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A COMPUTER SIMULATION STUDY OF BLOOD

CHOLINESTERASE INHIBITION IN

PARATHION TREATED RATS

by

Scott Christopher Hennes

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Toxicology

Approved:

UTAH STATE UNIVERSITY  
Logan, Utah

1980

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## ACKNOWLEDGMENTS

The work is finished. There are no more parameters to be computed, programs to be debugged, rats to be bled, or pages to be written. I would like to thank everyone who contributed to the attainment of this often-times elusive goal. I wish to thank: Dr. Joseph C. Street, who provided an atmosphere which fostered a sense of independence and confidence; Dr. Joe Douplik, who is an expert in exterminating resistant computer bugs; Susan Jones, who is now an expert in small animal surgery; my coworkers, who provided camaraderie as well as scientific catalysis.

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Scott Christopher Hennes



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## ABSTRACT

A Computer Simulation Study of Blood  
Cholinesterase Inhibition in  
Parathion Treated Rats

by

Scott Christopher Hennes, Master of Science

Utah State University, 1980

Major Professor: Dr. Joseph C. Street  
Department: Interdepartmental Program in Toxicology

A computer simulation model of blood cholinesterase inhibition in rats resulting from a single oral or dermal dose of parathion was developed and tested. The model consists of a set of non-linear differential equations describing the absorption, distribution and metabolism of parathion and the kinetics of inhibition and reactivation of erythrocyte and plasma cholinesterase. The equations are numerically integrated to produce the time-course of each of the cholinesterase activities of the blood. The model was tested for validity by a comparison of the activities predicted by the model to values determined experimentally in male rats. The model successfully simulated plasma cholinesterase activity after a four milligram per kilogram oral dose, and total blood cholinesterase activity after a one milligram per kilogram oral dose. Erythrocyte cholinesterase activity was closely predicted for the first three hours after a four milligram per kilogram oral dose, but inhibition was overestimated during the next two hours. Agreement between model output and observed erythrocyte cholinesterase activity was

good for the first twenty four hours after initiation of a fifteen milligram per kilogram dermal dose, but the rate of activity regeneration was overestimated. Estimated erythrocyte cholinesterase inhibition is sensitive to changes in the values of parameters controlling liver paraoxon metabolism and the phosphorylation reaction between paraoxon and the enzyme, but in general, the model is not sensitive to small perturbations of parameter values. The model has potential for interpreting human exposures to parathion and for investigating the relationships between various parameters and blood cholinesterase inhibition.

(80 pages)

## INTRODUCTION

Due to the hazardous nature of organophosphate insecticides, there has been widespread interest in developing a suitable index for human exposure to these chemicals. One of the most widely used indices is the extent of blood cholinesterase inhibition produced by a pesticide exposure. Blood cholinesterase inhibition is reasonably well correlated with the level of organophosphate exposure (Namba et al., 1971; Wills, 1972; and Long, 1975) and the response of these enzymes co-varies directly with the intensity and duration of absorption of the inhibiting chemical (Long, 1975). Blood cholinesterase depression is widely used in the monitoring and regulation of occupational exposure to organophosphates, such as the establishment of field reentry intervals for farmworkers (Gunther et al., 1977).

Interpretation of blood cholinesterase activity measurements could be aided by a quantitative description of the relationship between the dose and the time-course of the response. Two principal strategies exist for formulating this description: regression analysis and systems simulation.

Regression techniques have the advantage of simple mathematical formulation and inexpensive application of the resulting model. Serat (1973) presented a non-linear regression model relating plasma cholinesterase activity to total chemical exposure of farmworkers by use of a single first order differential equation. Use of this model requires either a fitting of the rate equation to actual data on human plasma cholinesterase activities after exposure to

a particular insecticide, or the use of a dermal LD50 in rats. The latter method requires an assumption that all insecticides have the same ratio of rat dermal LD50 to the total chemical exposure required to inhibit plasma cholinesterase in farmworkers by a given amount (Serat, 1975).

A systems analysis approach has several advantages (Innis, 1977). It utilizes system knowledge that takes forms other than data and is therefore more likely to yield useful approximations at some distance from the data base. It provides a rejectable set of hypotheses about mechanisms that operate within the system and also provides a simulated system upon which experiments can be performed. Garfinkel (1977) noted that simulation can quantify relationships among variables and show how important they are compared to other relationships in the same system, and in addition can aid in experimental design.

The objective of this study was to use the systems approach to develop a computer simulation model to predict plasma (ChE) and erythrocyte (AchE) cholinesterase inhibition in male rats resulting from parathion exposure, and to use the model to investigate the relationship between blood cholinesterase inhibition and the various parameters which control it. The model organism and the model insecticide were chosen because of the large literature data base that exists on the effects of parathion exposure upon rats.



## METHODS

### Model Structure

The construction of the model was based on the following assumptions:

- 1) Parathion is metabolized in the liver by two pathways: desulfuration, which yields paraoxon (Neal, 1967), and dearylation which yields p-nitrophenol and 0,0-diethyl phosphorothioic acid (Nakatsugawa and Dahn, 1967; Neal, 1967; Nakatsugawa et al., 1969). Liver parathion is exchanged with the blood by a flow-limited distribution process.
- 2) Blood parathion is exchanged with fat and muscle tissues (Gagne and Brodeur, 1972) by a flow limited distribution process.
- 3) Parathion is absorbed from the gut through two processes: gastric emptying and subsequent absorption from the intestine by passive diffusion (Romankiewicz and Reidenberg, 1978). Absorption from the skin is by a simple passive diffusion process (Dugard, 1977).
- 4) Paraoxon in the liver is subject to deethylation which yields monoethylparaoxon and to hydrolysis which yields diethylphosphoric acid and p-nitrophenol (Appleton and Nakatsugawa, 1977). Another route of paraoxon inactivation is the reaction with tissue proteins to form phosphorylated

proteins (Lauwerys and Murphy, 1969a, 1969b; Triolo et al., 1970; Benke and Murphy, 1974). Phosphorylated proteins can spontaneously reactivate (Levine and Murphy, 1977). Liver paraoxon is exchanged with the blood by a flow-limited distribution process.

- 5) Blood paraoxon is hydrolyzed to diethyl phosphoric acid and p-nitrophenol by plasma A-esterases (Aldridge, 1953), and inactivated by reactions with plasma proteins (Lauwerys and Murphy, 1969a; Silvestri, 1972; Schoenig, 1975), and blood cholinesterases (Aldridge and Reiner, 1972). Phosphorylated plasma proteins can spontaneously reactivate (Levine and Murphy, 1977). Paraoxon is exchanged with fat tissues (Gagne and Brodeur, 1972) by a flow limited distribution process.
- 6) Blood cholinesterases are inhibited by a reaction with paraoxon which yields a phosphorylated enzyme (Aldridge and Reiner, 1972). The enzymes may revert to the active form by hydrolysis to release diethyl phosphate or may undergo a deethylation of the phosphoryl moiety to form a non-reactivable (aged) enzyme (Hobbinger, 1963). Plasma cholinesterase is replaced by new enzyme synthesized in the liver (Wills, 1972; Murphy, 1975); erythrocyte cholinesterase is regenerated by turnover and replacement of the red blood cells (Berlin and Berk, 1975; Murphy, 1975).

The assumptions were formulated into a set of simultaneous differential equations and coded into FORTRAN. The five equations describing parathion dynamics were solved analytically by obtaining eigenvalues, eigenvectors and integration constants using numerical procedures (IMSL, 1979). The remaining eleven equations were integrated numerically by Gear's method for stiff equations (IMSL, 1979). Estimates of all model parameters except the dermal absorption rate constant were obtained from the literature. The dermal absorption constant was estimated by a least squares fitting of model output to erythrocyte cholinesterase activities obtained from rats during a 12 hour, 10 mg/kg dermal parathion exposure (described later in this section) by a gradient search technique (IMSL, 1979). Estimation procedures for parameters whose values were not explicitly available from the literature are detailed in Appendix B.

#### Model sensitivity analysis

Model sensitivity was studied by individually varying each parameter by  $\pm 25\%$  and observing the relative change in 6 selected model responses. The responses were minimum plasma and erythrocyte ChE activities, times when these minimum activities occurred, and maximum plasma parathion and paraoxon concentrations. A simulated oral dose of 2 mg/kg was used, and model responses were studied for a 13 hour interval.

Selected parameter interactions were studied by factorial arrangements of parameter value combinations using the previously listed model responses. The results were analyzed by analysis of variance for a completely randomized design involving a full

factorial treatment arrangement without replication, using the mean squares associated with the 3-way interaction to estimate experimental error. The 0.05 level of probability was used as the criterion of significance.

#### Dermal exposure study

Analytical standard grade parathion (99 + % pure) was provided by U.S. Environmental Protection Agency, Analytical Chemistry Branch (Research Triangle Park, N.C.). Adult male Sprague-Dawley rats (250 - 300 g) were purchased from Simonsen Labs Inc. (Gilroy, C.A.) and housed in individual metabolism cages.

The animals were divided into three treatment groups of five rats each. On the day before exposure the animals were anesthetized with ether and the hair on the dorsal, mid-lumbar region was removed with small animal clippers. Parathion, at 10 or 15 mg/kg, was applied to the shaved area in an acetone carrier of 150 - 200  $\mu$ l by a variable volume micropipette. The control group received only the acetone carrier. The rats were placed into restraining harness-and-collar assemblies as described by Bartek et al. (1972) and allowed access to food for the 12 hour duration of the exposure. After 12 hours the remaining parathion was removed by soap and water.

Blood was collected by tail clip in heparinized 100  $\mu$ l disposable micropipettes during ether anesthesia at 0, 2, 4, 8, 12, 24, 48, 72 and 120 hours following parathion administration. Erythrocytes and plasma were separated immediately by centrifuging for 5 minutes at 825 x gravity, prepared according to Serat et al.

(1977), and frozen for subsequent analysis by a modification of the pH Stat technique described by Serat et al. (1977). The titrant concentration was 0.003 M NaOH and sufficient NaOH was added to the saline diluent to raise the pH of the erythrocytes to nearly 8 before analysis.

## RESULTS

### Model Structure

The structure of the model is presented in a Forrester diagram in Fig. 1. The formulation of the model into a set of differential equations is given in Table 1. The accommodation of two exposure routes by the model required alternate sets of equations describing absorption since oral absorption consists of two consecutive first order processes transporting parathion into the hepatic portal system, but dermal absorption consists of one first order process transferring parathion directly into the systemic circulation.

Parameter and initial condition values used in the simulations are listed in Table 3. Parameters are those quantities whose values do not change during a model run; initial values are the starting amounts for variables whose values are altered during the course of a simulation.

### Model Validation

To test the validity of the model, results from model simulations were compared with results obtained from experimental investigations. The activities of AchE measured in rats dermally exposed for 12 hr to 15 mg/kg parathion are compared to model output from a computer simulation of the exposure in Fig. 2. Model output is in agreement with experimental results for the first 24 hr after initiation of exposure, with the exception of the 12 hr activity measurement. This point appears to be high in relation to the 8 hr and 24 hr activities (in the 10 mg/kg exposure, the 12 hr activity was less than the 24 hr activity). The simulated regeneration of AchE activity

The following table shows the results of the experiment. The first column is the number of trials, the second column is the number of correct responses, and the third column is the percentage of correct responses.

Number of trials	Number of correct responses	Percentage of correct responses
10	7	70%
20	14	70%
30	21	70%
40	28	70%
50	35	70%
60	42	70%
70	49	70%
80	56	70%
90	63	70%
100	70	70%

As can be seen from the table, the percentage of correct responses is constant at 70% for all numbers of trials. This suggests that the subject is performing the task at a constant level of accuracy.

