to the type and level of paraoxonase.

o Sex Differences

Sex related differences in susceptibility to ChE-I pesticides are most likely to occur for those compounds which require metabolic activation to produce ChE-I (e.g., parathion). (Doull, 1980). Agarwal et al. (1982) found that the percentage of ChE-I by parathion was 2.6, 1.2, and 2.7 times greater in female than male rats measured in plasma, erythrocytes, and brain, respectively, following a single oral dose. Paraoxon treatment, however, resulted in comparable inhibition of plasma, erythrocyte and brain ChE in both sexes. Castration increased the susceptibility of male rats to a similar level as females. Pre-treatment with testosterone enabled these castrated males to recover from this increased sensitivity, whereas estradiol enhanced their

sensitivity slightly. Gonadectomy had little effect on ChE levels in the females. Pre-treatment with testosterone decreased the sensitivity of ovariectomized females. The authors concluded that testosterone plays an important role in determining parathion toxicity.

While it is well established that the female rat is more susceptible to the acute toxic effects of parathion (e.g., LD_{50} and ChE-I) than male rats, this sex difference is not as obvious for chronic or subchronic ChE-I. For example, in a 2 year feeding study in rats prepared by Daly (1984) for Monsanto (in Ghali, 1985), no consistent pattern is observed.

Sex differences exist in absorption, distribution, and excretion of ChE-I pesticides also. For example, Khaak et al. (1984) found that less parathion was lost by evaporation from the skin of male than female rats, males having a larger percentage of the dose in their carcasses. Similar amounts in both sexes were excreted in the urine and feces. Although the amount absorbed from the skin was about the same over a 120 hour period in male and female rats, males absorbed parathion from the skin much more rapidly. Females absorbed more in heart and liver tissue than males.

The plasma and red blood cells of human males have higher ChE activities than human females (Wills, 1972). Serum ChE is significantly decreased in women using oral contraceptive pills (Robertson, 1967). How this affects susceptibility in humans is not clear, although in the rat, the female is more susceptible and has higher ChE activities than the male.

- Age

Kacew and Reasor (1984) report that "it is clear that neonates are more susceptible than adults to AChE inhibitors." Although the hepatic cytochrome P450 system which catalyzes some OPs to their active metabolites (e.g., parathion to paraoxon) is poorly developed in neonates, neonates are still more sensitive, apparently due to differences in detoxification, excretion, or redistribution. For example, the level of the enzyme that degrades malaoxon (carboxylic ester hydrolase) is less than the level of the activating enzyme for the first thirty days of life of the rat. By thirty days of age malaoxon inactivation is equal to its rate of production (Kacew and Reasor, 1984).

Besides such differences in detoxification ability, the specific activity of brain AChE increases from a minimum in the one day old rat, either due to an increase in the amount of AChE or its catalytic activity (Kacew and Reasor, 1984).

In the human, adult levels of AChE are not reached until three to five months of age. Even though blood from neonates has a higher proportion of young cells (which have higher activity than more mature cells) than adults, AChE activity in newborn circulating erythrocytes is less (Herz and Kaplan, 1973). Thus, for a given concentration of ChE-I pesticide, more AChE is expected to be inhibited in newborns compared to adults (Kacew and Reasor, 1984). This has been found to be the case with beef cattle given ChE-I pesticides to control parasites, according to Kacew and Reasor. Chlorpyrifos is thirty-fold more toxic to calves than adult cattle. Brodeur and Dubois (1963) compared the LD_{50}'s of sixteen ChE-I pesticides given intraperitoneally for

twenty-three day old weanling and adult rats. The acute toxicity was from one to five times greater for weanlings than adults, except for OMPA, for which adults were five fold more sensitive. On average, weanlings were twice as sensitive as adults. Mendoza and Shields (1977) compared the LD_{50}'s of rat pups treated with malathion (99.3%) by gastric intubation and found that one-day old pups were three times more sensitive than six day old pups and nine times more sensitive than eighteen day old pups. Similarly, the I_{50} (concentration of malathion required to inhibit ChE by fifty percent at specified conditions) of one day old pups measured for brain AChE was one-third, one-fourth, and one-eighth the amount in six day, twelve day, and eighteen day old pups, respectively. Lu, Jessup and LaVallee (1965) compared oral LD_{50}'s for malathion (99.6%) in rats of different ages and observed that newborns were twenty-eight times more sensitve than adults and seven times more sensitive than pre-weaning rats. They also observed that dividing the dose over four days reduced the toxicity in adults but increased the toxicity in pre-weaning rats.

Parathion, malathion and aldicarb all pass through the placenta and are toxic to the fetus. Malathion and parathion have been shown to have teratogenic effects [Calabrese (1978), Fish (1966), Hoffman and Eastin (1981), Wyttenbach and Thompson (1985), etc.]. Cambon et al. (1979a) found that AChE in the brain and blood (but not liver) of rat fetuses was consistently more inhibited than that of the dams treated by gastric intubation with aldicarb on the eighteenth day of gestation. Carbofuran consistently inhibited brain AChE more in the dam than in

the fetus, while for pirimicarb, no consistent pattern was observed. Cambon et al. (1976b) hypothesize that the reason for the observed differences in sensitivity are due to differences in fixation of carbamate derivatives on the fetal versus maternal isoenzymes.

The sensitivity of the elderly to ChE-I pesticides is less clear than for the very young and unborn. Rider et al. (1957) found that plasma ChE showed a small but definite increase with age in both sexes. Other investigators [Calloway et al (1951), Gage (1969)] did not find age to be a factor influencing the magnitude of variability of ChE in adults. Ando et al. (1984) found that serum ChE activity increased according to age in females, while it decreased slightly according to age in males. The ChE activity was higher in males than in females under fifty years of age, whereas the reverse was found in persons over fifty-five years of age. They also detected seasonal variation (higher activity in winter than summer) in females but not males. There does not appear to be any conclusive evidence indicating that adults of any specific age may be more sensitive to ChE-I pesticides.

o Nutrition

Nutritional deficiencies have been found in some cases to increase the susceptibility of test animals to ChE-I pesticides. Parathion, malathion and banol all produced greater ChE-I in rats on low protein diets than on high protein diets (Casterline et al. (1969a, b, 1971 a, b), Vaishwanar and Mallik (1984)). Parathion-induced serum ChE-I was more dependent on dietary protein levels for subchronically-exposed rats (28 days) than for acutely exposed animals (single dose) (Casterline and Williams, 1971). Behavioral changes were noted more often in rats on low protein diets exposed to parathion or banol than in unexposed rats on low protein diets or exposed rats on high protein diets (Casterline, Brodie and Sobotka, 1971). These behavioral changes consisted of a higher proportion of "No escape" rats (i.e., rats failing to press a lever to either avoid or escape a negative stimulus [electric shock] after training in a standard operant conditioning chamber). None of the diet-pesticide groups tested were associated with significant changes in avoidance only behavior (lever pressing during conditioned stimuli [light and sound] preceding the unconditioned stimulus [shock from electric grids]). In this experiment, although behavioral changes were noted, the activites of ChE (and monoamine oxidase (MAO)) in the cerebellum and cerebrum were not significantly affected by the low casein diet and/or the presence of a ChE-I pesticide. Since brain ChE can not be assayed until the end of the experiment (at 9 weeks in the parathion experiment and 10 weeks in the banol experiment), it is possible that inhibition may have occurred earlier in the experiment, preceding adaptation to chronic

exposure. In fact, brain ChE-I was noted in a similar 28 day experiment by the same investigator (Casterline and Williams, 1971). It is noteworthy that behavioral effects can be observed following subchronic exposure to a ChE-I pesticide even when brain AChE levels are not inhibited. Subchronic exposure to parathion was found to decrease serum and liver triacetinesterase (AliE) activities in protein-deprived animals as well as ChE activities, thus reducing the detoxification ability in those animals and making them highly susceptible to poisoning, even at low doses (Casterline and Williams, 1971).

In addition to protein (casein), varying dietary levels of calcium (Ca) and magnesium (Mg) also affected ChE-I by parathion and banol (Casterline and Williams, 1969a). Both high and low levels of these cations decreased serum and brain ChE-I by parathion and banol. Liver ChE-I was also decreased by parathion and banol, except by low magnesium which increased ChE-I by parathion. Serum and brain AliE by parathion and banol was unaffected or decreased by altered cation levels, while liver AliE-I by parathion and banol was significantly increased by high Mg or high Ca in the diet. These results are difficult to interpret, since they imply that low or high dietary concentrations of Mg or Ca might decrease susceptibility to parathion and banol since ChE-I by these compounds is reduced. However, the changes in ChE and AliE that occurred after pesticide administration did not influence the lethal action of the pesticides, except with the casein-free diet, where the mortality was increased.

Animals on food restricted diets, or deprived of water, were also shown to be more susceptible to subchronic (via diet) and acute exposures (via intraperitoneal injection) of parathion and paraoxon (Baetjer, 1983 and Villeneuve et al., 1978). Food restriction had a significantly greater effect than water deprivation on blood ChE-I by parathion, but not paraoxon.

In the subchronic study, food restriction increased plasma ChE-I elicited by parathion, but brain ChE was not inhibited by the doses of parathion used, either alone or with food restriction. Increased inhibition of plasma ChE in animals subjected to food restriction was not observed at the NOEL for the study.

o Pregnancy

During routine blood ChE monitoring at a pesticide industry, it was observed that a marked fall in plasma ChE occurred in pregnant women in their first trimester who had not been exposed to ChE-I pesticides (Howard et al., 1978). A more extensive survey by Evans and Wroe (1980) on 941 pregnant women distributed evenly throughout the 40 weeks of gestation revealed that a rapid fall occurred in the first trimester to a level which did not alter significantly during the remainder of pregnancy. Even lower values were observed in the 105 patients examined during the week following delivery. Three of the women surveyed possessed abnormal genotypes (i.e., E_1^a E_1^a, E_1^s E_1^s, and E_1^f E_1^f) and were excluded from the study. Nevertheless, some women were determined to be at risk to succinylcholine on the basis of ChE activity between 1.68 and 2 units/ml. The numbers of pregnant women at risk for each time period investigated

closely paralleled the changes in mean enzyme activity over time, with the highest risk of apnea and prolonged effects of succinylcholine occurring immediately after delivery.

How do low levels of plasma ChE occurring during pregnancy affect susceptibility to ChE-I pesticides? Recall that it was unclear whether individuals possessing non-normal genotypes and low levels of plasma ChE would be more susceptible to ChE-I pesticides due to the decreased ability of plasma ChE to hydrolyze cholines, or less susceptible since their plasma ChE was found to be less sensitive to fluoride, dibucaine, and some other ChE-I compounds. Weitman et al. (1983) found pregnant mice to be more susceptible to single doses of parathion and paraoxon than virgin female controls. In pregnant mice, signs of cholinergic stimulation (tremor, weakness, lacrimation, salivation) were more intense, brain and plasma ChE activites were lower, blood and brain concentrations of parathion and paraoxon were higher, and serum paraoxonase activities were lower, compared to controls. Whether pregnancy-induced alterations of hepatic function, ChE activity, serum protein binding, serum esterases or a combination of these are responsible for the enhanced susceptibility is unclear.

o Miscellaneous Health and Environmental Factors

The susceptibility to ChE-I pesticides can be affected by exposures to physical factors (e.g., cold), biological agents (e.g., viruses), and other toxic substances (e.g., pesticides, drugs).

Not only can the toxicity of ChE-I pesticides be altered in individuals with hypo- or hyperthermia, but ChE-I pesticides may

have hypothermic effects. Doull (1980) reports that hyperthermia increases the toxicity of parathion, while Chattopadhyay (1982) found that half the LD_{50} dose of parathion was lethal under cold temperature.

The percent whole blood ChE-I by DDVP was significantly less in cold-exposed rats than in rats at room temperature, but by parathion was significantly more at one-half the LD_{50} dose and unaltered at one-quarter of the LD_{50} dose. Chattopadhyay (1982) also noted hypothermic efects of OPs in rats under cold exposure. Body temperature decreased as the dose of OP increased, and the higher the ChE-I the lower was the body temperature of the animals under cold temperature.

Whole-body radiation produces a dose-dependent decrease in BuCh activity of the ilia in rats and mice, but there is no significant change in the acute toxicity of ChE-I pesticides in animals given lethal exposures of whole-body ionizing radiation (Doull, 1980).

Doses of parathion ordinarily considered sublethal were lethal to mice infected with the virus MCMV (murine cytomegalovirus), apparently due to a decrease in the ability of infected mice to detoxify parathion (Selgrade et al., 1984). MCMV is well established as a model for human cytomegalovirus, a ubiquitous herpes virus which infects a large portion of the population and remains with the host in a latent state for a lifetime.

The effects of human cytomegalovirus are usually subclinical or indistinct, but infections can be manifested congenitally, perinatally, in individuals who are immunosuppressed, and in some

other instances. Serum ChE was inhibited more by infected mice compared to uninfected mice.

Synergistic interactions among ChE-I pesticides are known to occur. Most of the studies of synergism among these compounds have tested for acute toxicity. According to Doull (1980), combinations of several OP pesticides fed at "recommended tolerance levels" failed to produce significant synergistic toxicity in chronic feeding studies.