Fig. 1. Forrester diagram of model structure. Represented are amounts of material ( $\square$ ), material transfers between compartments ( $\longrightarrow$ ), parameters ( $\ominus$ ), auxiliary variables computed from several parameters ( $\circ$ ), controls on rates of transfer ( $\bowtie$ ), and sources and sinks for material ( $\odot$ ). Parameter symbols are explained in Table 3.



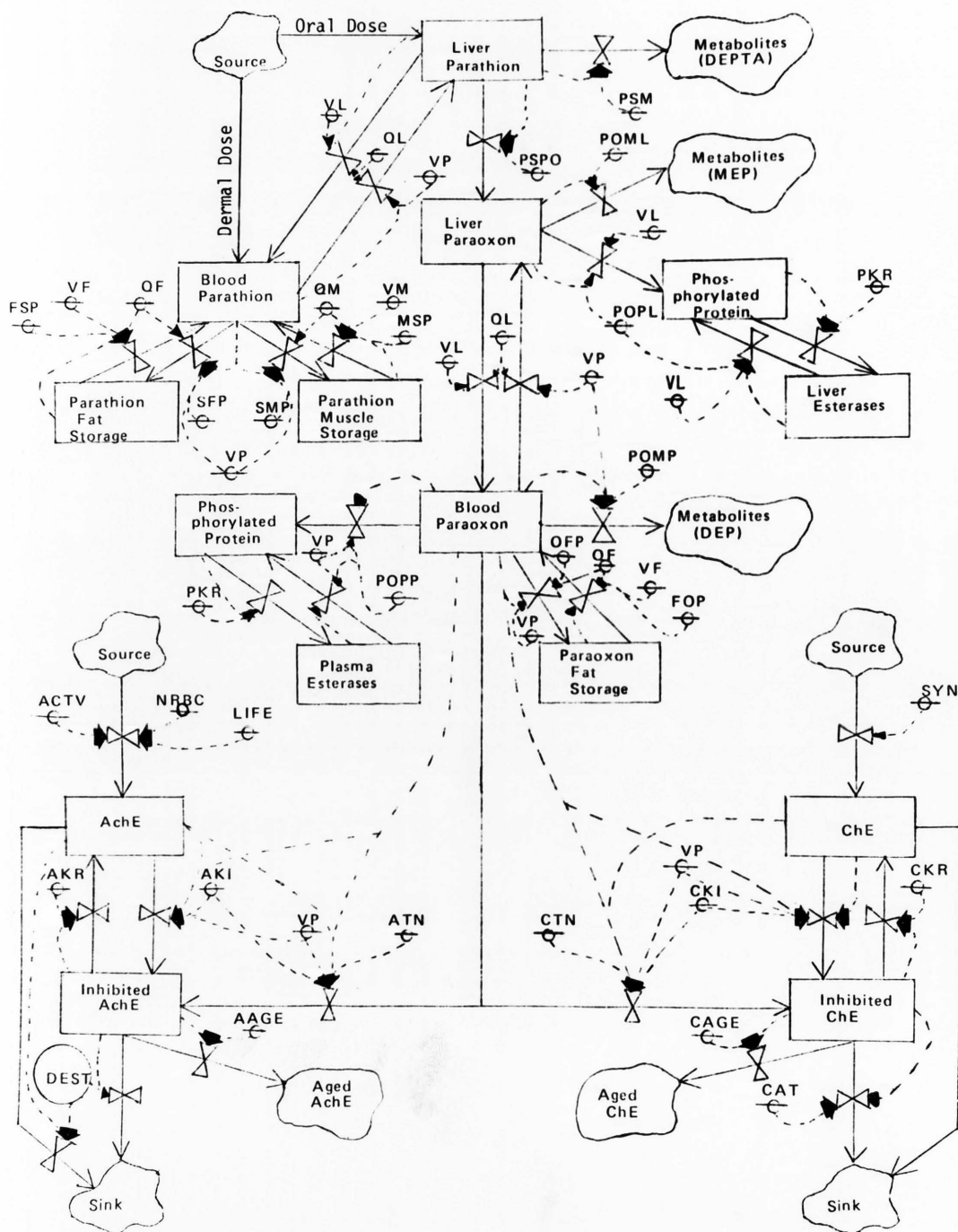


TABLE 1

Differential Equations Describing Model Processes<sup>a b</sup>


---

*Dermal Dose*

---

- 1) 
$$\frac{d \text{ PSL}}{dt} = - \left( \text{PSPO} + \text{PSM} + \frac{\text{QL}}{\text{VL}} \right) * \text{PSL} + \left( \frac{\text{QL}}{\text{VP}} \right) * \text{PSP}$$
- 2) 
$$\frac{d \text{ PSP}}{dt} = \left( \frac{\text{QL}}{\text{VL}} \right) * \text{PSL} - \left( \frac{\text{QF} * \text{SFP}}{\text{VP}} + \frac{\text{QM} * \text{SMP}}{\text{VP}} + \frac{\text{QL}}{\text{VP}} \right) * \text{PSP} \\ + \left( \frac{\text{QF} * \text{FSP}}{\text{VF}} \right) * \text{PSF} + \left( \frac{\text{QM} * \text{MSP}}{\text{VM}} \right) * \text{PSMU} + (\text{PSABS}) * \text{PSS}$$
- 3) 
$$\frac{d \text{ PSF}}{dt} = \left( \frac{\text{QF} * \text{SFP}}{\text{VP}} \right) * \text{PSP} - \left( \frac{\text{QF} * \text{FSP}}{\text{VF}} \right) * \text{PSF}$$
- 4) 
$$\frac{d \text{ PSMU}}{dt} = \left( \frac{\text{QM} * \text{SMP}}{\text{VP}} \right) * \text{PSP} - \left( \frac{\text{QM} * \text{MSP}}{\text{VM}} \right) * \text{PSMU}$$
- 5) 
$$\frac{d \text{ PSS}}{dt} = - (\text{PSABS}) * \text{PSS}$$
- 6) 
$$\frac{d \text{ POL}}{dt} = (\text{PSPO}) * \text{PSL} - \left( \text{POML} + \frac{\text{QL}}{\text{VL}} \right) * \text{POL} + \left( \frac{\text{QL}}{\text{VP}} \right) * \text{POP} \\ - \left( \frac{\text{POPL}}{\text{VL}} \right) * \text{ESTL} * \text{POL}$$
- 7) 
$$\frac{d \text{ POP}}{dt} = \left( \frac{\text{QL}}{\text{VL}} \right) * \text{POL} - \left( \frac{\text{POMP}}{\text{VP}} + \frac{\text{QL}}{\text{VP}} + \frac{\text{QF} * \text{OFP}}{\text{VP}} \right) * \text{POP} - \left( \frac{\text{POPP}}{\text{VP}} \right) * \text{ESTP} * \text{POP} \\ - \left( \frac{\text{AKI}}{\text{ATN} * \text{VP}} \right) * \text{ACHE} * \text{POP} - \left( \frac{\text{CKI}}{\text{CTN} * \text{VP}} \right) * \text{CHE} * \text{POP} + \left( \frac{\text{QF} * \text{FOP}}{\text{VF}} \right) * \text{POF}$$
- 8) 
$$\frac{d \text{ ESTL}}{dt} = - \left( \frac{\text{POPL}}{\text{VL}} \right) * \text{POL} * \text{ESTL} + (\text{PKR}) * \text{ESTLP}$$
- 9) 
$$\frac{d \text{ ESTLP}}{dt} = \left( \frac{\text{POPL}}{\text{VL}} \right) * \text{POL} * \text{ESTL} - (\text{PKR}) * \text{ESTLP}$$
- 10) 
$$\frac{d \text{ ESTP}}{dt} = - \left( \frac{\text{POPP}}{\text{VP}} \right) * \text{POP} * \text{ESTP} + (\text{PKR}) * \text{ESTPP}$$
- 11) 
$$\frac{d \text{ ESTPP}}{dt} = \left( \frac{\text{POPP}}{\text{VP}} \right) * \text{POP} * \text{ESTP} - (\text{PKR}) * \text{ESTPP}$$
- 12) 
$$\frac{d \text{ POF}}{dt} = \left( \frac{\text{QF} * \text{OFP}}{\text{VP}} \right) * \text{POP} - \left( \frac{\text{QF} * \text{FOP}}{\text{VF}} \right) * \text{POF}$$
- 13) 
$$\frac{d \text{ ACHE}}{dt} = \left( \frac{\text{NRBC} * \text{ACTV}}{\text{LIFE}} \right) - \left( \frac{\text{NRBC} * \text{ACTV}}{\text{LIFE} * \text{ACHEO}} \right) * \text{ACHE} - \left( \frac{\text{AKI}}{\text{VP}} \right) * \text{POP} * \text{ACHE} + (\text{AKR}) * \text{ACHEPR}$$
- 14) 
$$\frac{d \text{ ACHEPR}}{dt} = \left( \frac{\text{AKI}}{\text{VP}} \right) * \text{POP} * \text{ACHE} - \left( \text{AKR} + \text{AAGE} + \frac{\text{NRBC} * \text{ACTV}}{\text{LIFE} * \text{ACHEO}} \right) * \text{ACHEPR}$$
- 15) 
$$\frac{d \text{ CHE}}{dt} = (\text{SYN}) - (\text{CAT}) * \text{CHE} - \left( \frac{\text{CKI}}{\text{VP}} \right) * \text{POP} * \text{CHE} + (\text{CKR}) * \text{CHEPR}$$
- 16) 
$$\frac{d \text{ CHEPR}}{dt} = \left( \frac{\text{CKI}}{\text{VP}} \right) * \text{POP} * \text{CHE} - (\text{CKR} + \text{CAGE} + \text{CAT}) * \text{CHEPR}$$

---

TABLE 1, Continued

---

*Oral Dose*

---

1)  $\frac{d \text{ PSL}}{dt} = - \left( \text{PSPO} + \text{PSM} + \frac{\text{QL}}{\text{VL}} \right) * \text{PSL} + \left( \frac{\text{QL}}{\text{VP}} \right) * \text{PSP} + (\text{KA}) * \text{PSI}$

2)  $\frac{d \text{ PSP}}{dt} = \left( \frac{\text{QL}}{\text{VL}} \right) * \text{PSL} - \left( \frac{\text{QF} * \text{SFP}}{\text{VP}} + \frac{\text{QM} * \text{SMP}}{\text{VP}} + \frac{\text{QL}}{\text{VP}} \right) * \text{PSP} + \left( \frac{\text{QF} * \text{FSP}}{\text{VF}} \right) * \text{PSF}$   
 $+ \left( \frac{\text{QF} * \text{FSP}}{\text{VF}} \right) * \text{PSF} + \left( \frac{\text{QM} * \text{MSP}}{\text{VM}} \right) * \text{PSMU}$

5a)  $\frac{d \text{ PSI}}{dt} = - (\text{KA}) * \text{PSI} + (\text{KG}) * \text{PSST}$

5b)  $\frac{d \text{ PSST}}{dt} = - (\text{KG}) * \text{PSST}$

---

- a Variable Symbols are explained in Table 2  
 b Parameter symbols are explained in Table 3

TABLE 2

## State Variable Symbols

---

PSL	Liver Parathion	ESTP	Plasma Esterase
PSP	Plasma Parathion	ESTPP	Phosphorylated Plasma Esterase
PSF	Fat Parathion	POF	Fat Paraoxon
PSMU	Muscle Parathion	ACHE	Erythrocyte Cholinesterase
PSS	Skin Parathion	ACHEPR	Reactivatable Phosphorylated erythrocyte Cholinesterase
POL	Liver Paraoxon	CHE	Plasma Cholinesterase
POP	Plasma Paraoxon	CHEPR	Reactivatable Phosphorylated Plasma Cholinesterase
ESTL	Liver Esterase	PSI	Intestinal Parathion
ESTLP	Phosphorylated Liver Esterase	PSST	Stomach Parathion

---

TABLE 3

## Model Parameters and Initial Values

Symbol	Parameter or Initial Value	Value Used	Reference <sup>a</sup>
AACT	AchE Activity	1.7 units/ml	Wills (1972)
AAGE	Aging rate constant for AchE	$1.1 \times 10^{-5}$ units/cell	Estimated by non-linear optimization on data from Vandekar & Heath (1957)
ACTV <sup>b</sup>	Units of AchE per erythrocyte	$1.235 \times 10^{-10}$ units/cell	Calculated from enzyme activity and erythrocyte count
AKI	Bimolecular reaction constant for AchE-paraoxon	$210 \frac{1}{\text{min}} (\text{micromol/ml})^{-1}$	Estimated from Davison (1953)
AKR	Diethyl-phosphorylated AchE reactivation constant	$3.7 \times 10^{-4} \text{ min}^{-1}$	Estimated by non-linear optimization on data from Vandekar & Heath (1957)
ATN	Turnover number for AchE with acetylcholine	$1 \times 10^4$ units/micromol	Berry (1951)
CACT	ChE activity	0.620 units/ml	Wills (1972)
CAGE	Aging rate constant for ChE	$1.1 \times 10^{-5} \text{ min}^{-1}$	Adopted from AchE aging rate
CAT	ChE catabolism constant	$9.417 \times 10^{-5} \text{ min}^{-1}$	Estimated from Domschke <i>et. al.</i> (1970)
CKI	Bimolecular reaction constant for ChE-paraoxon	$82.5 \frac{1}{\text{min}} (\text{micromol/ml})^{-1}$	Estimated from Davison (1953)
CKR	Diethyl-phosphorylated ChE reactivation constant	$2.30 \times 10^{-3}$	Davison (1953)

TABLE 3, Continued

Symbol	Parameter or Initial Value	Value Used	Reference <sup>a</sup>
CTN	Turnover number for ChE with acetylcholine	$1 \times 10^4$ units/micromol	Adopted from AchE turnover number
DEST	Rate of metabolic destruction of AchE	$1.157 \times 10^{-5} \text{ min}^{-1}$	(Erythrocyte life span) <sup>-1</sup>
ESTL <sup>b</sup>	Phosphorylatable liver protein	$3.35 \times 10^{-2}$ micromol	Estimated from Lauwerys & Murphy (1969a)
ESTP <sup>b</sup>	Phosphorylatable plasma protein	$4.10 \times 10^{-2}$ micromol	Schoenig (1975)
KA	Absorption constant for parathion from the intestine	$6.064 \times 10^{-3} \text{ min}^{-1}$	Estimated by non-linear optimization on data from Schoenig (1975)
KG	Gastric emptying constant for corn oil	$5.058 \times 10^{-3} \text{ min}^{-1}$	
LIFE	Erythrocyte life span	86,400 min	Berlin & Berk (1975)
NRBC <sup>b</sup>	Erythrocyte number	$1.361 \times 10^{11}$	FASEB Biological Data Handbook (1971)
OFF	Distribution factors for	1.0	Estimated from Gagne & Brodeur (1972)
FOP	paraoxon in fat	1.0	
PSABS	Parathion dermal absorption constant	$1.45 \times 10^{-4} \text{ min}^{-1}$	Estimated by non-linear optimization on 10 mg/kg dermal dose AchE data
PKR	Phosphorylated protein reactivation constant	$2.30 \times 10^{-3} \text{ min}^{-1}$	Adopted from ChE reactivation
POML <sup>b</sup>	Paraoxon liver metabolism constant	$13.77 \text{ min}^{-1}$	Estimated from Appleton (1976)
POMP <sup>b</sup>	Paraoxon plasma metabolism constant	4.789 ml/min	Estimated by rat VM ÷ rabbit km Aldridge (1953)
POPL	Liver protein phosphorylation constant	$542 \text{ min}^{-1}$	Adopted from plasma protein phosphorylation constant

TABLE 3, Continued

Symbol	Parameter or Initial Value	Value Used	Reference <sup>a</sup>
POPP	Plasma protein phosphorylation constant	542 min <sup>-1</sup>	Estimated from Silvestri (1972)
PSM <sup>b</sup>	Parathion dearylation constant	5.79 min <sup>-1</sup>	Estimated from Schoenig (1975)
PSPO <sup>b</sup>	Parathion desulfuration constant	11.77 min <sup>-1</sup>	Estimated from Schoenig (1975)
QF <sup>b</sup>	Fat "plasma" flow rate	0.48 ml/min	Calculated by power law equations Lindstrom <i>et. al.</i> (1974) and adjusted for parathion distribution into RBC.
QL <sup>b</sup>	Liver "plasma" flow rate	10.52 ml/min	
QM <sup>b</sup>	Muscle "plasma" flow rate	5.69 ml/min	
SFP	Distribution factors for parathion in fat	1.378	Estimated by non-linear optimization on data from Gagne & Brodeur (1972)
FSP		$2.3 \times 10^{-3}$	
SMP	Distribution factors for parathion in muscle	1.0	
MSP		1.0	
SYN <sup>b</sup>	ChE synthesis rate	$1.89 \times 10^{-3}$ units/min	CAT * Total blood ChE activity
VF <sup>b</sup>	Fat volume	25.6 g	Calculated by equation from Gagne & Brodeur (1972)
VL <sup>b</sup>	Liver extracellular fluid volume	2.73 ml	Calculated by power law equations. Lindstrom <i>et. al.</i> (1974).
VM <sup>b</sup>		Muscle volume	
VP <sup>b</sup>	Plasma volume adjusted for parathion distribution into RBC	13.55 ml	Calculated by equation from Gagne & Brodeur (1974). Parathion distribution from Schoenig (1975)

<sup>a</sup>Procedures for obtaining estimated values are detailed in Appendix B

<sup>b</sup>Values which depend on rat body size are given for a 200 g rat

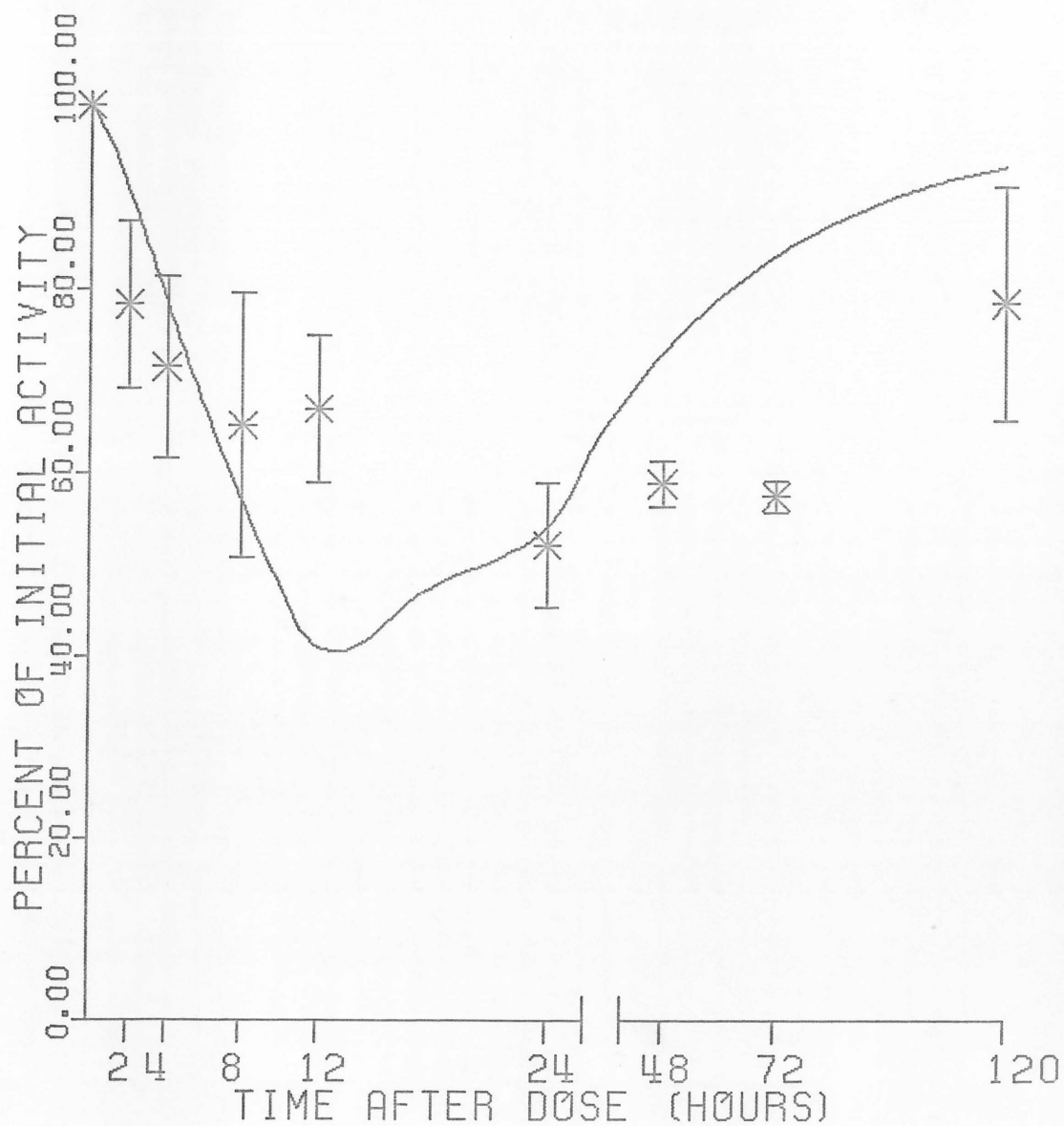


Fig. 2. Comparison of predicted AchE activities (—) with observed activities ( \* ) from a 12 hr, 15 mg/kg dermal parathion dose. Data points are means  $\pm$  1 SD (n=5 at 2,4, and 24 hr; n=4 at 12, 48, and 120 hr; n=3 at 8 and 72 hr).



appears to be more rapid than is experimentally observed.

The response of the model to a 4 mg/kg oral dose is compared to plasma (Fig. 3) and erythrocyte (Fig. 4) activities measured in male rats by Skalsky et al. (1979). The predictions of plasma activities are in excellent agreement with the experimental results. Model predictions are consistent with observed erythrocyte activities during the 3 hr period immediately following the dose. Over the next 2 hr interval the predicted activities were lower than observed values.

Whole blood cholinesterase activities reported by Jacobsen et al. (1973) for male rats orally dosed with 1 mg/kg parathion are presented with corresponding model-simulated activities in Fig. 5. Predicted and observed results are quite similar at one and four hours after dosing, but are different at 2.5 hr.