Malathion is potentiated by many OPs since it is detoxified by carboxylesterases that are inhibited by other OPs. Potentiation of malathion is known to vary across species. For example, TOTP (triorthotolyl phosphate) given at a dose which alone did not significantly affect brain AChE potentiated the anticholinesterase action of malathion by 29-fold in mice, 17-fold in quail, 100-fold in frog, 11-fold in sunfish and 12-fold in bullheads (Cohen and Murphy, 1970). Isomalathion and other impurities in technical malathion potentiate malathion and are believed responsible for an epidemic poisoning in Pakistan in 1976 during a malaria eradication program (Aldridge et al., 1979). Potentiation of malathion by these impurities is significantly less in the mouse compared with the rat (Umetsu et al., 1977).

Potentiation can also occur between drugs and ChE-I pesticides. For example, chlorpromazine, a tranquilizer, has been shown to potentiate dichlorvos toxicity in rats, producing about twice as much ChE-I as dichlorvos alone. Drugs that deplete glutathione (e.g., acetaminophen) may potentiate the toxicity of some OPs which are detoxified by glutathione transferases (Marquis, 1986).

In contrast, organochlorine (OC) insecticides protect against the acute toxicity of several OP insecticides. This is probably because OC's stimulate detoxification of OPs by liver microsomes and increase noncatalytic binding sites for OPs (Doull, 1980). Lead was observed not to potentiate parathion toxicity (Phillips et al., 1973). However, both lead and cadmium decreased AChE in rat brain following chronic exposure in the animals' drinking water (Marquis, 1986). Marquis (1986) summarized her review of heavy metal interaction with pesticides: "Clearly, the CNS is rendered more susceptible to the hazards of ChE inhibition in animals chronically intoxicated by heavy metals."

ChE levels are known to vary with certain disease conditions (Silver, 1974). A decrease in serum ChE levels has been associated with some forms of anemia, liver disease, carcinoma, epilepsy, eczema, rheumatic fever, typhus, tetanus, kwashiorkor, and tuberculosis, while an increase in serum ChE levels has been associated with diabetes, asthma, obesity, kidney disease, hyperthyroidism and hyperlipoproteinemia (abnormal lipid metabolism). Patients with mental abnormalities, including psychopathic patients, had raised levels of serum ChE more often than control subjects. A decrease in the ChE's of both serum and erythrocytes has been noted in cases of renal ischaemia. Spastic children have higher activities towards ACh in serum than either mongoloid or moronic children, who have activities within normal limits. ChE levels in erythrocytes are below normal in schizophrenics. According to a study reported by Silver, the stress associated

with sitting university examinations can cause an increase in ChE levels in whole blood from healthy students. Alcohol and nicotine have been shown to depress brain AChE levels. It is not clear whether or to what extent individuals with these diverse conditions may have altered susceptibility to ChE-I pesticides. Individuals with low ChE activities may be at high risk.

Methodology

Several reviews of the methods for determining ChE activity are available [Silver (1974), Wills (1972), Augustinsson (1971)]. These methods vary in their accuracy, complexity, efficiency, and units measured. The conditions to which the enzyme is subjected differ according to the method used, and thus the results obtained by different methods are not directly comparable (Silver, 1974). Temperature, pH, substrate, buffer, and contaminants (e.g. salts, detergents) can affect the measurement of ChE activity. Activities may be reported as the change in pH in a weak buffer solution due to the release of acetic acid from ACh (e.g., Michel method); the amount of CO_2 evolved from bicarbonate by acetic acid released from ACh (e.g. Warburg manometric method); the volume of sodium hydroxide needed to hold the pH of the reaction mixture constant (e.g. Hall and Lucas continuous titration or pH stat method); or the amount of substrate (e.g., ACh,. BzCh (benzoylcholine), BuCh) hydrolyzed. Reporting ChE activity in terms of micromoles of substrate hydrolyzed per ml per minute makes comparisons more valuable (Cornish, 1971). Analysis of ChE following inhibition by carbamates requires special methods,

since the complexes formed with ChEs by all but a few of the carbamates are readily dissociable by dilution (Wills, 1972).

As previously mentioned, it is important that ChE activity be assayed frequently to detect peak inhibition during chronic studies of ChE-I by pesticides. Different pesticides reach peak ChE-I at different times in different species. Also, a lack of ChE-I should not be considered as a true absence of effect when ChE is measured at only one site (e.g., plasma). For example Cornish (1971) cites a study with guthion in which brain ChE activity was inhibited by 60% while serum ChE was unaffected. Measuring only serum ChE would have resulted in a serious underestimation of the risk posed by guthion. Brain and blood contain both AChE and BuChE, so it is recommended that activities be determined separately. Reporting activities in terms of percent of mean control activity, while permitting comparisons between levels, does not provide information on the pre-exposure condition of the animal nor the variability involved. Even in studies using laboratory animals, where one would expect little variation, considerable variation may be present (e.g., see figures III 1-4). Gage (1967) has pointed out the limitations of comparing individual ChE activities to a population average since the coefficients of variation in control studies range from 10 to 25 percent. A much better control is a series of pre-exposure levels on the individual or animal to be studied. Reporting the variability encountered in a study provides more flexibility in the choice of dose-response assessment methods (see Section III of this report).

Table III-1 Review of Proposed Uncertainty Factors (UF)

UF	Proposed by	Applicability
10	NAS (1977)	Valid experimental data from studies on prolonged ingestion by man and when there is no evidence of carcinogenicity.
10	EPA (1986)	As above, except generalized to "prolonged exposure," (not just ingestion) and specified "average healthy humans." This factor is intended to account for the variation in sensitivity among the members of the human population.
10	EPA (Federal Register, 1981)	NOEL values based on ChE-I for organophosphorus and carbamate pesticides.
100	NAS (1977)	Cases where data on prolonged human studies are not available or are scanty (e.g. only acute exposures), where valid results on long term animal feeding studies are available with several species, and where there is no evidence of carcinogenicity.
100	EPA (1986)	As above, except generalized to exposures other than feeding/ingestion. This factor (10 x 10) is intended to account for the uncertainty in extrapolating animal data to the case of humans (10x) and for the variation in sensitivity among humans (10x). OR LOAEL instead of a NOAEL from valid experimental results from studies using prolonged exposure to average healthy humans.
100	FDA (Dourson and Stara, 1983)	Data from chronic animal studies. This factor is intended to account for intra-(human) and inter-(animal to human) species variability, and intrastrain variability in response to the toxicity of a chemical, allowance for sensitive human subpopulations due to illness as compared to healthy experimental animals, (as above), as well as possible synergistic action of any one of the many intentional or unintentional food additives or contaminants in the human diet.

100	FAO/WHO (1962, 1973 in Dourson and Stara, 1983)	To account for differences in body size of the laboratory animal vs. man, and differences in food requirements varying with age, sex, muscular expenditure, and environmental conditions, differences in water balance of exchange between the body and its environment, differences in hormonal functions and how they modify food intake, and differences in susceptibility among species. Establishes an "unconditional" ADI. ("Conditional" ADIs employ UFs > 100 due to uncertainties in animal data or in regard to the purity of the test substance).
100	WHO Expert Comm. for Pesticide Residues (Dourson and Stara, 1983)	To account for differences in susceptibility between animals and humans, variations in human sensitivities, small sample size, difficulties in estimating human intake, and the possibility of synergistic action among chemicals
1000	NAS (1977)	Cases where there are no long-term or acute human data, where animal data are scanty, and where there is no indication of carcinogenicity.
	EPA (1986)	Cases when extrapolating from less than chronic results on experimental animals when there are no useful long-term human data. This factor (100x10) is intended to account for the uncertainties cited with use of the 100x factor, as well as the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs (10x). OR Cases when using a LOAEL instead of a NOAEL from valid chronic animal studies where data on long-term human exposures are not available or are inadequate.
1000	FDA (Dourson and Stara, 1983)	Cases when extrapolating from subchronic animal NOELs or NOAELs, where data is available from two species.
2 ,000	FDA (Dourson and Stara, 1983)	As above, except where data are available for only one species.

10,000	EPA (1986)	Apply when extrapolating from less than chronic results in experimental animals when there are no useful long-term human data, and where using a LOAEL instead of a NOAEL.
additional 1-10x	EPA (1986)	Use professional judgment to determine this additional UF, depending on other aspects of the study not explicitly treated above; e.g., number of species tested and the slope of the dose-response curve. The default value for this UF, called the modifying factor (MF), is one.

Table III-2: Ratio of NOELs Implying Interspecies Variability of ChE-I*

Pesticide	Species Compared	Subchronic Study[a] Plasma	RBC	Brain	Chronic Study[b] Plasma	RBC	Brain
Parathion	Rat / Dog	≥0.4	≥0.4	---	≥2.5[c]	---	≥25.0[c]
Malathion	Rat / Human	---	21.7[d]	---			
Chlorthiophos	Rat / Dog	≥1	4	≤1.9	0.4[c]	3.2[c]	---
Metasystox	(Studies deficient)						
Ethion	Rat / Dog	6	---	---			
Chlorpyrifos	Rat / Monkey	1.9[e]	1.9[e]	---			
	Rat / Dog				10	1	1
Carbofuran	Mouse / Rat				3	3	3
	Mouse / Dog				6[f]	6[f]	---

* Comparisons should be interpreted cautiously, since methodology (other than length of study) may be different between compared studies. See Appendix III-3.

Pesticide	Species Compared	Subchronic Study Plasma	RBC	Brain	Chronic Study Plasma	RBC	Brain
Dimethoate	Rat / Dog	---	3.2	---			
	Rat / Human	---	3.5[g]	---			
Aldicarb sulfoxide	Dog / Rat	≥2[h]	---	---			

a Subchronic studies are 90 day feeding studies unless otherwise noted.
b Chronic studies are 2 year feeding studies unless otherwise noted.
c Duration of dog study is one year, rat study is 2 years.
d Duration of human study is 47 days, rat study is 33 days.
e Doses in monkey study administered by gavage.
f Duration of both studies are 6 months.
g Dose in rat study administered by intraperitoneal injection.
h "It does not appear that the doses were established" in rat study, and plasma ChE-I was measured in dog study after 1 month, although it was a 3 month study.

Table III-3: Ratio of NOEL's Implying Interspecies Variability of ChE-I, from Edson (1964) Data*

Pesticide	Species Compared	Study Length (days)	RBC	Plasma	Considerations
Schradan	pig / rat	102 / 37	1	5[a,b] (1,25)[c]	Rats dosed by intraperitoneal injection. Only 2 female pigs/dose group.
	pig / rat	102 / up to 273	8	2[a] (10)[b]	Only 2 female pigs/dose group. Maximum ChE-I at 3rd week in rat.
	pig / human	102 / 44	1.4	---	Human ChE activity reported on "whole blood" basis, but levels in RBC were noted to be more sensitive than plasma; thus comparison is for RBC. Dose considered as human NOEL by Edson caused 25% ChE-I. Human doses reported in mg; individual body weights not given, but average body weight inferred as 70 kg.
	human / rat	44 / up to 273	5.6[a] (1.1)[b]		See above.
Dimefox	pig / rat	133 / up to 287	2.4	.5[a] (4)[b] (1.6,.1)[c]	Only 2 female pigs/ dose group. All rats female. ChE-I reached a maximum after 4 weeks for both species. Variable dosing schedule for pig.

Pesticide	Species Compared	Study Length (days)	RBC	Plasma	Considerations
(Dimefox)	pig / human	133 / 70	3		Same as pig/rat, dimefox and pig/human, schradan considerations (above), except human doses reported on mg/kg basis.
	rat / human	up to 287 / 70	1.5		See above.
Parathion	pig / rat	up to 122 / 84	48	960[a] (96[b])	Dose considered as rat NOEL by Edson caused 54% ChE-I. (RBC ratio is 480 if consider this dose the LOEL.) Maximum ChE-I at 4th week in rats and 6th week in pigs. Variable dosing schedule in pig.
	pig / human	up to 122 / up to 70	24	480	Variable dosing schedules in human and pig. Human doses reported in mg; individual body weights not given but average body weight inferred to be 68 kg. Doses multiplied by 5/7 since given 5 days/week. As above, measured in whole blood, but this time levels in plasma were noted to be more sensitive (LOEL caused 16% RBC ChE-I and 37% plasma ChE-I). Only 2 female pigs/dose group. Maximum ChE-I at 7th week in humans and 6th week in pigs.

Pesticide	Species Compared	Study Length (days)	RBC	Plasma	Considerations
Parathion	human / rat	up to 70 / 84	20[a] (2[b])	---	Same as above.

* Determined in feeding/oral studies unless otherwise noted

a Ratio obtained by considering dose(s) described as causing "slight" ChE-I as LOEL(s) (i.e., "slight" ChE-I is considered an effect).

b Ratio obtained by considering dose(s) described as causing "slight" ChE-I as NOEL(s) (i.e., "slight" ChE is not considered an effect).

c Ratio obtained by considering dose(s) described as causing "slight" ChE-I as a NOEL in one species and a LOEL in the other species.