#### Sensitivity Analysis

Model parameters and initial values were varied individually by  $\pm 25\%$  to study the relationship between each input and selected model responses. Response changes were measured as percentages of the control (with all parameter values as given in Table 3) response. Average responses were computed from the absolute values of the responses to increased and decreased input alterations, and these are presented in Table 4 as fractions of the input perturbation i.e. 25%. Only six indicators changed by amounts equal to or greater than the input change. These were: maximum AchE inhibition and maximum blood parathion and paraoxon concentrations in response to changes in dose; maximum AchE inhibition and maximum blood paraoxon concentration in response to changes in liver paraoxon metabolism constant (POML); and

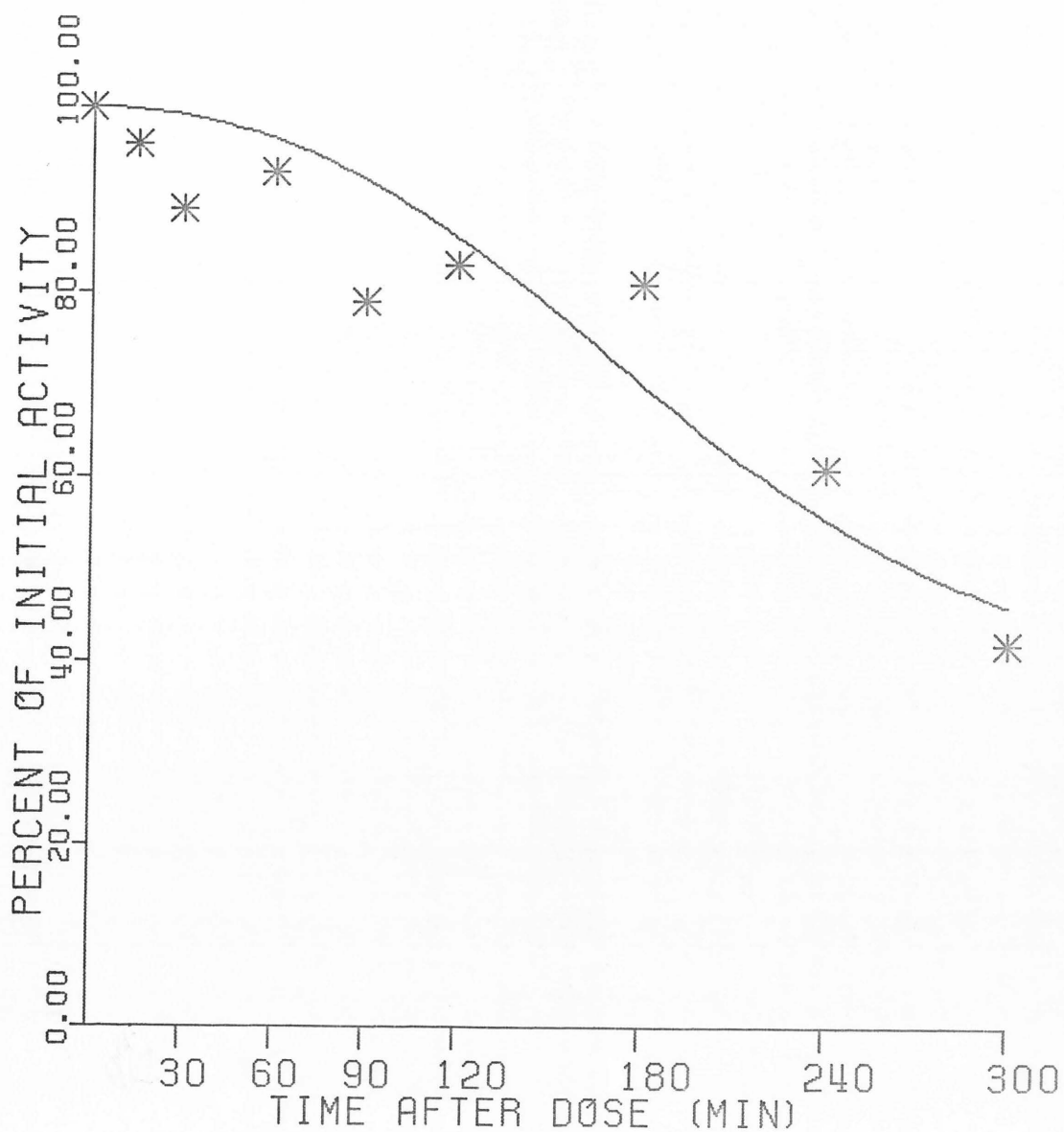


Fig. 3. Comparison of predicted ChE activities (—) with observed activities (\*) in male rats following a 4 mg/kg oral parathion dose. Data points are mean values (n=5) from Skalsky et al. (1979).

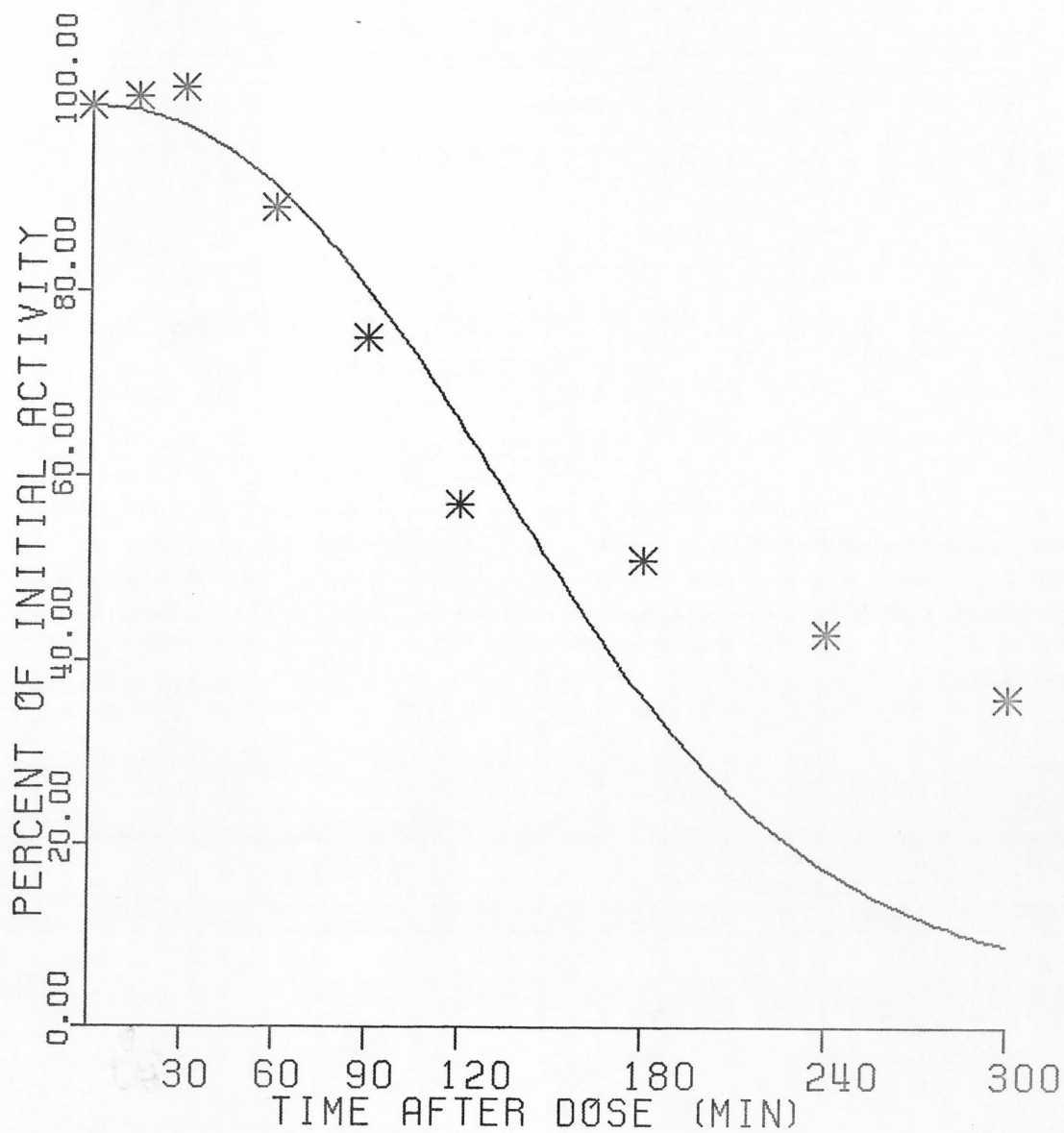


Fig. 4. Comparison of predicted AchE activities (—) with observed activities (\*) in male rats following a 4 mg/kg oral parathion dose. Data points are mean values (n=5) from Skalsky et al. (1979).

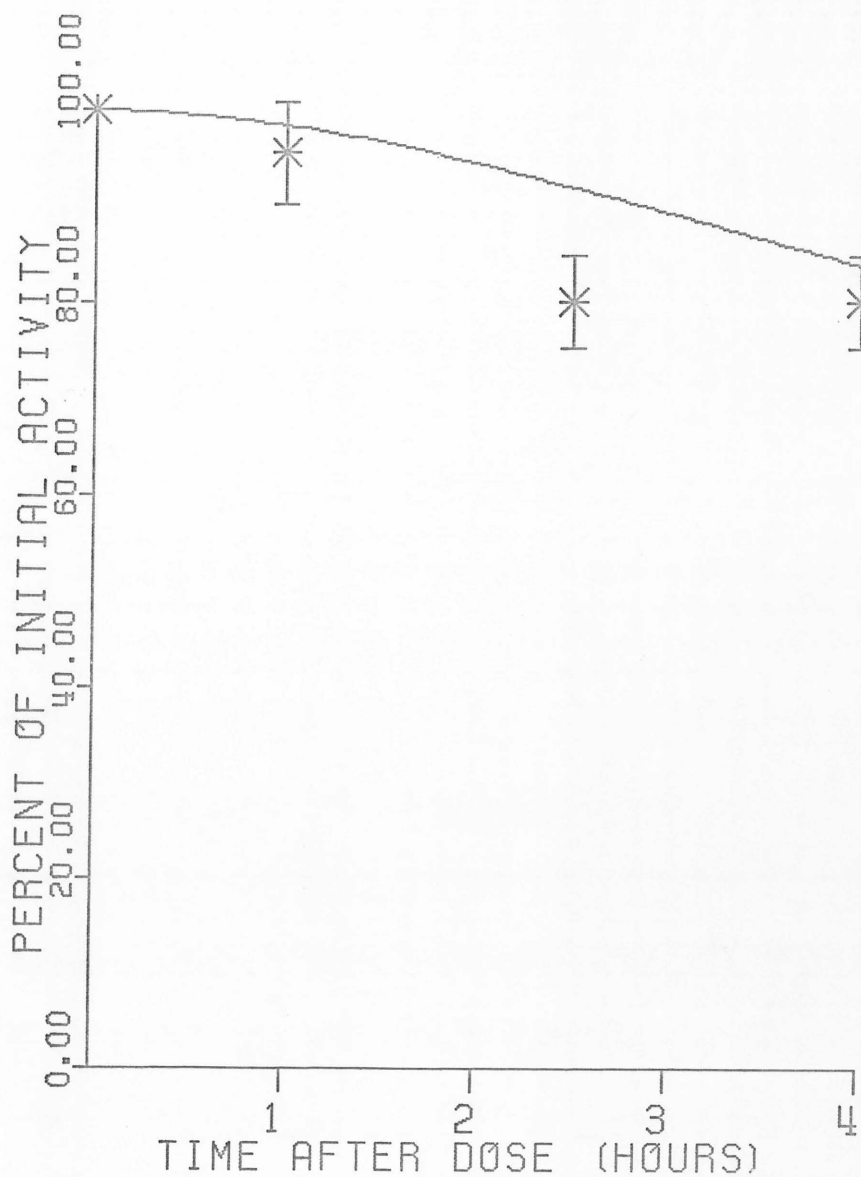


Fig. 5. Comparison of predicted whole blood cholinesterase activities (—) with observed activities in male rats (\*) following a 1 mg/kg oral parathion dose. Data points are means  $\pm$  1 SE (n=8) from Jacobsen et al. (1973).

TABLE 4

Effects of Altering Model Parameters and Input Variables on Selected Model Output<sup>a b</sup>

ALTERED MODEL PARAMETER OR INPUT VARIABLE	RATIO OF OUTPUT CHANGE: INPUT CHANGE					
	MAXIMUM ACHE INHIBITION	MAXIMUM CHE INHIBITION	TIME OF MAXIMUM ACHE INHIBITION	TIME OF MAXIMUM CHE INHIBITION	MAXIMUM BLOOD PARATHION CONCENTRATION	MAXIMUM BLOOD PARAOXON CONCENTRATION
AACT	0.11	0.03	0.05	0.04	0.00	0.12
AAGE	0.01	0.01	0.00	0.06	0.00	0.05
AKI	1.18	0.01	0.16	0.06	0.00	0.06
AKR	0.27	0.00	0.19	0.06	0.00	0.04
ATN	0.12	0.04	0.05	0.06	0.00	0.03
BW	0.05	0.01	0.03	0.06	0.13	0.09
CAGE	0.01	0.01	0.00	0.04	0.00	0.11
CACT	0.02	0.01	0.00	0.08	0.00	0.11
CAT	0.01	0.01	0.00	0.06	0.00	0.11
CKI	0.02	0.31	0.00	0.08	0.00	0.10
CKR	0.01	0.15	0.02	0.23	0.00	0.11
CTN	0.03	0.01	0.00	0.02	0.00	0.08
DOSE	1.89	0.50	0.31	0.08	1.00	1.66
ESTL	0.14	0.04	0.03	0.02	0.00	0.12
ESTP	0.66	0.18	0.10	0.08	0.00	0.55
KA1	0.31	0.12	0.42	0.40	0.49	0.56
KG2	0.30	0.12	0.42	0.40	0.49	0.57
LIFE	0.02	0.00	0.03	0.06	0.00	0.10
LIV	0.15	0.04	0.03	0.06	0.00	0.11
OFP	0.02	0.01	0.02	0.06	0.00	0.08
FOP	0.02	0.01	0.02	0.04	0.00	0.09
PKR	0.28	0.07	0.03	0.11	0.00	0.18
POML	1.46	0.42	0.23	0.08	0.00	1.43
POMP	0.33	0.09	0.08	0.08	0.00	0.26
POPL	0.02	0.00	0.08	0.08	0.00	0.08
POPP	0.21	0.05	0.02	0.06	0.00	0.09
PSMI	0.61	0.17	0.08	0.08	0.33	0.66
PSPO	0.67	0.17	0.13	0.06	0.68	0.62
SFP	0.03	0.01	0.02	0.06	0.07	0.08
FSP	0.01	0.01	0.00	0.06	0.01	0.11
SMP	0.01	0.01	0.00	0.11	0.02	0.06
MSP	0.00	0.00	0.03	0.06	0.04	0.08

<sup>a</sup>Parameter symbols are explained in Table 3.<sup>b</sup>Changes are expressed as average percent difference from the value obtained with unperturbed parameter values. Input change is  $\pm 25\%$ .

maximum AchE inhibition in response to changes in AchE-paraoxon bimolecular reaction constant (AKI).

To determine whether parameter errors can interact to yield greater than additive effects on model output, a factorial arrangement of parameter alterations was employed in an investigation of responses of the selected indicators. The parameters which caused the largest changes in the indicators when varied individually (DOSE, POML, AKI) were chosen to maximize the chance of detecting interactions (Snedecor and Cochran, 1967). Two levels of AKI and POML ( $\pm 25\%$  of unperturbed value) and three levels of DOSE (1.5, 2.0 and 2.5 mg/kg) were employed. Two significant interactions were found, POML X DOSE on ChE minimum activity and on maximum blood paraoxon concentration, and these are presented as differences in the dose-response relationship at the two levels of POML (Fig. 6). These two interactions also yielded statistically significant quadratic effects of DOSE but, as evidenced by the straight lines in Fig. 6, these have no practical significance and are the result of the very small error terms produced by simulation.

The interactions of the metabolism rate constants were also investigated. A 2X2X2 factorial arrangement of POML, PSM, and PSPO was used and two significant interactions were found. These interactions, POML X PSPO and POML X PSM on maximum paraoxon concentration, are presented in Fig. 7.

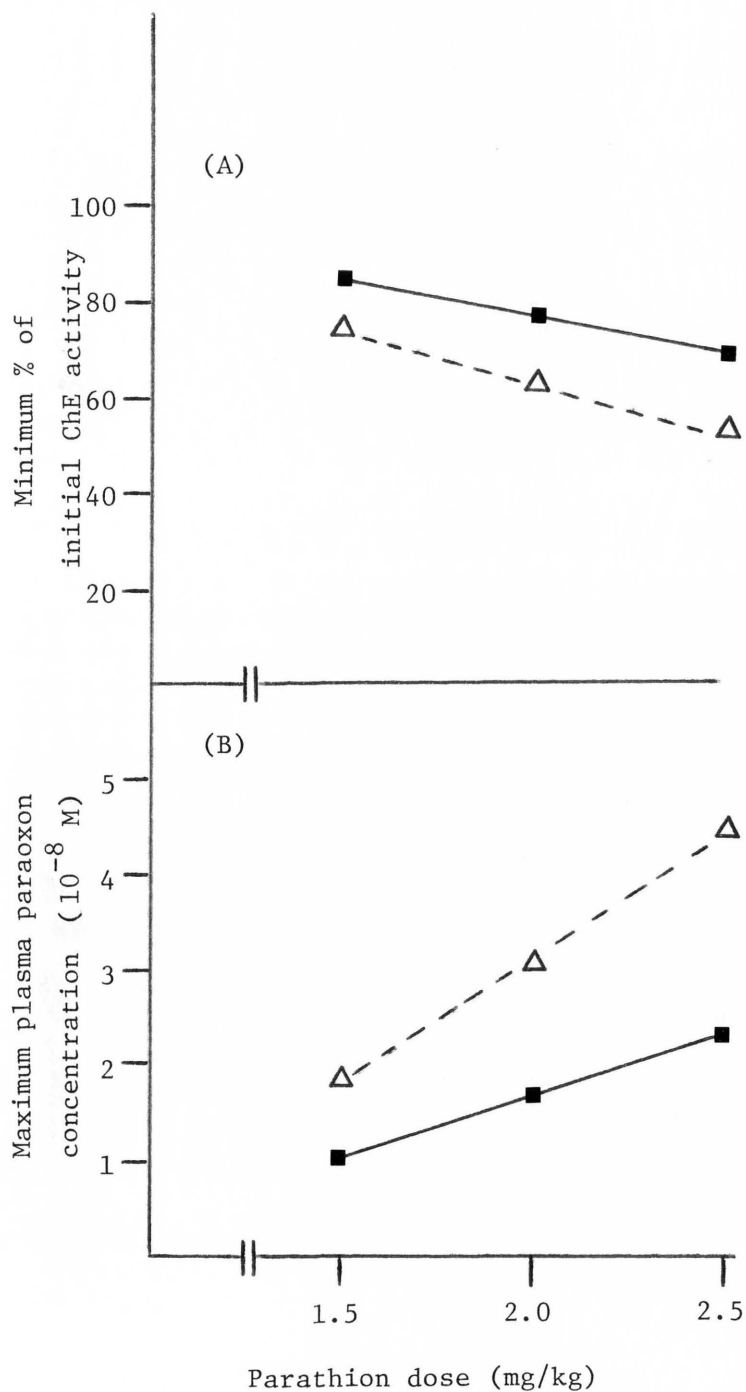


Fig. 6. Effects of dose on (A) minimum ChE activity and (B) maximum plasma paraoxon concentration at POML = +25% (■—■), and POML = -25% (△—△) of the control value. Responses are from a 13 hr simulation of an oral parathion dose in a hypothetical 200 g rat.

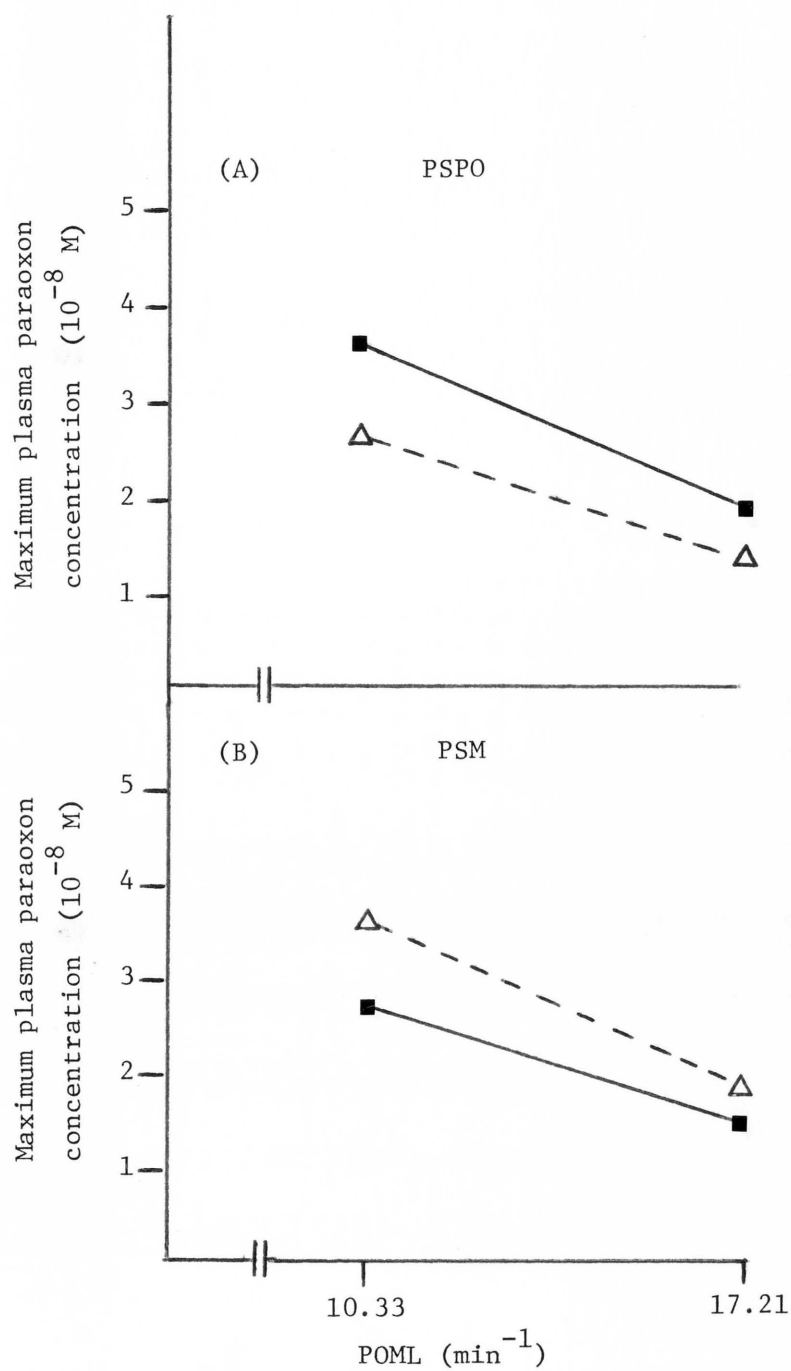


Fig. 7. Effects of POML on maximum paraoxon concentration with (A) PSPO and (B) PSM at +25% (■—■), and -25% (Δ--Δ) of their respective control values. Responses are from a 13 hr simulation of an oral parathion dose in a hypothetical 200 g rat.



DISCUSSION

A model predicting blood cholinesterase inhibition following parathion exposure was constructed on the basis of a set of simplifying assumptions concerning parathion metabolism and distribution, and cholinesterase inhibition and regeneration. Although the model did not give an exact simulation of the data at all times, it did describe the proper dynamics of inhibition with the conditions under which it was tested. The model adequately predicted AchE activities for the first 24 hours with a 12 hour dermal parathion dose. Model output closely agreed with plasma cholinesterase activities over the entire time span following an oral dose, and corresponded well with AchE activities for the first three hours. Total blood cholinesterase activity following a low oral dose was predicted well by the model.