Table III-4: Examples of Species Differences in Metabolism of ChE-Inhibiting Pesticides*

Pesticide	Species Compared	Factor Measured	Interspecies Variation
malathion	human / rat(male)	rate of metabolism (% degraded in liver homogenate/h/20mg)	14.0 / 7.1 = 2
parathion	rat(male) / human	"	99.8 / 98 = 1
carbaryl	rat(male) / human	"	63.1 / 14.8 = 4
malaoxon	rat(male) / human(male)	percentage hydrolyzed (based on AChE-I) after incubation of pesticide with sera	61 ±9 / 1 ±2 = 61
malaoxon	dog(male) / human(male)	"	6 ±2 / 1 ±2 = 6
malaoxon	human(female) / human(male)	"	5 ±2 / 1 ±2 = 5
malaoxon	cat(male) / human(male)	"	100 ±0 / 1 ±2 = 100
paraoxon	rat(male) / human(male)	"	32 ±7 / 22 ±4 = 1
paraoxon	dog(male) / human(male)	"	94 ±4 / 22 ±4 = 4
paraoxon	human(male) / human(female)	"	22 ±4 / 13 ±7 = 2

* From Calabrese(1983) and Hollingworth (1971)

Pesticide	Species Compared	Factor Measured	Interspecies Variation
paraoxon	rat(male) / human	I_{50} (concentration required to effect a 50% reduction) for plasma ChE (10^{-7}M)	1.8 / 1.1 = 2
paraoxon	human / dog	"	1.1 / 0.3 = 4
paraoxon	pig / human	"	7.9 / 1.1 = 7

EXPLANATION OF FIGURES

Figures III-1 through III-4 are plots of the data given in Appendix III-2, Table 2, of mean activities (± standard deviation) of plasma, erythrocyte (RBC) and brain cholinesterase in dogs fed parathion for one year. Activities are shown after two, four and twelve months for males and famales.

Figure III-1: RBC and Brain ChE in Male Dogs

Figure III-2: RBC and Brain ChE in Female Dogs

Figure III-3: Plasma ChE in Male Dogs

Figure III-4: Plasma ChE in Female Dogs

Figures III-5 through III-10 are semi-log plots of the same data described above (not brain ChE).

Figure III-5: RBC and Plasma ChE in Males (measured at 12 months)

Figure III-6: RBC and Plasma ChE in Females (measured at 12 months)

Figure III-7: RBC and Plasma ChE in Males (measured at 4 months)

Figure III-8: RBC and Plasma ChE in Females (measured at 4 months)

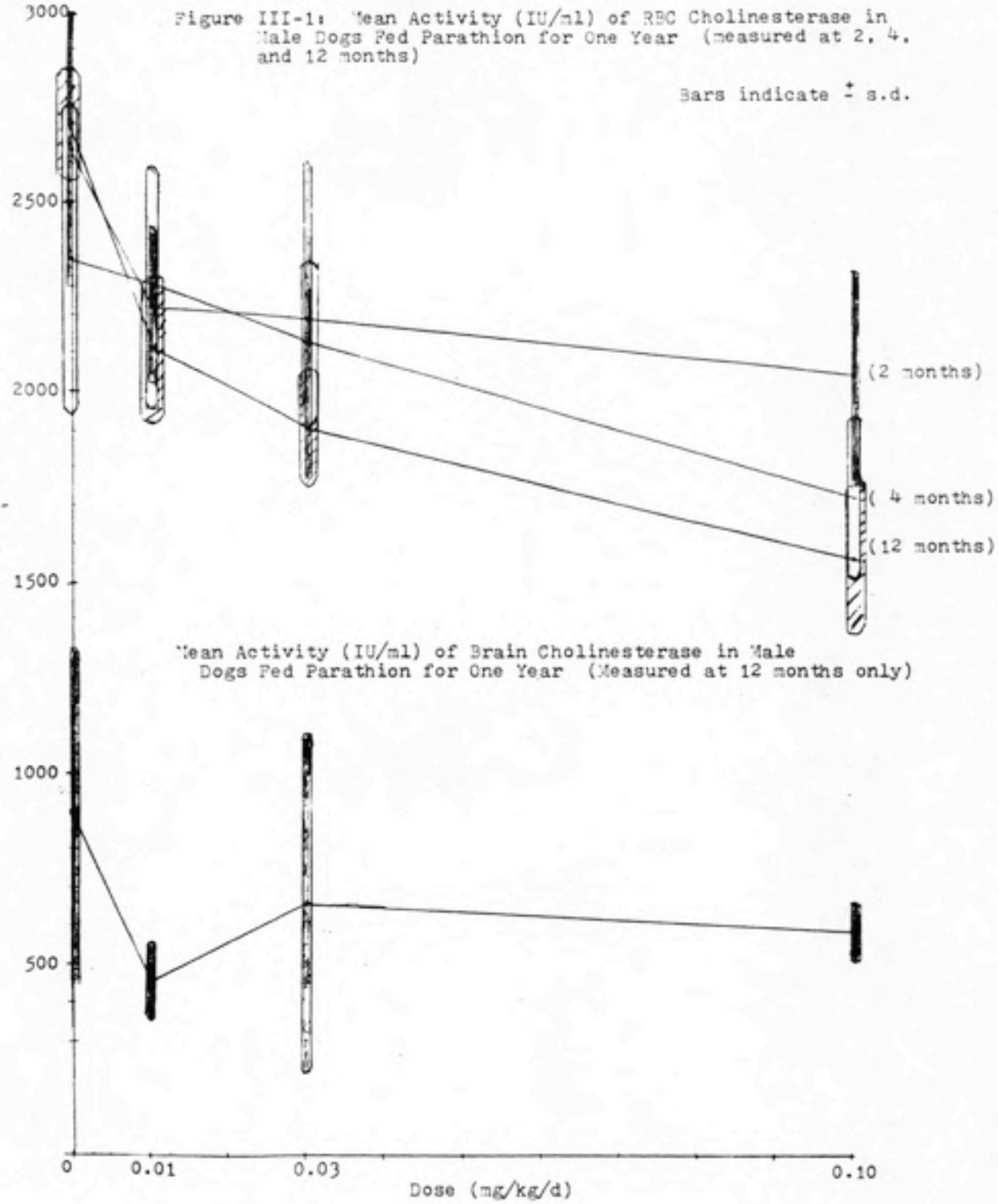
Figure III-9: RBC and Plasma ChE in Males (measured at 2 months)

Figure III-10: RBC and Plasma ChE in Females (measured at 2 months)

Figure III-11 is a semi-log plot of the data given in Appendix III-2, Table 1 of mean activities (as percent of control) of plasma and RBC cholinesterase in rats fed parathion for two years. Activities are shown at termination (26 months for males and 28 months for females).

Figure III-1: Mean Activity (IU/ml) of RBC Cholinesterase in Male Dogs Fed Parathion for One Year (measured at 2, 4, and 12 months)

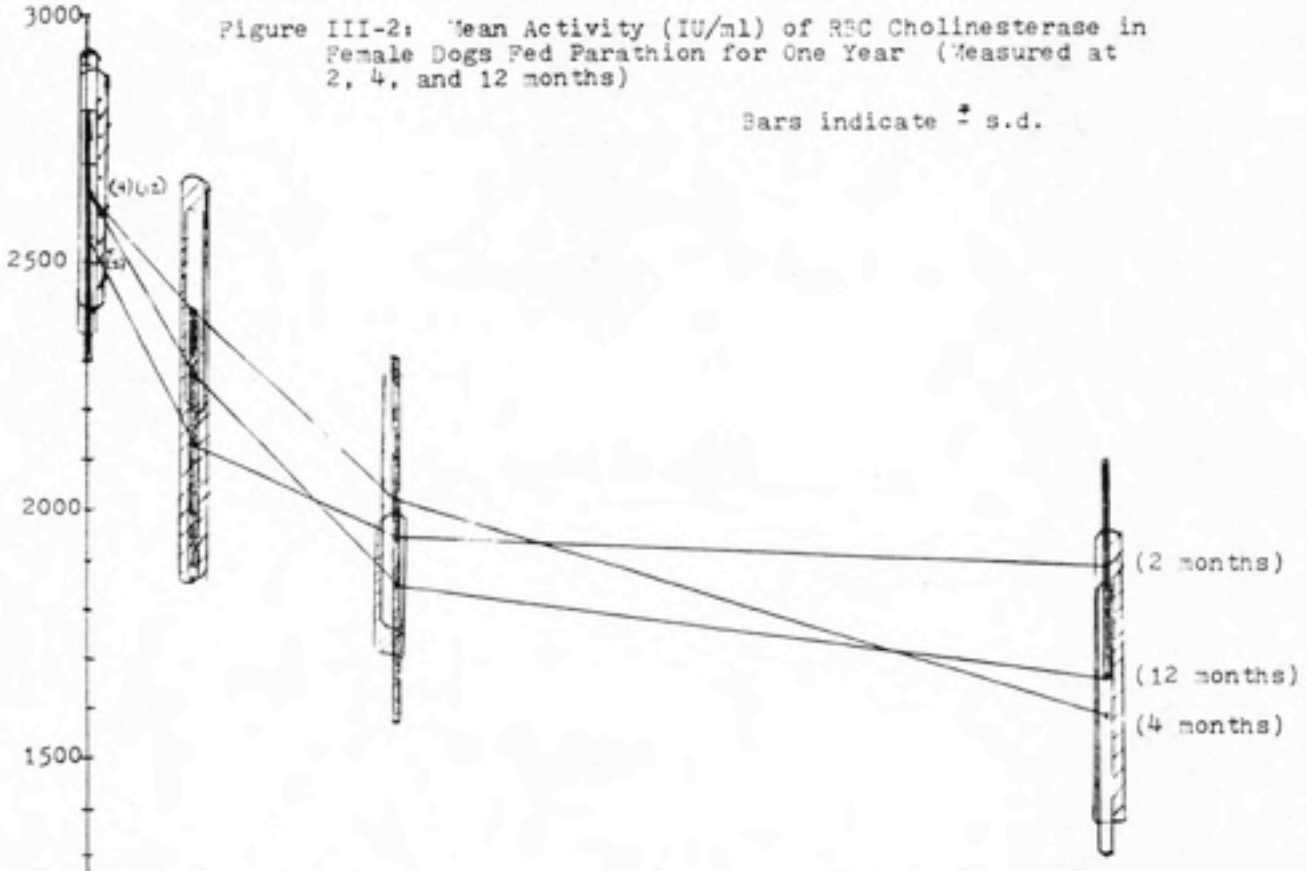
Bars indicate ± s.d.



Mean Activity (IU/ml) of Brain Cholinesterase in Male Dogs Fed Parathion for One Year (Measured at 12 months only)

Figure III-2: Mean Activity (IU/ml) of RBC Cholinesterase in Female Dogs Fed Parathion for One Year (Measured at 2, 4, and 12 months)

Bars indicate ± s.d.



Mean Activity (IU/ml) of Brain Cholinesterase in Female Dogs Fed Parathion for One Year (Measured at 12 months only)

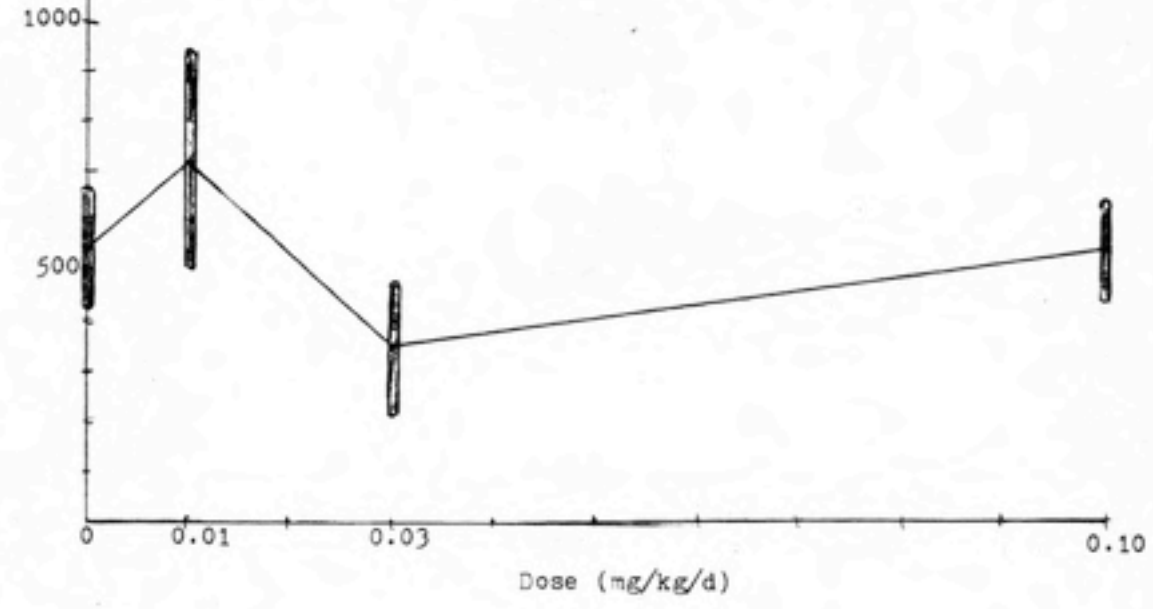


Figure III-3: Mean Activity (IU/ml) of Plasma Cholinesterase in Male Dogs Fed Parathion for One Year (Measured at 2,4 and 12 months)