Many of the parameters required for the model have not been adequately investigated experimentally, and therefore had to be estimated for the purposes of this study. A comprehensive mathematical description of the type presented here reveals areas in need of additional research effort for a better understanding of the operation of such a system. A model sensitivity analysis can be the guide to the relative degree of effort that should be expended on the investigation of the various parameters.

Disagreement between the model and experimental data was observed for AchE regeneration upon termination of the dermal dose. This may have resulted from an improper aging rate constant. Data from Vandekar and Heath (1957) on AchE activity regeneration after exposure

to a diethyl-phosphorylating compound (0,0-diethyl S-ethylsulphonio-ethylmethyl phosphorothiolate) were used to estimate the aging rate by a least squares fitting procedure. Although diethyl phosphorylated AchE is known to undergo an aging reaction (Aldridge and Reiner, 1972; Mehratra and Singh, 1972), observed AchE reactivation in vivo was log-linear to virtually complete reactivation at 28 days, indicating little or no aging; use of this data set probably caused an underestimation of the aging rate. When the aging rate reported for rat brain cholinesterase (Mehrotra and Singh, 1972) was substituted into the model (a 100 fold increase in the parameter value) the simulated activity regeneration underestimated the observed results. Interpretation of these results is complicated by uncertainties about the completeness of skin parathion removal by soap and water washing, although the agreement between predicted and observed values at 24 hour indicates an adequate cessation of exposure.

In the later stages of AchE inhibition following an oral dose, the model predicted lower activities than were actually observed. This could be due to an underestimate of the paraoxon liver metabolism constant. The constant was estimated from data derived from a study of paraoxon metabolism resulting from a "first pass" infusion of paraoxon into the liver (Appleton, 1976), a situation which omits the effect of the subcellular proximity of the paraoxon formation and degradation enzyme systems in promoting degradation (Nakatsugawa and Morelli, 1976). In addition, the effect of P450 binding by reactive metabolites of parathion (Neal, et al., 1977) on the in vivo desulfuration constant needs to be evaluated.

A sensitivity analysis provides a measure of the model's behavior or "robustness" (Levins, 1966) and an indication of the accuracy required in various input data (Wiens and Innis, 1974). From the sensitivity analysis of the oral cholinesterase inhibition model (Table 4), only six out of 192 measured responses exhibited a relative change equal to or greater than the relative change in the input value, and three of these were due to changes in dose. More than 90% of the responses had relative changes less than half of the relative change in the input value. In addition, only four significant interactions between simultaneous parameter alterations were detected out of 48 tested, and these four were rather small. This demonstrates the general insensitivity of the model to errors in most input variable values and suggests that the model may be relatively "robust".

The levels of all sensitivity indicators responded more strongly to changes in dose level than to changes in any of the parameter values. The times for maximum AchE and ChE inhibition were most responsive to changes in gastric emptying (KG) and intestinal absorption (KA) constants. Maximum AchE inhibition and maximum blood paraoxon concentration were the most sensitive indicators to input changes; AchE bimolecular reaction constant (AKI) and paraoxon liver metabolism constant (POML) alterations elicited larger responses than any of the other parameter changes, with paraoxon concentration responding strongly to the latter and AchE inhibition responding strongly to both. None of the indicators were very sensitive to changes in parameters controlling parathion and paraoxon tissue distribution.

The use of blood cholinesterase inhibition measurements to evaluate exposure to organophosphate insecticides, and to determine farmworker reentry periods could be facilitated by the use of a predictive model such as the one presented here. A quantitative description of the relationship between dose and the time-course of the response would allow estimation of the dose from the measurement of enzyme activities at several times. The coupling of a model describing environmental dynamics of insecticides with a model describing blood cholinesterase inhibition would facilitate evaluation of the multitude of factors which influence the time needed for a sprayed field to become safe enough for human entry. An effort to adapt this model to human exposure to parathion is now being conducted.

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APPENDIXES

Appendix AReview of LiteratureOrganophosphate exposure index

Due to the hazardous nature of organophosphate insecticides, there has been widespread interest in developing a suitable index for human exposure to these chemicals. One of the most widely used indices is the amount of cholinesterase inhibition produced by a pesticide exposure. Since organophosphates produce their toxic effects by inhibiting cholinesterases, measurements of the activities of these enzymes serve as basis for an index of the absorbed dose and of a biochemical response to the dose.

The cholinesterase-containing tissue that is most accessible for monitoring is the blood. There are two distinct types of cholinesterases in the blood, erythrocyte cholinesterase (AChE) and plasma cholinesterase (ChE), both of which may be measured to yield an exposure index (Matsumura, 1975). Since the cholinesterase in erythrocytes is considered to be identical to the enzyme in nerve tissue, AChE measurements are usually considered to be a better index of toxicity (Murphy, 1975). The amount of blood cholinesterase inhibition is, however, only an indirect measure of the extent of inhibition at the sites of toxic action, which are the cholinergic neuronal synapses.

Blood cholinesterase inhibition is reasonably well correlated with the level of organophosphate exposure (Namba et al., 1971; Wills, 1972; Long, 1975). Long (1975) reported that the response of these enzymes co-varies directly with the intensity and duration

of absorption of the inhibiting chemical and that the specificity is excellent in relation to AchE and fair in relation to ChE. Blood cholinesterase depression is widely used in the monitoring and regulation of occupational exposure to organophosphates, such as the establishment of field reentry intervals for farmworkers (Gunther et al., 1977).

#### Quantitative models

An equation for calculating safe reentry periods based on acceptable levels of cholinesterase depression has been reported (Serat, 1973). The equation combines a rate expression for the loss of pesticide residues from the field with an equation describing the rate of change of cholinesterase activity with cumulative pesticide exposure. The model assumes that cholinesterase activity declines by a first order process in relation to pesticide residues. Data relating enzyme activity of exposed workers to the cumulative pesticide residues to which they were exposed are needed to calculate the first order rate constant. An extension of this model has been proposed which allows the calculation of reentry intervals without data from human exposure (Serat et al., 1975). The model is based on the assumption that all pesticides have the same ratio of the amount of pesticide exposure needed to lower ChE activity by a given amount to the dermal LD<sub>50</sub> of female rats for the particular pesticide. A comprehensive literature search did not reveal any additional predictive models of cholinesterase inhibition.

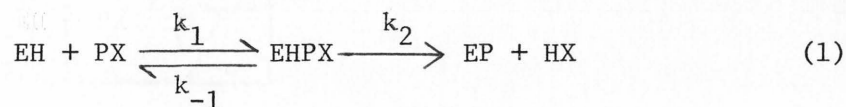
Lindstrom et al. (1974) presented a simulation model of dieldrin distribution in mammals that was based on physiologically

meaningful parameters (e.g. blood flow rates, biotransformation rates and compartment sizes). They concluded that such a model is applicable to analysis of data, testing of hypotheses, examination of experimental design and exposure of areas of inadequate knowledge which require further research.

Garfinkel (1977) stated that simulation is valuable in interpreting the behavior of complex biological systems. It can help quantify the observed relationships among variables and show how important they are in comparison to other relationships in the same system. Simulation can also help in experimental design such as in planning the quantitative manipulation of a system.

#### Cholinesterase Inhibition

The reaction of organophosphates with cholinesterases has been described as active-site directed irreversible inhibition (Main, 1973). The reaction scheme may be represented as:



where EH is the active, protonated enzyme, PX is an organophosphate inhibitor, EHPX is an enzyme-inhibitor binding complex of the Michaelis type, and EP is the irreversibly inhibited enzyme.

A kinetic treatment using Michaelis-Menten assumptions yields a rate equation of:

$$\frac{d\text{EP}}{dt} = \frac{k_2([\text{E}]_t - [\text{EP}]) [\text{PX}]}{K_D + [\text{PX}]} \quad (2)$$

where  $K_D$  is the inhibitor concentration required to yield half the maximum rate of production of EP, and  $[\text{E}]_t$  is the total concen-

tration enzyme.  $K_D = \frac{k_{-1}}{k_1}$  with an assumption of rapid equilibrium

formation of EHPX;  $K_D = \frac{k_{-1} + k_2}{k_1}$  with a steady-state formation

assumption.

Due to the low blood concentration of acetylcholine, the protective effect of the competing substrate reaction against enzyme phosphorylation is negligible in vivo. This can be demonstrated by an analysis of the equation presented by Hart and O'Brien (1973) for enzyme phosphorylation in the presence of substrate:

$$\frac{d[EP]}{dt} = \left[ \frac{k_2 [PX] ([E]_t - [EP])}{K_D + [PX]} \right] \left[ \frac{1 - \frac{[S]}{K_m + [S]}}{1 - \left[ \frac{[S]}{K_m + [S]} \right] \left[ \frac{[PX]}{K_D + [PX]} \right]} \right]$$

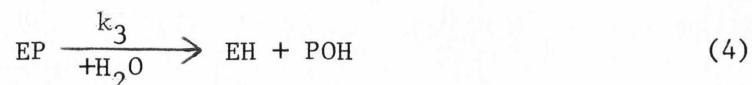
The first term on the right side of the equation is the rate expression for phosphorylation in the absence of substrate; the second term is the modification of the phosphorylation rate produced by the substrate reaction. The effect of blood acetylcholine can be seen by substituting appropriate values for S (substrate) and  $K_m$  (substrate Michaelis constant).

Using a  $K_m$  value of  $10^{-4}$  M, which is the  $K_m$  for rat brain AchE (Ho and Ellman, 1969), and a S value of  $6.84 \times 10^{-11}$  M (Lipshits and Kratinova, 1977) the numerator acquires a value of 0.999999. The size of the denominator varies according to the concentration of PX. If PX is very large,  $\frac{[PX]}{K_D + [PX]}$  will approach one, and the denominator will approach the numerator. If PX is

very small,  $\frac{[PX]}{K_D + [PX]}$  will approach zero, and the denominator will approach one. Therefore, the value of the second term can be no smaller than 0.999999, which is an insignificant diminution of the phosphorylation rate.

#### Cholinesterase activity regeneration

Inhibited cholinesterase may revert to the active form by hydrolysis of the diethyl phosphorylated enzyme (Aldridge, 1972). The reaction releases diethyl phosphate, which is not inhibitory. The rate of hydrolysis is dependent on the type of cholinesterase, the temperature and pH of the reaction medium, and the nature of phosphorylating group. The reaction may be represented as:



The rates of return of enzyme activity have been shown to be the same in vivo as they are in vitro (Davison, 1953; Vandekar and Heath, 1957; Blaber and Creasy, 1960). The amount of reactivation that can be attained is inversely related to the elapsed time after phosphorylation. This is due to the "aging" of the enzyme, which corresponds to a dealkylation of the dialkoxy phosphorylated cholinesterase (Murphy, 1975). The reaction is acid catalyzed and follows first order kinetics (Hobbinger, 1963).

The rate of aging of diisopropyl fluorophosphate (DFP) inhibited cholinesterase is so rapid that the return of cholinesterase activity is due to resynthesis of new enzyme rather than to enzyme reactivation (Murphy, 1975). Plasma cholinesterase is replaced by new enzyme

synthesized in the liver (Murphy, 1975; Wills, 1972) while erythrocyte cholinesterase is regenerated by turnover and replacement of the red cells themselves (Murphy, 1975; Berlin and Berk, 1975).

The rate of resynthesis of plasma cholinesterase does not appear to be affected by paraoxon. Domschke et al. (1970) reported a stimulatory effect of soman on ChE synthesis by rat livers, but this effect was not observed with paraoxon, dimethoate, or DFP. No reports of the influence of parathion on the rate of erythrocyte turnover have been found, but DFP is routinely used to measure red cell life span in vivo, and it yields results similar to those obtained by other methods (Berlin and Berk, 1975).