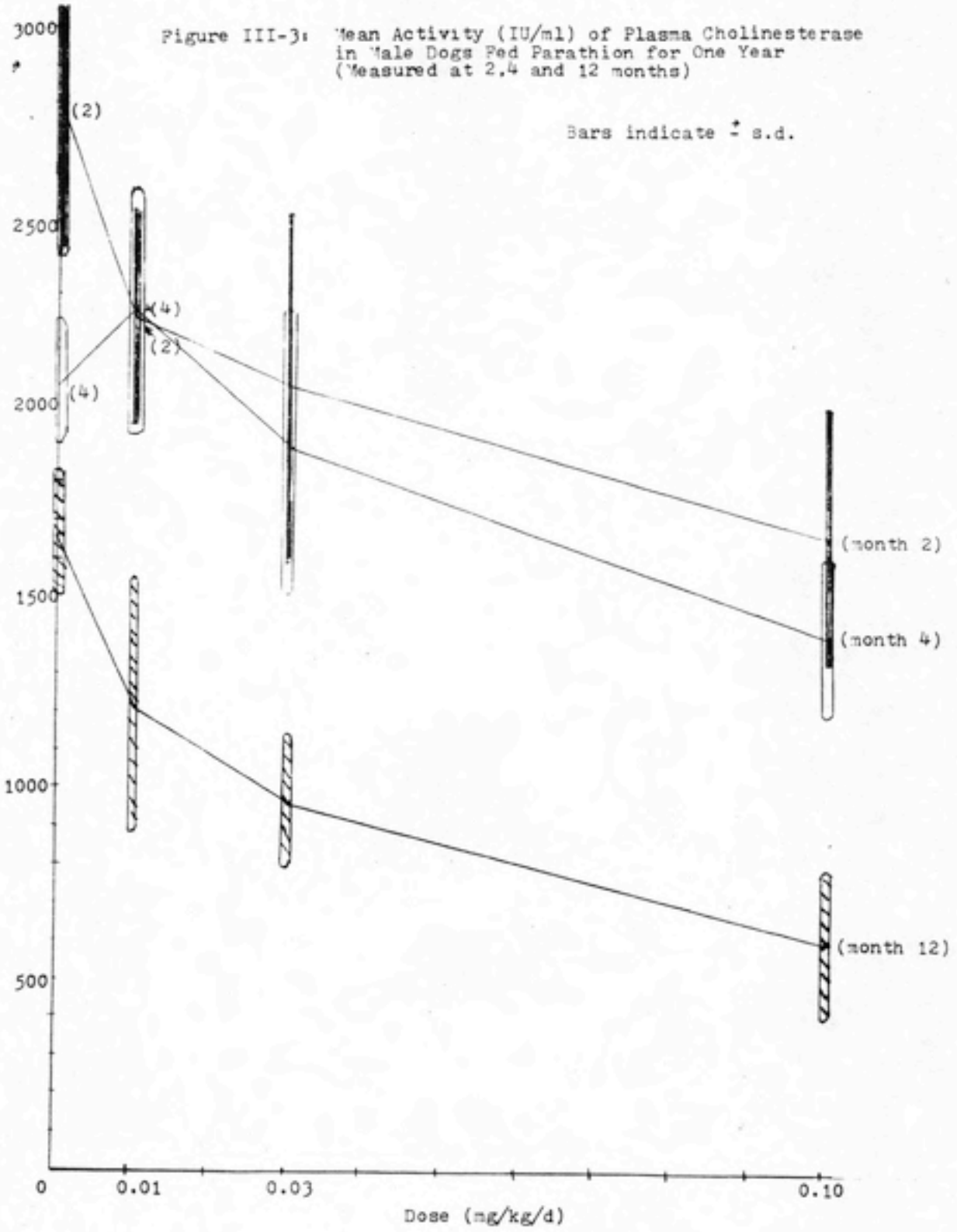


Figure III-4: Mean Activity (IU/ml) of Plasma Cholinesterase in Female Dogs Fed Parathion for One Year (Measured at 2,4, and 12 months)

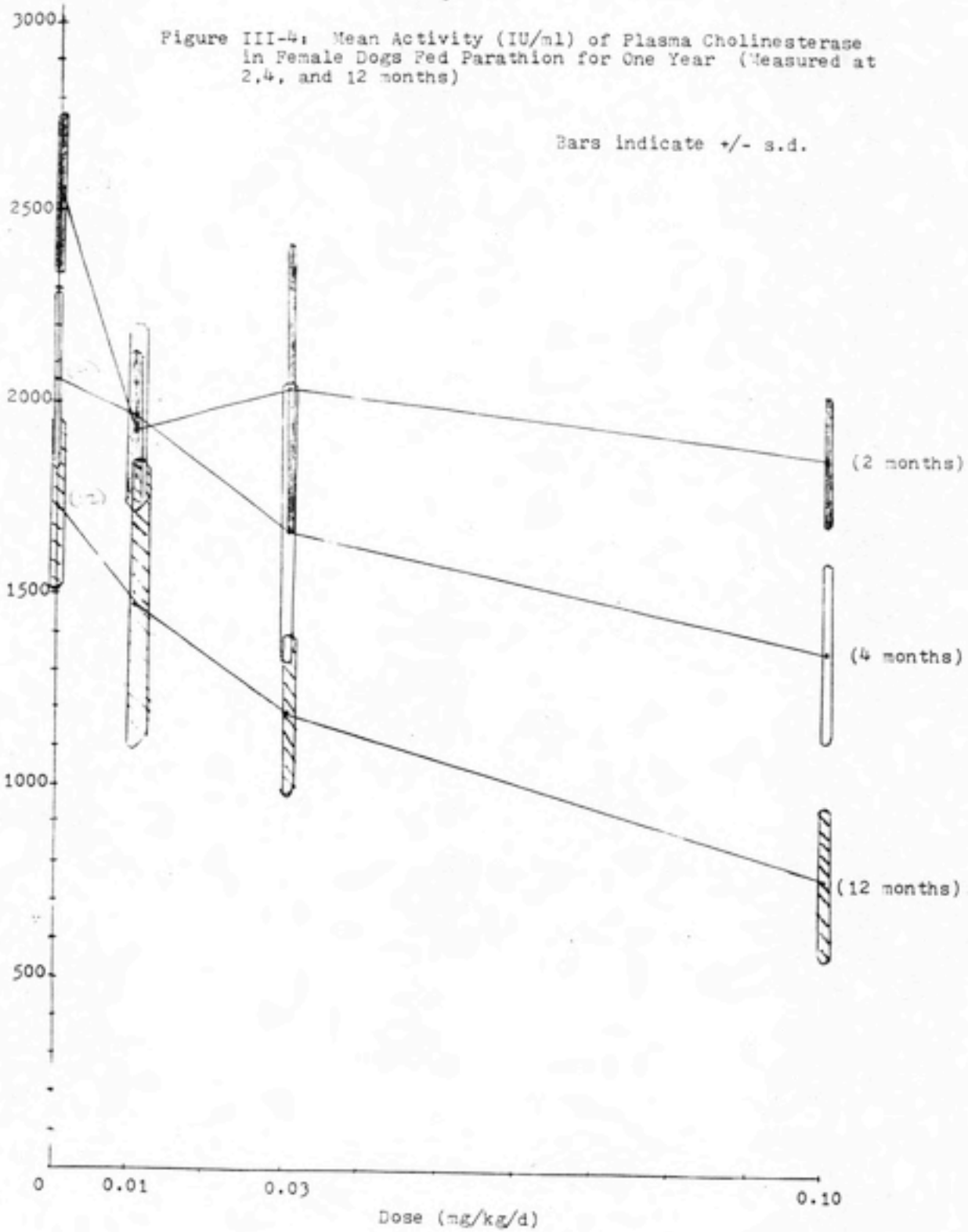


Figure III-5

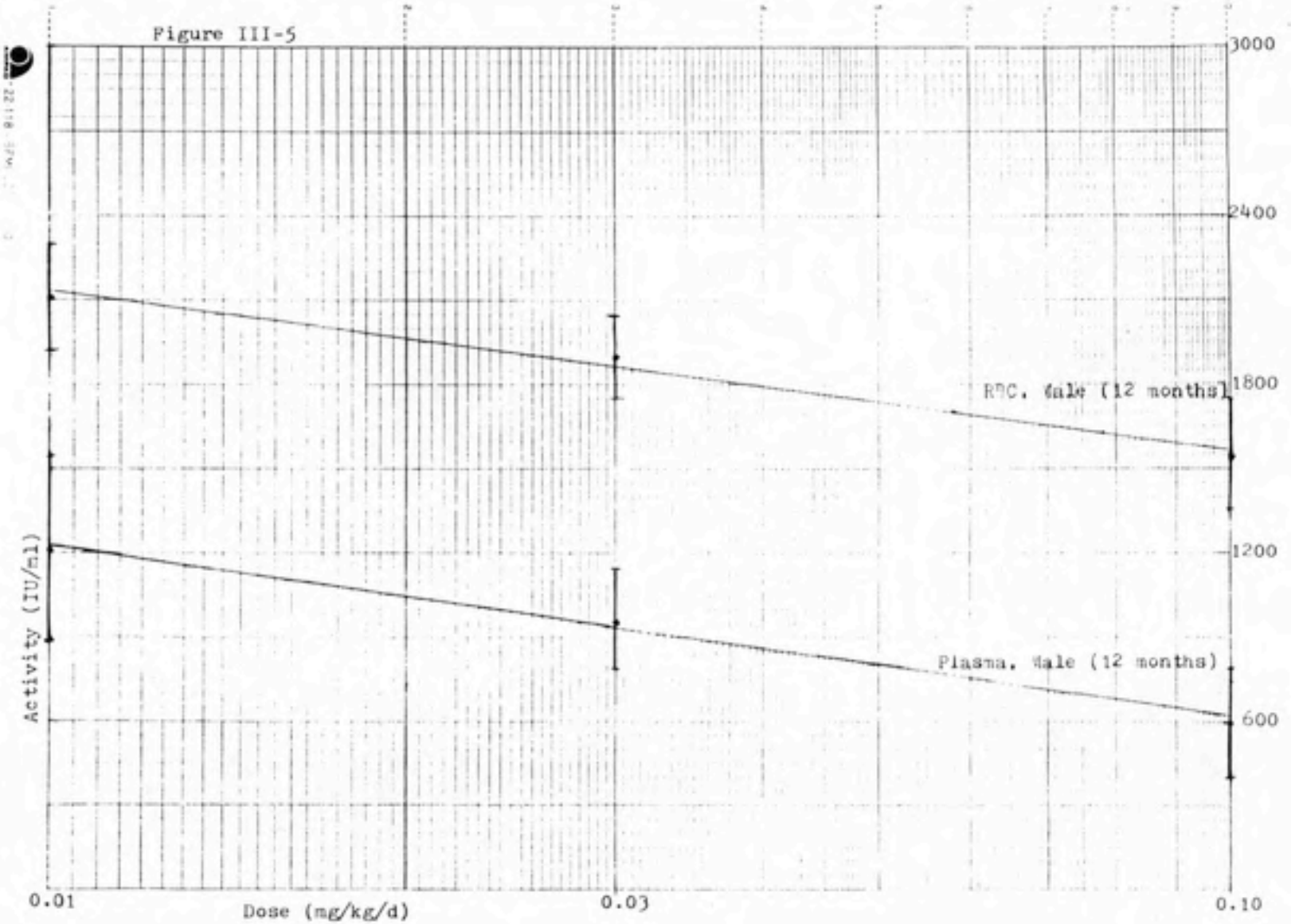

3000
2400
1800
1200
600
RBC, Male (12 months)
Plasma, Male (12 months)
Activity (IU/ml)
0.01
Dose (mg/kg/d)
0.03
0.10

Figure III-6

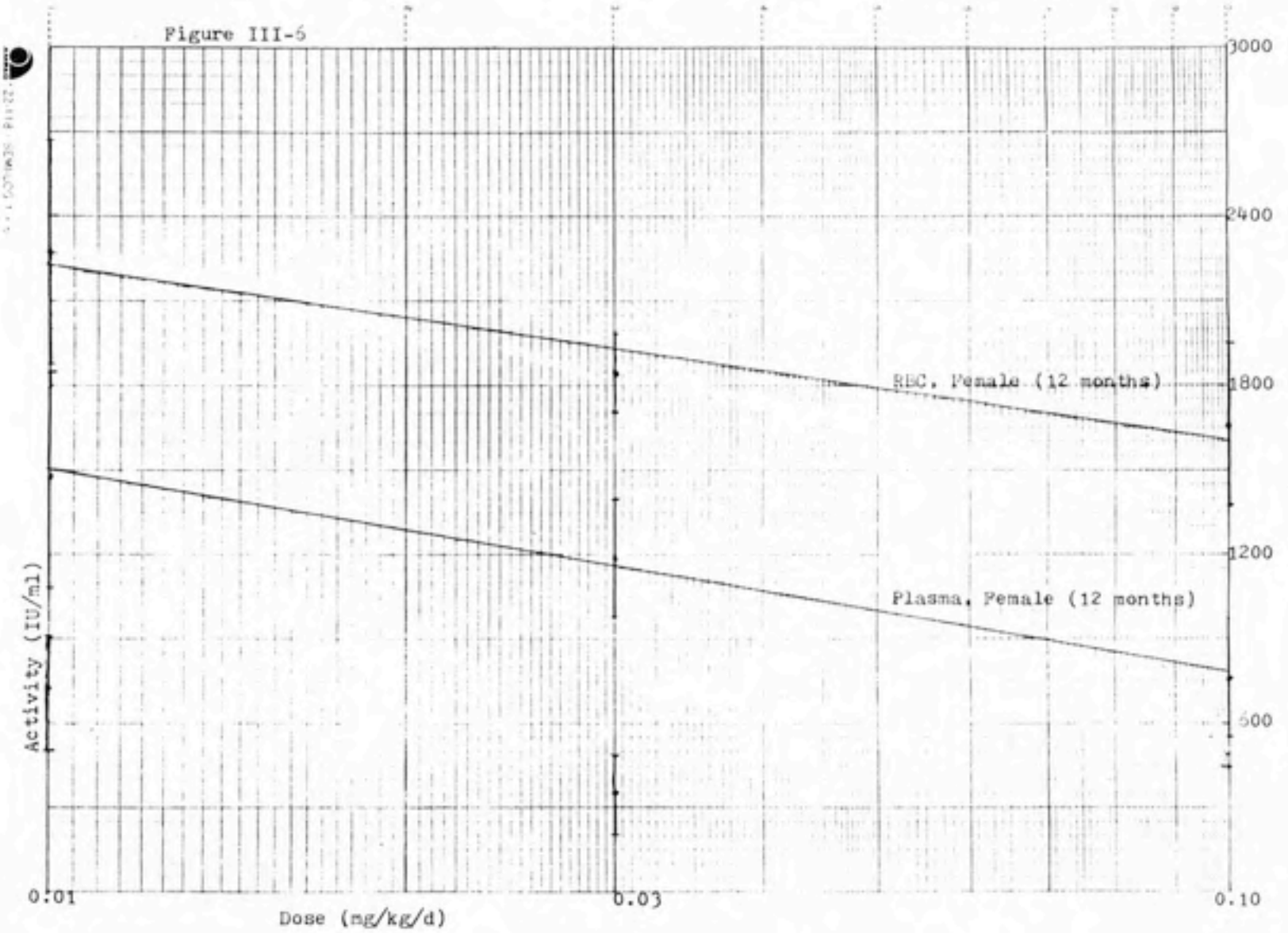

Activity (IU/ml)
3000
2400
1800
1200
600
RBC, Female (12 months)
Plasma, Female (12 months)
0.01
0.03
0.10
Dose (mg/kg/d)

Figure III-7

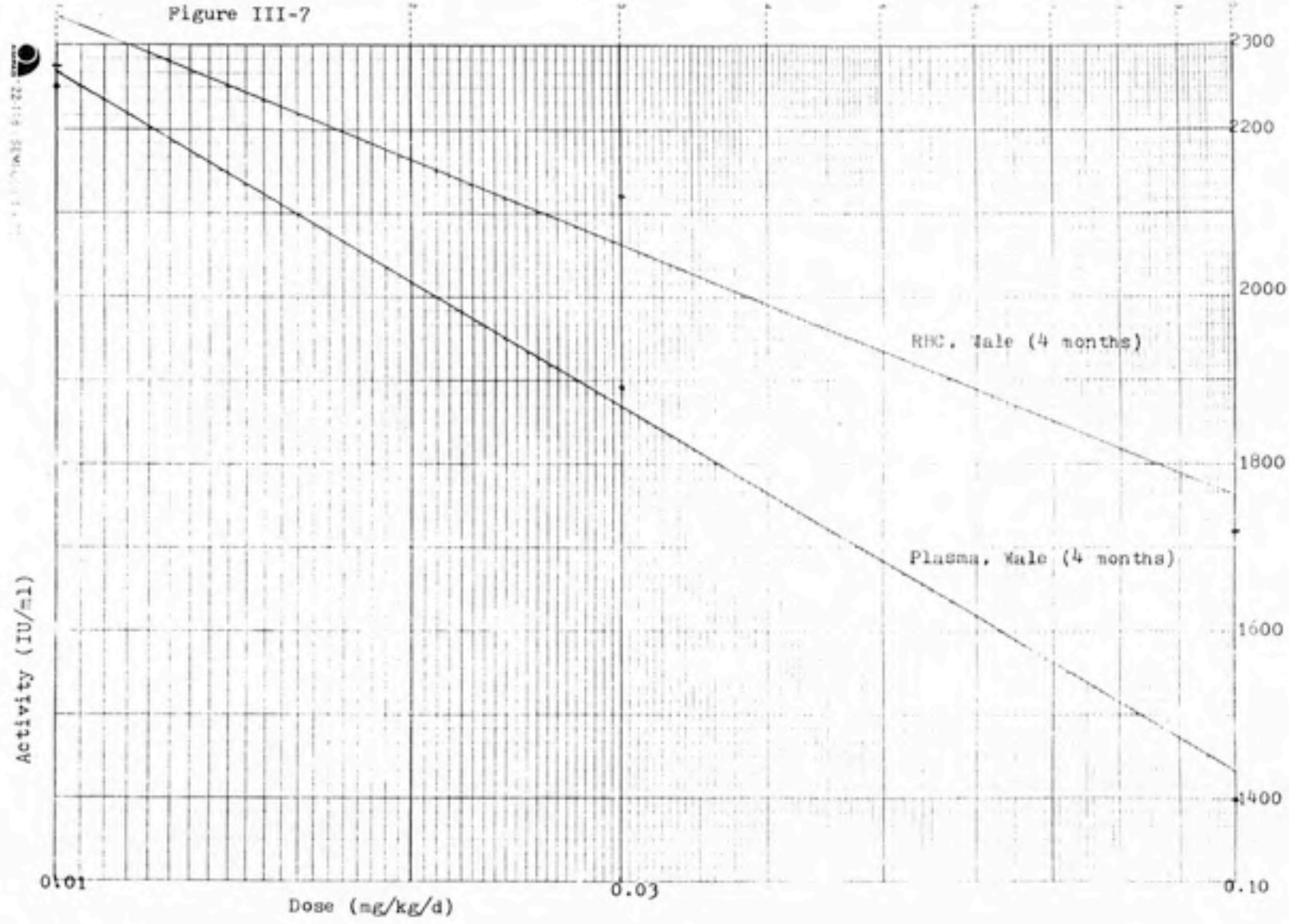

2300
2200
2000
1800
1600
1400
RHC, Male (4 months)
Plasma, Male (4 months)
Activity (IU/ml)
0.01
0.03
0.10
Dose (mg/kg/d)

Figure III-8

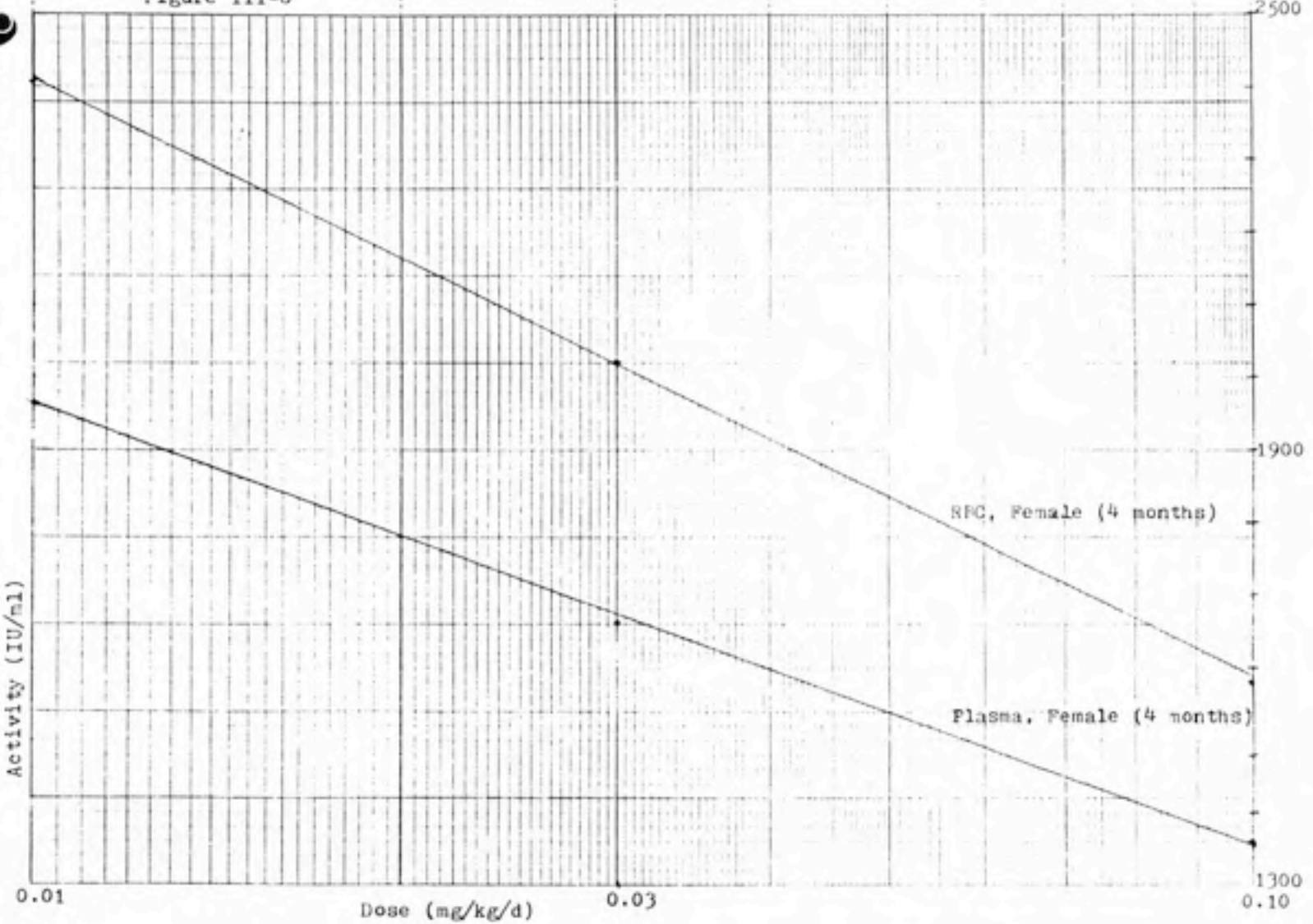

2500
1900
1300
RBC, Female (4 months)
Plasma, Female (4 months)
Activity (IU/ml)
0.01
0.03
0.10
Dose (mg/kg/d)

Figure III-9

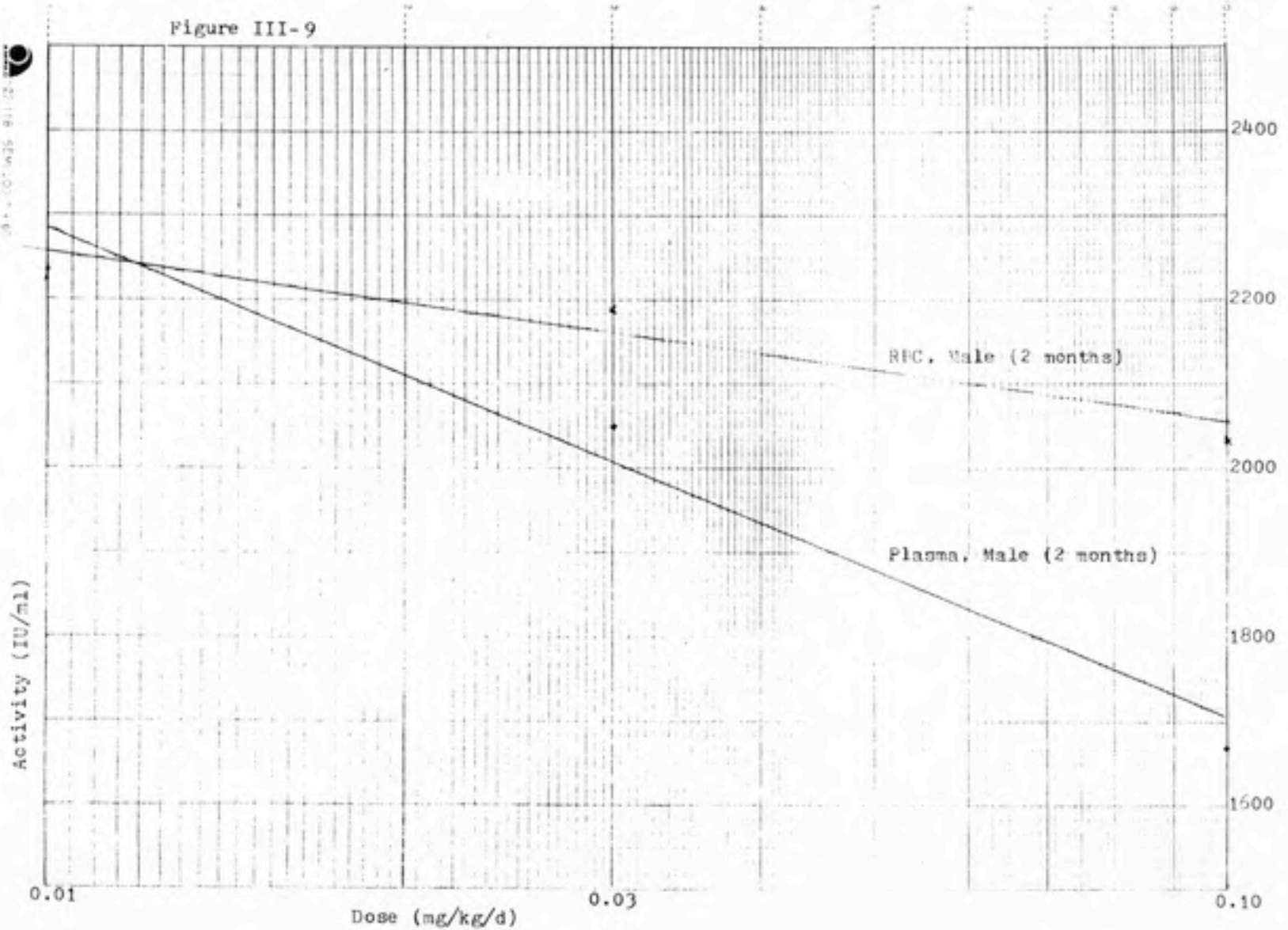

2400
2200
2000
1800
1600
RBC, Male (2 months)
Plasma, Male (2 months)
Activity (IU/ml)
0.01
0.03
0.10
Dose (mg/kg/d)