#### Parathion metabolism

Desulfuration of parathion to form paraoxon has been shown to be the result of mixed function oxidase activity (MFO) (Nakatsugawa and Dahm, 1967). The liver is quantitatively the most important site of paraoxon formation (Neal, 1967a), however, small amounts are produced by the brain and lung which may be very important in relation to toxicity (Poore and Neal, 1972). Erythrocyte cholinesterase inhibition appears to be related to the amount of paraoxon produced by the liver, while brain AchE inhibition appears to be related to the amount of oxon produced by the brain (Poore, 1972).

The aryl-phosphorus bond of parathion can be cleaved to form diethylphosphorothioate and p-nitrophenol (Nakatsugawa and Dahm, 1967; Neal, 1967a), and this has been established as the major route of direct degradation of parathion (Nakatsugawa et al., 1969; Gagne

and Brodeur, 1972). This reaction can be catalyzed by a MFO enzyme that appears to be distinct from the enzyme that catalyzes desulfuration (Neal, 1967b). It is also catalyzed by glutathione-S-aryl transferase, a soluble liver enzyme (Hollingworth, 1973). The oxidative pathway appears to dominate, since parathion has been shown to be a poor substrate for glutathione transferase enzymes (Hutson et al., 1972; Benke and Murphy, 1975; Levine and Murphy, 1977). Plasma does not contain a parathion desulfurase or a parathionase (Alary and Brodeur, 1969).

#### Paraoxon metabolism

In contrast to parathion metabolism, a large number of processes are involved in the degradation of paraoxon. The major pathway was previously considered to be the hydrolysis of the aryl-phosphate bond by paraoxonases (Plapp and Casida, 1958; Neal, 1967a) and by glutathione S-aryl transferase (Hollingworth et al., 1973; Benke and Murphy, 1975). Recent studies by Appleton (1976) of in situ hepatic metabolism of parathion and paraoxon indicated that hydrolytic mechanisms functioned at a much lower rate than oxidative deethylation. He concluded that O-deethylation is the primary hepatic system of paraoxon degradation following parathion administration in the male rat. The role of liver paraoxonase may have been overestimated in the past because of: the reliance on in vitro procedures which may have allowed the expression of hydrolytic activity which was not available in vivo, the instability of the O-deethylase enzymes, inappropriate analytical procedures (the TLC procedures commonly employed do not adequately resolve monoethyl



paraoxon and diethyl phosphoric acid) or misinterpretation of urinary diethyl phosphoric acid levels which may be largely attributable to extrahepatic processes.

Another group of investigators has questioned the role of liver paraoxonase activity in paraoxon metabolism in vivo. Lauwerys and Murphy (1969a) found that paraoxonase activity is the primary mode of paraoxon inactivation by rat liver and plasma in vitro only when high paraoxon concentrations ( $>10^{-4}$  M) were used. With lower concentrations of paraoxon ( $10^{-7}$  M), inactivation is primarily the result of tissue binding (enzymatic hydrolysis accounts for less than 1% of the total inactivation by 10 mg of rat liver at  $10^{-7}$  M paraoxon). This inactivation is completed very rapidly ( $<2.5$  minutes) and the amount that is inactivated is not affected by a four-fold increase in the concentration of paraoxon. The lower paraoxon concentration is thought to be more realistic estimate of in vivo levels (Triolo *et al.*, 1970; Gagne and Brodeur, 1972). The binding of paraoxon by liver and plasma has been shown to be an important protective mechanism against paraoxon toxicity. Selective blocking of these sites by tri-*O*-tolyl phosphate potentiates the toxicity and the inhibition of AchE and ChE (Lauwerys and Murphy, 1969b). The binding sites have not been identified, but there is suggestive evidence that they may be nonspecific tissue esterases (Murphy, 1975).

These enzyme systems could reasonably be expected to regain their activity by synthesis and reactivation, and this may be important with multiple exposures to parathion. These rates are not known at this time. The reactivation rates for liver and plasma aliesterase have been measured in mice, and they are much higher than the

corresponding rates for liver and plasma cholinesterase (Levine and Murphy, 1977). Aliesterases comprise a major fraction of the total plasma esterase activity in rats (Wills, 1972) and they do not undergo an aging reaction (Jansz, 1959).

#### Parathion and paraoxon distribution

The distribution of parathion and paraoxon within the rat body has not been well characterized. A review of the literature revealed only one distribution study of parathion. Gagne and Brodeur (1972) studied the fate of  $^{32}\text{P}$ -parathion in adult male rats following an i.v. injection. The total radioactive material recovered from the liver, kidneys and brain represented small fractions of the administered dose. The peak amounts, expressed as percentages of the dose were 3%, 1 - 2.5%, and 0.03% respectively. Relatively important fractions of the administered dose were recovered from the skeletal muscles as shown by the peak value of 10.5%. The only substances found in the abdominal fat tissue were parathion and paraoxon. Maximal quantities were reached at 60 minutes and represented 9.2% of the injected dose.

The fat tissue may serve as a reservoir for parathion, accumulating it in the early stages, and releasing it to the blood in the later stages of distribution (Gagne and Brodeur, 1972). A fairly good temporal correlation was found between the rise of parathion in the liver and its disappearance in abdominal fat tissue. The concentration of parathion in fat tissue was much higher than that in the plasma, but the paraoxon concentration was somewhat lower in fat than in plasma. The parathion concentration in muscle was lower

than that in the plasma, and the paraoxon concentration was not given. The interaction between parathion and lipoproteins of rat blood has been investigated (Skalsky and Guthrie, 1977). Parathion appears to bind to these proteins in a non-specific hydrophobic manner. Parathion therefore appears to be bound and transported by blood proteins, but the significance of this finding in relation to toxicity is unknown.

Excretion of unmetabolized parathion and paraoxon appears to be negligible. They have been reported as traces (0.5-2.0%) of excreted materials in urine (Gagne and Brodeur, 1972). Fecal matter contained only 1.6% of the administered labelled parathion dose after 96 hours in a study by Appleton (1976).

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Appendix B

Parameter and Initial Condition Calculations

In vivo liver metabolism constants

Derivation.

$$(PSP)_{Out} = F(PSP)_{In} \quad (1)$$

Mass balance for in situ metabolism studies:

$$\frac{d(PSL)}{dt} = QL (PSP)_{In} - F QL(PSP)_{In} - k_{Met} (PSL) \quad (2)$$

At steady state:

$$0 = QL[(PSP)_{In} - F(PSP)_{In}] - k_{Met} (PSL) \quad (3)$$

$$k_{Met} = QL(PS)_{In} [1 - F] \frac{1}{(PSL)} \quad (4)$$

Assuming that the concentration in liver capillary blood is the same as the concentration leaving the liver (Gillette and Pang, 1977), and that there is a liver volume in equilibrium with the capillary blood:

$$\frac{PSL}{VL} = \frac{PSP_{Out}}{VP} \quad (5)$$

$$PSL = \frac{VL}{VP} PSP_{Out} \quad (6)$$

Substituting for  $PS_{Out}$ :

$$PSL = \frac{VL}{VP} VP F(PSP)_{In} = VL F(PSP)_{In} \quad (7)$$

Substituting for PSL in Equation (4):

$$k_{Met} = \frac{QL(PSP)_{In} [1 - F]}{VL (PSP)_{In} F} \quad (8)$$

$$k_{Met} = \frac{QL}{VL} \left[ \frac{1 - F}{F} \right] \quad (9)$$

Abbreviations used.

( ) Concentration

PSP	Plasma parathion
PSL	Liver parathion
QL	Liver plasma flow rate
F	Fraction of parathion entering liver which exits unmetabolized
$k_{Met}$	Observed metabolism constant
E	Hepatic extraction ratio
$Cl_o$	Apparent oral systemic blood clearance of total drug
C	Concentration

Discussion. Equation (9) is equivalent to an expression for apparent oral total drug clearance when all of the drug is metabolized by the liver, presented by Wilkinson and Shand (1975).

$$Cl_o = QL \left[ \frac{E}{1 - E} \right] \quad (\text{Wilkinson and Shand, 1975}) \quad (10)$$

$$E = 1 - F \quad (11)$$

Substituting for E in Equation (10):

$$Cl_o = QL \left[ \frac{1 - F}{F} \right] \quad (12)$$

It is also equivalent to an equation given by Gillette and Pang (1977) for a drug constantly infused into the blood just before it enters the organ in an isolated organ perfusion system in which blood recirculates (a situation equivalent to an oral administration of a drug which is metabolized in the liver and measured in systemic blood).

$$F = \frac{C_{Out}}{C_{In}} \quad (13)$$

$$\text{Clearance} = QL \left[ \frac{C_{In} - C_{Out}}{C_{Out}} \right] \quad (\text{Gillette and Pang, 1977}) \quad (14)$$



$$\text{Clearance} = QL \left[ \frac{C_{\text{In}}}{C_{\text{Out}}} - 1 \right] \quad (15)$$

Substituting identity for F into Equation (15):

$$\text{Clearance} = QL \left[ \frac{1}{F} - 1 \right] \quad (16)$$

$$\text{Clearance} = QL \left[ \frac{1 - F}{F} \right] \quad (17)$$

These two equations are expressed as clearance (ml/min) in contrast to Equation (9) given above which is expressed as a rate constant ( $\text{min}^{-1}$ ). This distinction disappears when the rate of metabolism is computed because the plasma concentration used with the clearance equations is the same as the liver concentration used to derive Equation (9). This occurs because, with an oral dose, the site of systemic blood sampling is after the tissue but before the site of administration (Gillette and Pang, 1977). The rate equations can be shown to be equal:

$$\frac{d \text{PSP}}{dt} = QL \left[ \frac{1 - F}{F} \right] * \frac{\text{PSP}}{VP} = QL \left[ \frac{1 - F}{F} \right] \frac{1}{VL} * \text{PSL} \quad (18)$$

$$\frac{d \text{PSP}}{dt} = \left[ \text{apparent clearance} \right] * \left[ \text{plasma concentration} \right] = k_{\text{Met}} * \left[ \text{liver amount} \right] \quad (19)$$

Calculations. Schoenig (1975) reported the results of an in situ hepatic metabolism study in which a steady-state input concentration of parathion was achieved from oral absorption. The data were presented as hepatic portal vein (HPV) and inferior vena cava (IVC) parathion concentrations, along with percent metabolism via the diethyl phosphoric acid (DEPA) pathway. Data from the two dose levels were averaged and then used to compute an overall parathion metabolism constant. This constant was divided into the dearylation and desulfuration pathways by use of the value for the DEPA pathway

contribution.

$$\frac{1 - F}{F} = \frac{.82}{.18} = 4.56 \quad (20)$$

$$\text{PSPO} = \frac{QL}{VL} (0.67) (4.56) = (3.05) \frac{QL}{VL} \quad (21)$$

$$\text{PSM} = \frac{QL}{VL} (0.33) (4.56) = (1.50) \frac{QL}{VL} \quad (22)$$

Similar data were furnished by Appleton (1976) for hepatic paraoxon metabolism. He presented the amount of metabolites and parent compound that exited the liver 1 to 2 minutes after a simulated intraperitoneal injection of 2 mg/kg paraoxon (direct application to exposed gut tissue and mesentery). The data were expressed as percentages of the total radioactivity exiting the liver. The fraction of the total radioactivity that was paraoxon represents F.

$$\frac{1 - F}{F} = \frac{.781}{.219} = 3.57 \quad (23)$$

$$\text{POML} = 3.57 \frac{QL}{VL} \quad (24)$$

#### Plasma cholinesterase catabolism constant

Derivation.

$$\frac{d \text{CHE}}{dt} = \text{SYN} - [\text{CAT} * \text{CHE}] \quad (\text{Rehchigl, 1971}) \quad (25)$$

Solving the differential equation:

$$\text{CHE} (t) = \frac{\text{SYN}}{\text{CAT}} - \left[ \frac{\text{SYN}}{\text{CAT}} \right] e^{-\text{CAT} * t} \quad (26)$$

At steady state, from Equation (25):

$$\text{SYN} = \text{CAT} * \text{CHE}_{ss} \quad (27)$$

Substituting for SYN in Equation (26):

$$\text{CHE} (t) = \frac{\text{CAT} * \text{CHE}_{ss}}{\text{CAT}} \left[ 1 - e^{-\text{CAT} * t} \right] \quad (28)$$

$$\frac{\text{CHE}(t)}{\text{CHE}_{ss}} = 1 - e^{-\text{CAT} * t} \quad (29)$$

Solve for CAT:

$$\text{CAT} = -\ln \left[ 1 - \frac{\text{CHE}(t)}{\text{CHE}_{ss}} \right] t^{-1} \quad (30)$$

Converting percent activity to fractional activity:

$$\text{CAT} = -\ln \left[ 1 - \frac{\% \text{ activity}}{100} \right] t^{-1} \quad (31)$$

Abbreviations used.

t Time

$\text{CHE}_{ss}$  Steady state plasma cholinesterase activity

Discussion. Domschke *et al.* (1970) measured the return of plasma cholinesterase over 48 hours following a high dose of DFP. Since diisopropyl phosphorylated cholinesterases do not reactivate due to a high aging rate, the return of activity is solely by synthesis of new enzyme (Murphy, 1975). Inhibition was 100% at four hours, but recovery had begun by 8 hours.