Figure III-10

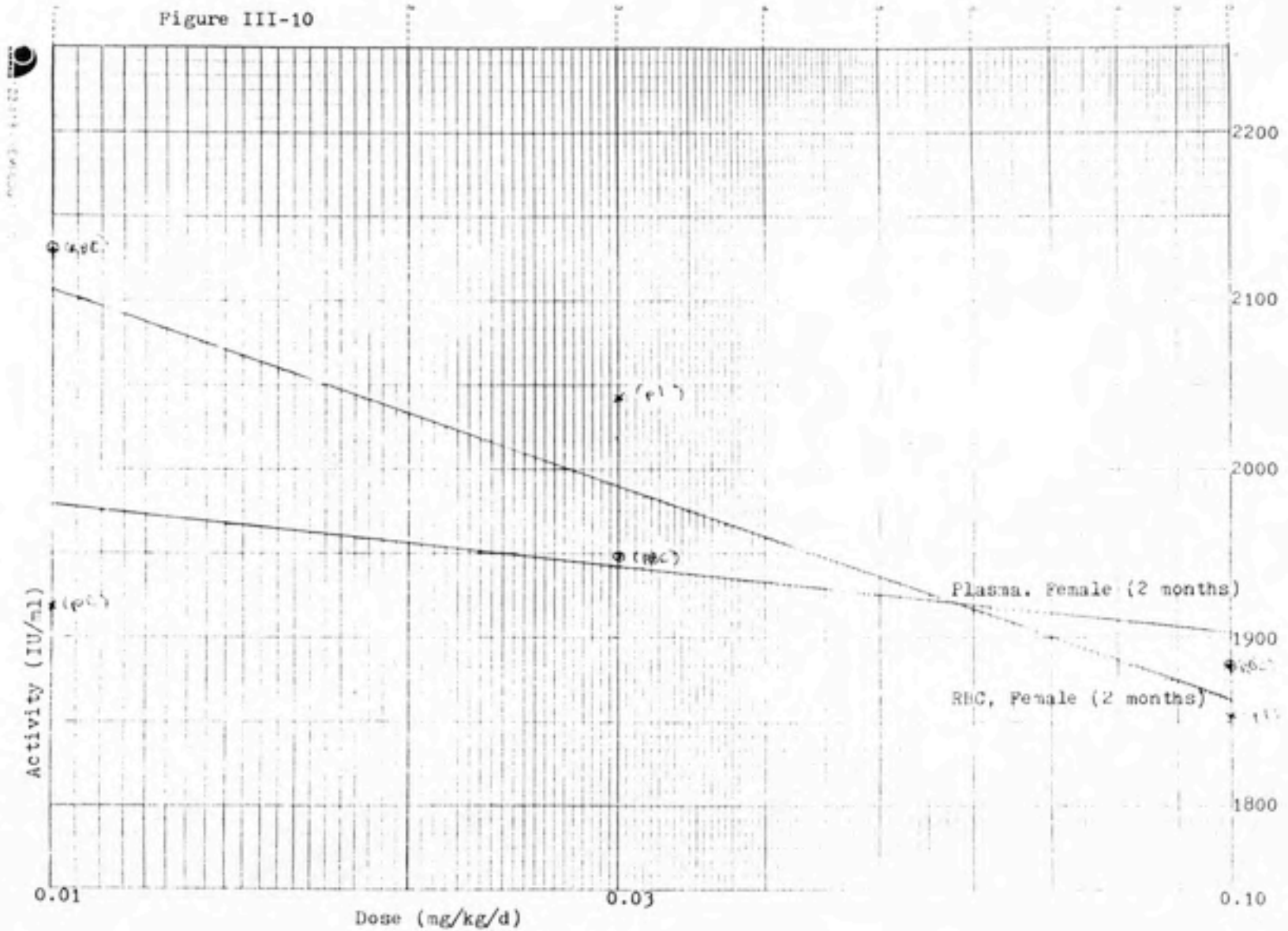
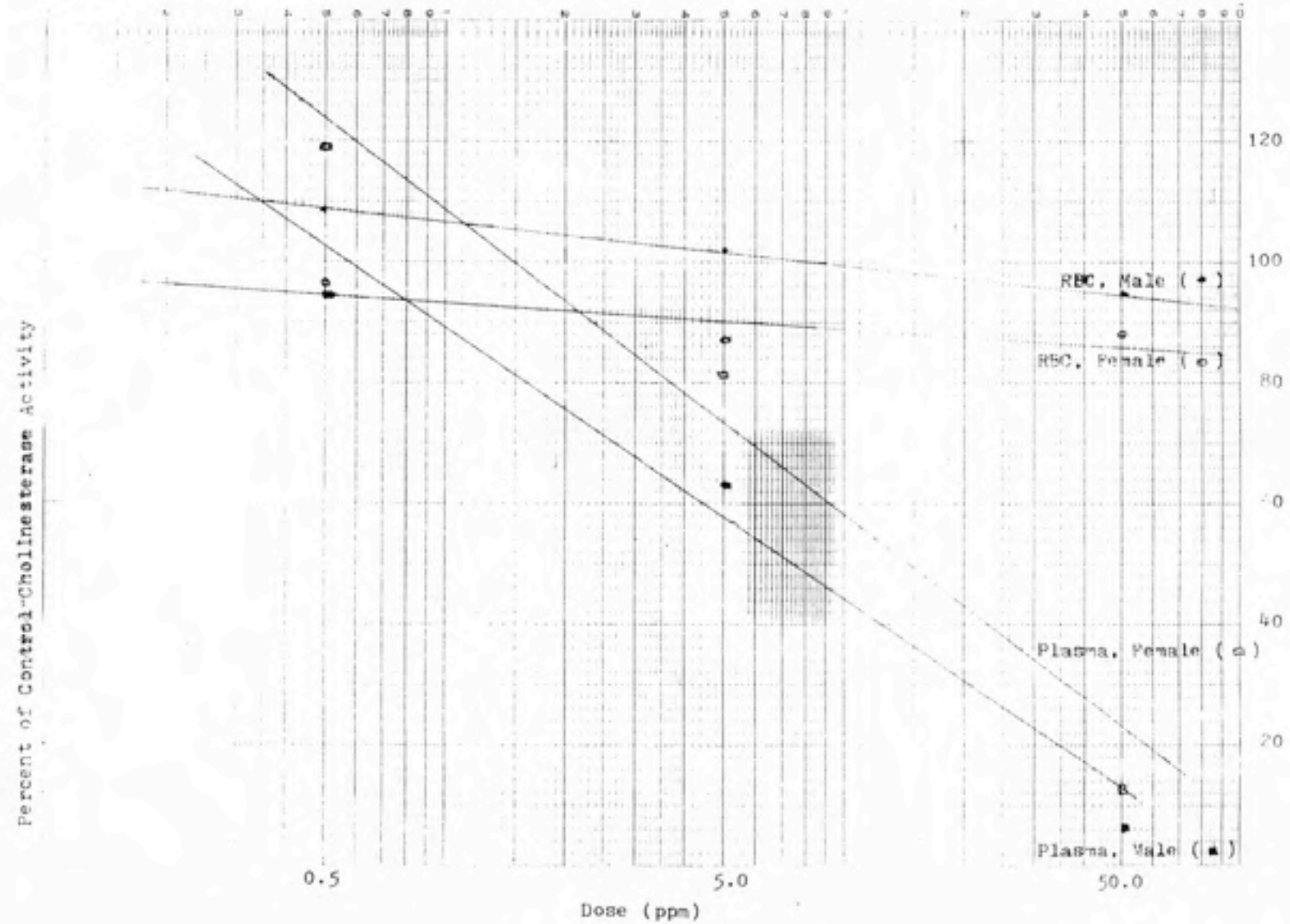


Figure III-11: Mean Cholinesterase Activity in Rats Fed Parathion (measured at 25 - 28 months)



Appendix III-1: Approaches to Dose Response Assessment of Parathion

1. NOEL-UF Approach

A. Current EPA ADI

Current ADI of 0.005 mg/kg/day was set at the 1965 Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residue. "It appears ... that this level has been established on the basis of a "NOEL" of 1.0 ppm or 0.05 mg/kg/day, for ChE-I, generated from a long-term feeding study in the rat, using a 10X safety factor. No data evaluation records are available, and no reference has been made to this study in the Toxicology Branch files. Thus the validity of the study could not be determined." [emphasis added] (Ghali, 1985)

B. ADI Using Most Sensitive Species

Can not be determined since NOEL was less than 0.4 ppm (less than 0.01 mg/kg/day). Data is from chronic feeding study in dogs (Pharmacopatics Res. Inc., Report No. 7828, 8/20/81).

C. ADI Using Most Sensitive Species for which a NOEL was Observed

Using a 2 year feeding study in rats (Biodynamics, Inc. Report No. BD78-0005, 1/23/84), [data given in Appendix III-2]

NOEL = 0.5ppm = 0.25mg/kg/day

based on ChE-I significantly different from control value ($p < 0.05$) at next highest dose (5ppm).

This occurred in plasma ChE of females at 12 months (but not other times measured) and in RBC (at 6 and 12 months) and plasma (at 18 months) in males (measured ChE at 6, 12, 18 months and at termination)

Uncertainty Factor Choices and Resultant ADIs:

1. 10x (based on EPA OPP) = 0.0025 mg/kg/d
2. 100x (based on NAS, EPA, FDA, FAO/WHO, WHO) = 0.0025 mg/kg/d

2. BD-UF Approach

A. ADI Using Most Sensitive Species*

The model $y = \beta_0 + \beta_1$ (ln dose) for dose > 0 was fitted to the data in Appendix III-2 to obtain the BD, defined here as the dose causing a 10% inhibition of ChE. This model was chosen for its simplicity and because the data fit a straight line when log dose was plotted against either plasma or RBC ChE activity (1U/ml) for each time period measured (Figures III-5 to III-10). Such log dose plots are common in toxicology (Doull, 1980). Other data of ChE-I by parathion supported this model (Figure III-11). The main disadvantage to this model is that it can not directly accomodate the response at a zero dose (since ln(0) is undefined), and it must be assumed that the dose-response

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* Since this was also the only data set for which standard errors were available, it was the only data set to which the BD-UF approach could be applied.

relationship remains linear to the BD. However, the control response value is indirectly incorporated in the estimation of the confidence limits around the response at the BD.

Using the data on male beagle dogs fed parathion (plasma ChE measured after 12 months of feeding), the estimated dose causing a 10% inhibition of plasma ChE (ED_{90}) is 4.2×10^{-3}. The 95% upper and lower confidence limits are 3.7×10^{-2} and 1.1×10^{-4}. According to Crump the BD is the statistical lower confidence limit to ED_{90}, or 1.1×10^{-4}. To determine a RfD, an UF must be applied.

3. Linear Extrapolation

A. ADI Using Most Sensitive Species*

Using the same data on plasma ChE-I measured at the 12th month for beagle dogs fed parathion, the ED_{90} was calculated (from 2A) as 4.2×10^{-3}. The upper confidence limit on the amount of plasma ChE-I associated with this dose is U=43.5. If it is desired to control the response to 5% ChE-I (R = 0.05) compared to controls, then an UF = U/R = 43.5/0.05 = 870 is required. The RFD would then be $4.2 \times 10^{-3}/870 = 4.8 \times 10^{-6}$, based on plasma ChE-I measured at twelve months in male beagle dogs. Determining an ED_{80} or ED_{70} and using R = .10 or .20 would result in an RfD closer to that obtained by the NOEL-UF method.

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* Since this was also the only data set for which standard errors were available, it was the only data set that linear extrapolation could be applied.

Following are ED_{90}'s and RfD's for plasma and RBC ChE-I measured at two, four, and twelve months for males and female beagle dogs (based on R = .05, as above):

Characteristics (Sex, site, time ChE-I measured)	ED_{90}	Confidence Limits (95%) for ED_{90}	U=Upper Confidence Limit on Response at ED_{90}	UF=U/R	"RfD"= ED_{90}/UF
Male, plasma, 12	4.2×10^{-3}	$(1.1 \times 10^{-4}, 3.7 \times 10^{-2})$	43.5	870	4.8×10^{-6}
Male, plasma, 2	2.9×10^{-2}	$(7.0 \times 10^{-3}, 8.1 \times 10^{-2})$	68.4	1368	2.1×10^{-5}
Male, plasma, 2	3.5×10^{-3}	$(4.2 \times 10^{-6}, 7.5 \times 10^{-2})$	50.8	1016	3.4×10^{-6}
Female, plasma 12	8.6×10^{-3}	$(4.7 \times 10^{-4}, 5.2 \times 10^{-2})$	48.3	966	8.9×10^{-6}
Female, plasma, 4	1.5×10^{-2}	$(9.3 \times 10^{-4}, 7.9 \times 10^{-2})$	62.5	1252	1.2×10^{-5}
Female, plasma, 2	1.7×10^{-7}	$(\leq 0, 9.4 \times 10^{-1})$	--	--	--
Male, RBC, 12	3.0×10^{-3}	$(1.1 \times 10^{-4}, 2.4 \times 10^{-2})$	66.7	1334	2.2×10^{-6}
Male, RBC, 4	2.5×10^{-2}	$(2.6 \times 10^{-4}, 1.9 \times 10^{-1})$	56.9	1138	2.2×10^{-5}
Male, RBC, 2	2.0×10^{-3}	$(2.8 \times 10^{-19}, 4.0 \times 10^{-1})$	51.7	1034	1.9×10^{-6}
Female, RBC, 12	3.2×10^{-3}	$(3.1 \times 10^{-6}, 7.5 \times 10^{-2})$	50.5	1010	3.2×10^{-6}
Female, RBC, 4	1.2×10^{-2}	$(1.4 \times 10^{-3}, 4.8 \times 10^{-2})$	66.9	1338	9.0×10^{-6}
Female, RBC, 2	1.9×10^{-3}	$(1.3 \times 10^{-13}, 2.7 \times 10^{-1})$	49.0	980	1.9×10^{-6}

Appendix III-2: Data on ChE-I by Parathion

TABLE 1. Mean Cholinesterase Activity in Rats Fed Parathion (Percent of Control)

Dose Level (ppm)	6 Months		12 Months		18 Months		Termination[a]		
	Plasma	RBC	Plasma	RBC	Plasma	RBC	Plasma	RBC	Brain
Males									
0.5	100	100	90	95	78	100	94	109	103
5.0	71	87**	80	86*	41*	94	63	102	92
50.0	14**	91**	10**	88	7**	100	6**	95	22**
Females									
0.5	103	101	91	97	100	98	119	96	96
5.0	82	97	63*	90	64	91	81	87	98
50.0	11**	91	6**	86*	8**	88	13**	88	18**

*Significantly different from control value at $p < 0.05$.

**Significantly different from control value at $p < 0.01$.

[a] Terminal sacrifice was at 26 months for males and 28 months for females.

Citation: Daly, I. (1984). Ethyl parathion chronic feeding study. Report No. 77-2055 prepared by Biodynamics, Inc. for Monsanto Company. January 23, 1984. EPA Accession No. 252702-705. In Ghali, George Z. (1985). Parathion Registration Standard, EPA

Appendix III-2: Data on ChE-I by Parathion

TABLE 2. Mean Activities of Plasma Erythrocyte and Brain Cholinesterase in Dogs Fed Ethyl Parathion for One Year

Group/Dose (mg/kg/d)	Plasma cholinesterase (IU/ml) at month 2	Plasma cholinesterase (IU/ml) at month 4	Plasma cholinesterase (IU/ml) at month 12	RBC cholinesterase (IU/ml) at month 2	RBC cholinesterase (IU/ml) at month 4	RBC cholinesterase (IU/ml) at month 12	Brain cholinesterase at month 12
Males							
0	2790±385[a] (100)	2071±162 (100)	1665±158 (100)	2634±354 (100)	2346±404 (100)	2699±145 (100)	899±432 (100)
0.01	2238±286* (80)	2250±324 (109)	1218±328* (73)	2228±208* (84)	2274±315 (97)	2111±189* (78)	459±89 (51)
0.03	2050±458* (73)	1891±377 (91)	968±173* (68)	2188±416* (83)	2122±213 (90)	1904±150* (70.5)	662±438 (74)
0.1	1667±331* (60)	1399±208* (67)	601±193* (46)	2031±275* (77)	1718±204* (73)	1558±198* (58)	586±70 (65)
Females							
0	2567±184 (100)	2064±231 (100)	1735±219 (100)	2552±253 (100)	2643±287 (100)	2654±238 (100)	545±124 (100)
0.01	1924±211* (75)	1965±255 (95)	1470±380 (85)	2136±276* (83)	2407±199 (91)	2272±407* (86)	728±216 (134)
0.03	2041±376* (79)	1663±386* (81)	1184±208* (68)	1947±369* (76)	2019±259* (76)	1848±134* (70)	347±134* (64)
0.1	1856±173* (72)	1353±231* (64)	763±212* (34)	1882±219* (74)	1583±269* (60)	1665±286* (63)	545±97 (100)

[a] Mean value and deviation; the values in paratheses are cholinesterase activities expressed as percent of control.

* Significantly different from control value at $p \leq 0.05$ (Dunnetts' test).

Citation: Ahmed, F. E., Sagartz, J.W., Tegeris, A. S., et al. One year feeding study in dogs. (Unpublished Study No. PRL-77-115 prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Co., St. Louis, MO; dated August 20, 1981.) Accession Nos. 246639, 246642-43. In Ghali, George Z. (1985). Parathion Registration Standard, EPA

Appendix III-3: Data on Cholinesterase Inhibition by Pesticides

Data from EPA's "Caswell files" in the Office of Pesticide Programs (OPP) were reviewed for the following pesticides: parathion, malathion, aldicarb, chlorthiophos, chlorpyrifos, dimethoate, ethion, metasystox, and carbofuran. These pesticides were selected since all have been tested in humans. The quality of the available data for metasystox was too poor to use for further analysis. The following information was abstracted for each study: study type and length (e.g., one year feeding), species, ChE NOEL in plasma, RBC, and brain (if measured), administration of dose (e.g., solution dissolved in 25 ml corn oil), method of ChE measurement (e.g., Michel's potentiometric), purity/grade of pesticide, time of measurement of ChE, number (age) of subjects, doses tested, and other pertinent information (e.g., study conducted by IBT; reevaluation pending). Information on method of ChE measurement was frequently not reported.

The reviews described above are too lengthy to be included here, but the original worksheets and typed summaries prepared by EPA's Risk Assessment Forum are available from the author.

IV. Exposure Assessment

EPA's general approach and framework for conducting exposure assessments is outlined in its "Proposed Guidelines for Exposure Assessment" (Federal Register, 1984). Exposure assessments generally cover five principal topics: (1) sources, (2) exposure pathways and environmental fate, (3) monitored or estimated concentration levels, (4) exposed populations, and (5) integrated exposure analysis (combines (3) and (4) to give exposure profiles). Since an exposure assessment is part of the overall risk assessment, it should be coordinated with the findings of the previous two steps of the risk assessment. For ChE-I pesticides, this means that both acute and chronic exposure scenarios and oral, dermal, and inhalation routes of exposure should be considered. (Although this review has focused on oral toxicity, the most common route of exposure for the general public, most ChE-I pesticides are readily absorbed through the skin and lung and can pose a hazard through these routes of exposure as well.)

ChE-I pesticides have a wide variety of uses, so that exposures may occur in a variety of ways (Blum and Manzo, 1985). Some ChE-I insecticides are systemic (i.e. absorbed and distributed throughout plants) and can be ingested either in their original form or as a breakdown product when the plant is ingested. Malathion, parathion, and diazinon are examples of systemic insecticides. Other ChE-I pesticides (e.g. fenchlorphos) are used to control parasites of domestic animals, and may be given orally or dermally. Nematocides (e.g. prophos, zinophos) are introduced into the soil through the water system. Other ChE-I pesticides are used as fungicides (e.g. edinphos,

phosbutyl), herbicides (e.g., bensulide), rodenticides (e.g. gophocide), insect repellants (e.g. o-n-butyl-o-cyclohexenyl-N, N-diethylphosphoramidate), and insecticide synergists (e.g. propyl-2-propynylphenylphosphonate) (Blum and Manzo, 1985).

Both agricultural and non-agricultural uses of ChE-1 pesticides are significant. Of the thirty million pounds of insecticides used on corn in 1982, 95% (28.4 million) were either OPs or carbamates. Almost half of the insecticides used in 1982 on thirteen major field and forage crops in thirty-three states were used on corn. Another 1.5 million pounds of parathion was used on wheat (Marquis, 1986). Four of the top ten pesticides used by homeowners were ChE-I pesticides, according to EPA (GAO, 1986). These include diazinon, chlorpyrifos, carbaryl and malathion. These four are also among the top twenty-five pesticides used by professional applicators for nonagricultural purposes, according to the 1984 National Urban Pesticide Applicators Survey (NUPAS). Parathion was ranked as thirty-third. (GAO, 1986).

A recent GAO report (1986) points out why non-agricultural uses of pesticides are of particular concern:

- There is no approach for controlling exposures resulting from the use of nonagricultural products that is comparable to the tolerance setting process for agricultural pesticides. The potential for cumulative exposure is generally not determined when a nonagricultural pesticide product is considered for registration by EPA.

- People who apply pesticides around their homes, or hire others to do so, are poorly informed about the risks associated with pesticide use. Information contained on pesticide labels is limited, and information provided in advertisements or literature from manufacturers, distributors or professional applicators is at best limited, and frequently unlawful. Rarely do any

of these sources available to the homeowner discuss chronic health effects or gaps in the database.

- o People may be exposed to non-agricultural pesticides in public places (e.g. schools, offices, hotels, restaurants) without their knowledge or against their will. This is of particular concern to people who are hypersensitive to chemicals.

- o Some professional applicators, particularly those that apply pesticides only to their employers' properties (known as "not for hire" applicators) are not required to be tested to ensure that they are competent to apply pesticides.

In addition, many persons identified as potentially highly susceptible to ChE-I pesticides may spend much of their time at home, or other public places where nonagricultural pesticides might be used. For example, infants, children, pregnant women and their unborn children, and ill persons may spend much of their time at home.

As with nonagricultural uses, agricultural uses of ChE-I pesticides can result in occupational exposures and exposures to the general public (primarily through food and water). Occupational exposures may be estimated by direct or indirect approaches. Direct methods sample pesticides as the workers encounter them, by estimating the amount that could contact the skin or be inhaled. Indirect methods measure the pesticide or its metabolites in human fluids or via some other indicator of physiological effect (Hayes, Wise and Weir, 1980).

The measurement of ChE-I in blood has been frequently used an an indirect method of estimating exposure of workers to ChE-I pesticides. However, as Wills (1972) points out, the finding of a normal ChE activity in a worker suspected of receiving exposure to ChE-I pesticides does not necessarily mean that no exposure

has occurred, because some ChE-I pesticides have little effect on blood ChE (even in doses near the lethal dose), and because regeneration of blood ChE's (particularly BuCh) may be rapid enough so that ChE activity in the blood is within normal limits despite significant inhibition within some neuroeffector systems. Also, some workers may have low ChE activities due to genetic or nutritional factors. The Subcommittee on Pesticides of the Permanent Commission and International Association on Occupational Health recommended in 1976 that other biological methods (e.g. electromyograms, or EMGs) be investigated to assess exposure in addition to ChE-I (Zavon, 1976).

A number of researchers [e.g. Gage (1967), Cornish (1971), Zavon (1976), Vandekar (1980)] have suggested levels of ChE-I which could be considered indicative of unsatisfactory occupational exposures. These range from twenty to fifty percent ChE-I. In an attempt to answer the question, "What level of ChE-I should be considered to be of regulatory significance?", many EPA reviewers have argued that these levels should be considered as estimates of a biological threshold, and used to define the cut-off for a NOEL. It should be remembered, however, that these figures were originally developed to apply to healthy adult (usually male) workers, and probably do not apply to others in the general population. Gage (1967) clearly shows the intended purpose of these levels: "The method [i.e., of using a threshold limit of ChE-I to 'indicate the existence of unsatisfactory working conditions'] would fall into disrepute if men were too frequently taken off work or if there were an interruption of production or of application processes when no clinical symptoms

were apparent, nor any evidence of a breakdown in safety precautions." While 20-50% ChE-I may be an acceptable level of ChE-I for healthy adult workers who voluntarily work in areas where exposures are known to occur, it should not be misinterpreted as a population threshold (defined as the lowest of the thresholds of the individuals within a population (EPA, 1986)).

In addition to indirect and direct approaches to monitoring exposure, models may also be used to estimate exposures. For example, Guy, Hadgraft and Maibach (1985) developed a kinetic model of chemical absorption via human skin which they applied to the study of percutaneous absorption of malathion.

Potential exposures to the general public through the diet can be estimated using EPA's Tolerance Assessment System (TAS). The TAS can be used to estimate the distribution of exposure to a pesticide among individuals who eat a particular food commodity. A pesticide tolerance is the maximum permissible concentration of a pesticide allowed in or on raw agricultural commodities and processed foods. The TAS can (and should) be used to ensure that infants, children and others do not face high dietary risks from ChE-I pesticides. Vegetarians and others eating large quantities of fruits and vegetables have been known to exhibit chronic mild anti-ChE poisoning (Ratner et al., 1983).

The size, distribution, and other characteristics of the subpopulations of high sensitivity should be studied. In contrast to some highly sensitive subgroups (e.g., children), those possessing non-typical serum ChE activities are not readily identifiable. Different races appear to have different

frequencies of the non-typical ChE's. According to Silver (1974), Caucasian populations investigated from Canada, U.S., and various European countries, as well as Australian aborigines, generally comprise about three to four percent heterozygotes possessing both the normal and atypical variant (i.e., $E_1^{u}E_1^{a}$). The atypical variant is absent or very rare in the population of Tristan de Cunha, certain of the Eskimo and Red Indian tribes, Icelanders, American and African blacks, Japanese, and other Oriental populations. Other nontypical variants are more rare (less than one percent). According to Udsin (1970) an Israeli population studied had a high frequency of the E_1^{a} phenotype. The frequency of heterozygotes in Israel varies from 0.7% among North African Jews to 3.1% among European Ashkenazi Jews to 9.7% among Jews from Iraq and Iran. Udsin also reported that a population of southern Eskimos studied had a high incidence of atypical (E_1^{a}) homozygotes, heterozygotes, and "silent" gene individuals.

V. Risk Characterization

A. Uncertainty

In this, the final stage of the risk assessment, information about the hazard of the substance and the potential for human exposure from the last three stages is combined to estimate the extent of public health risk. The dose-response data and assessment approach chosen is discussed, as is the data describing the population groups for which exposure estimates are most meaningful. Perhaps most important, the uncertainties in characterizing the risk are presented and their implications discussed.

Presenting the uncertainties and the corresponding assumptions and judgments made to deal with those uncertainties is crucial since:

1) this makes it easier to go back and revise the risk estimate if this is necessitated by new data or research clarifying the uncertainty
2) it influences decisions made by the risk manager.

Classifying uncertainties helps to structure the presentation and thus increases the risk manager's efficiency in using this information to make decisions. One such classification scheme is that adapted from McGarity's (1979) discussion of the types of science policy issues involved in regulating carcinogens by EPA (and OSHA). His discussion is applicable to science policy issues associated with the regulation of other hazardous substances, including ChE-inhibiting pesticides. These science policy issues originate from uncertainties that are associated with the risk assessment procedure. McGarity's categorization contains four types of science policy issues, which really lie on

a continuum between issues of pure scientific fact and pure policy, and which are paralleled by four types of uncertainty (using the same descriptors as McGarity):

1) "Trans-scientific" uncertainty -- uncertainty which theoretically could be resolved, but for practical or moral reasons, can not be. For example, one could demonstrate with a high degree of accuracy the shape of the dose-response curve for persons exposed to ChE-inhibiting pesticides by using hundreds of volunteers at scores of dosage levels, but this would not be practical or ethical.

2) Uncertainty due to insufficient data -- uncertainty which could be resolved if more time and resources were spent. For example, more data is needed to understand how individuals with genetically non-typical ChE respond to ChE-inhibiting pesticides.

3) Uncertainty resulting from varying scientific interpretation -- For example, some EPA scientists (and scientists in the research community) interpret the NOEL from the same study of ChE-inhibiting pesticides differently. Some feel that 20% inhibition of RBC or plasma ChE compared to control values should be considered a response, whereas others feel a greater inhibition in plasma is necessary. Some feel that a statistical measure of significance should be used, rather

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* The term "transscientific" was coined by Alvin Weinburg (1972) in "Science and Trans Science," Minerva 10: 209

than an absolute percent inhibition. Others feel that the inhibition should only be compared to pre-exposure values of ChE in the same animal rather than to values in the control group.

4) Uncertainty resulting from disagreement over inferences -- This category encompasses many of the uncertainties in using animals as models for dose-response assessment in humans, and of using a non-random sample of humans as predictors for the population at large.

Questions relating to the amount society should pay for protection from risks associated with these compounds, or the extent to which regulatory approaches should protect sensitive subpopulations, involve political, legal, ethical and economic uncertainties associated with risk management, which lie outside the scope of this paper.

McGarity's classification of uncertainty is useful since it simplifies both the analysis of uncertainty and the science policy decisions for describing, expressing and/or resolving uncertainty. McGarity advocates a results-oriented approach to dealing with these issues in light of the fact that they are not scientifically resolvable at the time the decision must be made. This is not to say that factual accuracy or scientific reasoning is disregarded; to the contrary, additional data and more sophisticated analyses can resolve or at least clarify or narrow the range of uncertainty. Indeed, a results-oriented approach should be the outcome of a consistent and rational risk assessment policy.

Such a classification scheme also helps the risk manager to decide what approaches to take for resolving the uncertainty in the future. For example, additional research to generate more data would be much more useful for type 2 uncertainties than for type 3. A better approach for handling type 3 uncertainties would be activities directed towards consensus building. For type 1 uncertainties there is a declining rate of return on investments to narrow the range of uncertainty.

Appendix V-1 provides examples of these different types of uncertainties encountered throughout the risk assessment of ChE-I pesticides.

With regard to ChE-I per se, most of the uncertainties result from differences in interpretation and disagreement over inferences (type 3 and 4 uncertainties). This is not surprising, since ChE-I has been known to be associated with OPs and carbamates for a long time, and extensive research has been done in this area. In contrast, a much larger proportion of the uncertainty in regard to neurobehavioral effects is of type 2, resulting from inadequate data.

B. Summary and Recommendations

In concluding, let us re-examine the components that were originally proposed for analysis in this review:

- What degree of ChE-I is significant (i.e., to be regarded as an effect)?

The uncertainties regarding the role of ChE's (particularly in the blood) and the influence of other factors (e.g., rate of ChE-I, degree of inhibition of arylesterases, as well as age, diet, etc.) on the toxicity of ChE-I pesticides makes this a

difficult question to answer. Although a large degree of ChE-I in the blood (e.g., 70%) is usually accompanied by unmistakable signs of poisoning, the effects associated with smaller levels of inhibition (particularly those observed with chronic exposures) are less clear. Since ChE-I is a graded response, with some inhibition occurring even at very low doses, it is difficult to determine a level which is considered to be the biological threshold for an individual. Although levels of ChE-I have been determined to be "significant" in occupational settings (i.e., if that level is exceeded the worker is removed from the exposure), these levels are not necessarily appropriate as estimates of a threshold for the general population, including sensitive subgroups.

Several approaches to developing a scientific policy for this component were presented. Since the determination of a population threshold for exposures to ChE-I pesticides is a trans-scientific problem, it is necessary that a level of ChE-I believed to be adverse be determined as a matter of science policy. Various considerations in setting such a level (e.g., site of ChE, how measured) were given. The choice of dose-response assessment method influences the importance that this uncertainty (regarding what level of ChE-I is biologically significant) has on the risk assessment (i.e., this is more important with the NOEL-UF approach than with other approaches).

- What dose-response assessment methods should be used to extrapolate from experimental doses to exposure doses?

The advantages and disadvantages of the four principal

methods were discussed. Although the NOEL-UF approach has historically been used, it has several drawbacks, particularly in that it neglects dose-response information, discourages large sensitive studies, and can not always be used (e.g., if NOEL is less than the lowest dose tested). Various options to remedy these shortcomings were suggested. It is recommended that the wealth of information on the mechanism of action of ChE-I pesticides be exploited to develop mathematical models as an alternative to the NOEL-UF approach. Registrants should be encouraged to provide data in which the variability is reported, so that alternative methods of dose-response assessment can be used. A risk assessment policy that allows some flexibility in the choice of dose-response assessment methods will result in a more efficient use of available data.

- What uncertainty factor should be employed for interspecies variation of ChE-I between animals and humans?

Investigating the interspecies variability in an admittedly small sample indicated that the NOEL in one species was on average five fold different than in another species (measured at the same site), and ranged from one to twenty-five in studies of comparable length. These results should be viewed cum grano salis since methodological factors in addition to species differences may have accounted for the observed variability. The routes of administration were comparable except where noted. In an attempt to reduce the variability due to differences in methodology, studies reported by one researcher (Edson, 1964) of schradan, dimefox, and parathion in the pig, rat and human were compared. Inconsistencies in the reporting of the data also

render these results debatable. The interspecies variability observed for schradan and dimefox was comparable to that reported above, although the variability for parathion was much greater, particularly when ChE-I was measured in plasma.

The traditional UF of ten for interspecies variability appears to be adequate in most cases, but does not fully account for differences in sensitivity between humans and test animals for some ChE-I pesticides (e.g., malathion and parathion).

- What uncertainty factor should be employed for intraspecies variation of ChE-I?

Age was identified as a significant factor affecting intraspecies variability to ChE-I pesticides. Young cattle and rats are up to thirty times more sensitive than the adults of their species. Nutrition and gender also influence intraspecies variability. Pregnancy, genetically-determined atypical ChE's and low activity paraoxonase, and a variety of health conditions are likely to increase sensitivity to ChE-I pesticides. A variety of drugs, chemicals and other agents are known to potentiate or contribute to the toxicity of some ChE-I pesticides.

It is not clear whether the use of the term "intraspecies variation" in reference to UF's is supposed to account for potential exposures to the agents which can potentiate or otherwise increase toxicity. It is recommended that for those pesticides where synergism or potentiation by other environmental agents is likely to occur (e.g., malathion) that an additional MF be applied.

The traditional UF of ten is not adequate to account for the increased sensitivities of special subgroups in the general popu-

lation, particularly infants. Clearly, EPA's use of an UF as small as ten (Federal Register, 1981) to account for both inter- and intraspecies variability is not justified. Either a larger UF is required, or a dose-response assessment method which does not employ inter- and intraspecies variation UF's should be used.

- What special considerations should be included in exposure assessments for ChE-I pesticides?

The size, location and extent of exposure of the potential subgroups identified should be determined. Nonagricultural uses of ChE-I pesticides deserve special attention since exposures may be involuntary and/or may result in greater exposures to certain sensitive subgroups (e.g., small children, pregnant women, or ill persons in the home).

- What are the uncertainties in estimating the extent of health effects associated with these compounds? How should they be estimated and presented to Agency decision makers?

Appendix V-1 presents some of the major uncertainties in assessing the risks of ChE-I pesticides. It is recommended that uncertainties be presented in an organized format according to McGarity's classification of type of uncertainty. This enables the decision maker to quickly ascertain the nature of the uncertainties and what to do about them. Information bearing on a particular uncertainty is more likely to be utilized when uncertainties are explicitly identified in this way.

Appendix V-1: Examples of Uncertainty in the Risk Assessment of ChE-I Pesticides

Type*	Uncertainty	Explanation
Hazard Identification		
2	What is the role of ChE in RBC's? In plasma?	Various hypotheses have been suggested but none proven (Silver, 1974)
3	What amount of ChE-I in blood is an adverse effect?	Some scientists (e.g. Rider, 1961) interpret a decrease of ChE activity over 25% below controls as an adverse effect. Others interpret a decrease of ChE activity to be adverse if it is significantly different ($p < .05$) than pre-exposure values (telephone conversation, 6/5/86, OPP scientist)
4	How should the rate of ChE-I be used to establish an adverse effect?	Rate (as well as magnitude) of ChE-I has been identified as contributing to toxicity, but currently only the magnitude of ChE-I is used as a measure of toxicity.
3	Does a study have special characteristics that lead one to question its results?	For example, a dosing schedule in a study of malathion in humans was unusual: eight mg was administered to five subjects for thirty-two days, then no treatment was given for three weeks, and then sixteen mg was given to the same subjects for forty-seven days. The first exposure may have altered the response observed with the second exposure.
4	If so, how should the results of that study be interpreted?	
2	Does the change in ChE activity associated with a variety of conditions (e.g., pregnancy, oral contraceptives, schizophrenia, liver disease, anemia, genetically non-typical ChE) have an appreciable effect on sensitivity to ChE-I's?	It is hypothesized that individuals with decreased ChE levels are at high risk to ChE-I pesticides, but there is insufficient data to establish this.
4	What relative weights should be given to studies with differing results?	In cancer policy, a study in which a dose is associated with an adverse effect (e.g., a tumor) is weighted more than a comparable study finding

Type*	Uncertainty	Explanation
		no effect at that dose, since it is inferred that the latter study must have been less sensitive than the former. For ChE-I pesticides, a study in which a dose is found to exhibit no effect is weighted more than a comparable study (same species) showing an effect at that dose. The highest dose showing no effect is considered the NOEL for that species.
Dose-Response Assessment		
3	What method of dose-response assessment should be used?	Currently, the NOEL-UF approach is used by EPA, although some scientists (e.g. Crump) advocate other methods (e.g., BD-UF approach).
3	What UF should be selected to reduce a NOEL observed in an animal study to a RfD for humans?	Different offices within EPA (e.g., OPP, ODW) have differed over the selection of an appropriate UF (e.g., 10 or 100).
1	What is the population threshold for a ChE-I pesticide?	The true population threshold for a ChE-I pesticide can not be determined with certainty, since to do so would involve exposing a multitude of people to the pesticide, an impractical and socially unacceptable proposal.
3	What criteria should be used to determine a NOEL for ChE-I?	Different scientists use different criteria (e.g., magnitude of ChE-I, site where ChE is measured, dose-response trend) to determine a NOEL.
3	If data are available on more than one species, how should they be used?	Although the use of the most sensitive species is stated to be current EPA science policy (EPA, 1986), this is not strictly true. The dog is the most sensitive species to ChE-I by parathion, but is not used since the NOEL is below the lowest dose tested in the available dog study.

Type*	Uncertainty	Explanation

Exposure Assessment

3	What methods of estimating exposures of potential high risk groups are most accurate?	Different researchers favor different methods, even under the same conditions (e.g., direct vs. indirect monitoring methods).
2	How should one estimate the size, location, and other characteristics of the population of individuals with genetically non-typical ChE?	Individuals with genetic variants of ChE are not readily identifiable.
4	How should dietary habits and other variations in lifestyle be taken into account?	Dieting, fasting, alcohol and cigarette use, etc. can potentially contribute to increased susceptibility to ChE-I pesticides.
2	How should one estimate the size and nature of the populations likely to be exposed through non-agricultural uses of ChE-I pesticides?	Data on exposures from nonagricultural uses of ChE-I pesticides (particularly to the general public) is generally insufficient, largely because there is no regulatory approach for controlling these exposures, as there is for dietary exposures (GAO, 1986)

Risk Characterization

3	What are the biological uncertainties in estimating the extent of health effects from exposures to ChE-I pesticides? How should they be estimated and presented to decision makers?	The difference between fact and opinion can be a matter of dispute. The way in which uncertainties are estimated and presented may bias the decision maker.
	What are the statistical uncertainties in estimating the extent of health effects from exposures to ChE-I pesticides? How should they be estimated and presented to decision makers?	Statistical uncertainties are often not estimated when assessing risks of ChE-I pesticides. The way in which uncertainties are presented may bias the decision maker.

Type*	Uncertainty	Explanation
3	Which population groups should be the primary targets for protection?	Should "average" consumers or high risk groups be targeted? Risk management considerations factor into the resolution of these questions.
4	Which provide the most meaningful expression of the health risk?	

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* Type codes (see text pp. V-2, 3):

1: Trans-scientific uncertainty
2: Uncertainty due to insufficient data
3: Uncertainty due to varying scientific interpretation
4: Uncertainty due to disagreement over inferences

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