The activity at 48 hours was used to calculate CAT. Four hours was chosen as the time for initiation of regeneration in the absence of further inhibition.

Calculation.

$$\text{CAT} = -\ln \left[ 1 - \frac{21.6}{100} \right] \left[ \frac{1}{48 - 4} \right] = 5.65 \times 10^{-3} \text{ hr}^{-1} \quad (33)$$

Plasma protein phosphorylation constant

Derivation. The second order rate equation:

$$\frac{-d(\text{EST})}{dt} = k(\text{EST})(\text{PX})$$

Integrating the rate equation:

$$k = \left[ \frac{1}{(\text{EST})_o - (\text{PX})_o} \right] \ln \left[ \frac{(\text{EST})(\text{PX})_o}{(\text{EST})_o(\text{PX})} \right] \left[ \frac{1}{t} \right] \quad (34)$$

Abbreviations used.

- ( ) Concentration  
EST Phosphorylatable plasma proteins  
k Second order rate constant  
PX Organophosphate inhibitor  
t Time

Discussion. Silvestri (1972) measured amounts of paraoxon inactivated by rat plasma in vitro. Two concentrations of paraoxon were used and measurements were made after one and fifteen minute incubations.

The initial protein concentration was estimated by using a value for plasma concentration of phosphorylatable sites obtained from Schoenig (1975). Silvestri's (1972) paraoxon inactivation data were converted to reaction volume concentrations from a "per ml plasma" basis by use of volume manipulation information provided in the text.

The data were plotted by use of Equation (34) in the manner suggested by Daniels and Alberty (1975), but a non-linear plot resulted. The fifteen minute data yielded lower estimates of K than did the one minute data.

The estimates of K provided by the data from the one minute incubations at both concentrations were averaged to provide the estimate of POPP. The higher estimate of K provided by the one minute incubations was more consistent with results from in vivo studies which implied that plasma protein phosphorylation was more rapid than AchE phosphorylation. These findings were: 1) 8% of

plasma binding sites remained at 4 hours after a 3 mg/kg i.p. injection (Lauwerys and Murphy, 1969a); 2) 20% of erythrocyte cholinesterase activity remained at 4 hours after a 5 mg/kg i.p. injection (Murphy, 1969).

Calculations. Concentrations are in micromoles/liter.

0.40 micogram/ml paraoxon:

$$k = \frac{1}{4.2987 - 1.4534} \ln \left[ \frac{3.114 * 1.4534}{4.2987 * 0.2662} \right] = .483 \text{ uM}^{-1} \text{ min}^{-1} \quad (35)$$

0.80 microgram/ml paraoxon:

$$k = \frac{1}{4.2987 - 2.907} \ln \left[ \frac{1.9684 * 2.907}{4.2987 * 0.5766} \right] = .601 \text{ uM}^{-1} \text{ min}^{-1} \quad (36)$$

$$\text{POPP} = 542 \text{ (micromole/ml)}^{-1} \text{ min}^{-1} \quad (37)$$

### Bimolecular reaction constants

Derivation.

$$\frac{-d \text{ (EH)}}{dt} = k_i \text{ (EH) (PX)} \quad (\text{Matsumura, 1975}) \quad (38)$$

Assuming pseudo-first order kinetics, and integrating  $t=0$  to  $t$  and the enzyme level  $(\text{EH})_0$  to  $(\text{EH})_t$ :

$$\ln \left[ \frac{(\text{EH})_0}{(\text{EH})_t} \right] = k_i \text{ (PX) } t \quad (39)$$

Solving for  $k_i$  and substituting for the enzyme concentration ratio:

$$k_i = \frac{1}{(\text{PX})_t} \ln \left[ \frac{100}{P} \right] \quad (40)$$

If (PX) is the inhibitor concentration causing 50% inhibition of enzyme

$$k_i = \frac{\ln 2}{I_{50} t} \quad (41)$$

Abbreviations used.

( )	Concentration
EH	Cholinesterase enzyme
PX	Organophosphate inhibitor
$k_i$	Bimolecular reaction constant
t	Time
P	% enzyme activity
$I_{50}$	Inhibitor concentration causing 50% enzyme inhibition
EP	Phosphorylated enzyme
$K_A$	Affinity constant for enzyme-inhibitor complex formation

Discussion. The reaction between cholinesterase and paraoxon is actually a reversible association followed by a non-reversible phosphorylation rather than a simple bimolecular reaction as described in the previous section. The kinetics become bimolecular with an assumption that  $K_A \gg (PX)$ . The necessary constants were not available from the literature; therefore, the rigorous kinetic equation could not be used in this project.  $I_{50}$  data from Davison (1953) were used to calculate  $k_i$  values for both enzymes.

Calculations.

$$\begin{aligned} AKI &= \frac{0.693}{[1.1 \times 10^{-7} M][30 \text{ min}]} = 2.1 \times 10^5 \text{ M}^{-1} \text{ min}^{-1} \\ &= 210 (\text{micromoles/ml})^{-1} \text{ min}^{-1} \end{aligned} \quad (42)$$

$$\begin{aligned} CKI &= \frac{0.693}{[2.8 \times 10^{-7} M][30 \text{ min}]} = 8.25 \times 10^4 \text{ M}^{-1} \text{ min}^{-1} \\ &= 82.5 (\text{micromoles/ml})^{-1} \text{ min}^{-1} \end{aligned} \quad (43)$$

### Plasma volume and flow rates

Parathion distribution between plasma and erythrocytes was studied by Schoenig (1975). With whole blood concentrations ranging over three orders of magnitude, the fraction of parathion in the plasma was consistently 78%.

Drugs contained within erythrocytes are available for rapid exchange with the plasma (Wilkinson and Shand, 1975); consequently blood parathion behaves as if parathion were contained in a homogeneous plasma having a larger volume than the actual plasma volume. The plasma concentration of parathion was computed in the model by dividing total blood parathion by plasma volume and then multiplying by a factor of 0.78. The plasma flow rates in tissues were increased by a factor of  $1/0.78$  to accommodate the larger apparent plasma volume.

### Liver volume

The mass of liver that was represented by VL was the portion of liver water that was assumed to be in equilibrium with parathion in the liver capillaries. The liver extracellular fluid (ECF) volume was chosen for VL because lipid-soluble molecules enter the interstitial fluid almost instantaneously during their passage through tissue capillaries and cell membranes restrict the passage of drugs to a greater extent than do the capillary membranes (Goldstein *et al.*, 1974). This choice was further supported by the results of a parathion distribution study (Gagne and Brodeur, 1972) in which liver and plasma parathion concentrations were roughly equal when liver parathion was divided by liver ECF volume.

Calculation.

$$VL = [\text{Liver mass}] * \left[ \frac{\text{Liver fractional water content}}{\text{water content}} \right] * \left[ \frac{\text{ECF-plasma}}{\text{Total body water}} \right] \quad (44)$$

$$VL = [\text{Liver mass}] * [0.75] * [0.35] \quad (45)$$

The fractional water content of rat liver was obtained from the FASEB Biological Handbook (1971); the ECF, plasma and total body water volumes were adopted from human data (Guyton, 1971).

Phosphorylatable liver protein

Paraoxon inactivation by rat liver homogenates has been investigated at low concentrations of paraoxon (Lauwerys and Murphy, 1969a, 1969b). One gram of liver homogenate can inactivate 15.2 nanomoles of paraoxon by a binding mechanism (Lauwerys and Murphy, 1969a). There are no data on the amount of paraoxon bound per gram of liver in vivo.

The availability of binding sites in vivo was estimated by multiplying the in vitro availability by the fractional liver mass that represents the volume of liver that is hypothesized to be in equilibrium with plasma parathion.

Paraoxon fat distribution constants

The relationship between the concentrations of paraoxon in fat and plasma is similar to the relationship between the concentrations of parathion in muscle and plasma (Gagne and Brodeur, 1972). Therefore the values of FOP and OFP were adopted from the corresponding values of MSP and SMP.

Parameter optimization strategy

Discussion. Parameters were optimized by use of a modified



Levenberg-Marquardt algorithm for finding the minimum of the sum of squares of the differences between model output and observed data (IMSL, 1979). Parameter values were constrained by estimated upper and lower bounds (IMSL, 1979). In some cases, starting points for the optimization algorithm were generated by use of a program which systematically placed points to optimize their dispersion within chosen bounds (IMSL, 1979).

The general procedure was: 1) to obtain a data set of observed values corresponding to the model response(s) of interest; 2) to allow a set of model parameters which control the response(s) to vary simultaneously within specified bounds; and 3) to explore this parameter space to find the combination of values which minimizes the difference(s) between model output and observed data.

The following parameters were optimized in this fashion: AAGE, AKR, KA, KG, PSABS, FSP, SFP, MSP, and SMP.

#### Acetylcholinesterase aging and reactivation constants

##### Derivation.

$$\frac{d \text{ACHE}}{dt} = C50 + [\text{AKR} * \text{ACHEPR}] - [\text{DEST} * \text{ACHER}] \quad (46)$$

$$\frac{d \text{ACHER}}{dt} = \text{AKR} * \text{ACHEPR} \quad (47)$$

$$\frac{d \text{ACHEPR}}{dt} = -C15 * \text{ACHEPR} \quad (48)$$

Solving the system by an eigensystem procedure:

$$\text{ACHE} = \text{ACHEI} + [C50 * t] + \text{ACHEPRI} \left[ \frac{-\text{DEST} * \text{AKR} * t}{C15} + [1 - e^{-C15 * t}] \right] \left[ \frac{[\text{DEST} * \text{AKR} / C15 + \text{AKR}]}{C15} \right] \quad (49)$$

$$ACHER = \frac{ACHEPRI * AKR}{C15} \left[ 1 - e^{-C15 * t} \right] \quad (50)$$

$$ACHEPR = ACHEPRI * e^{-C15 * t} \quad (51)$$

Abbreviations used.

ACHE	Total amount of active enzyme at time = t
ACHER	Amount of active enzyme at time = t that exists as a result of reactivation of inhibited enzyme after time = 0
ACHEPR	Amount of reactivatable inhibited enzyme at time = t
ACHEI	Total amount of active enzyme at time = 0
ACHEPRI	Amount of reactivatable inhibited enzyme at time = 0
ACHEO	Total amount of enzyme
C15	AKR + AAGE + DEST
C50	$\left[ \frac{NRBC * ACTV}{LIFE} \right] - [DEST * ACHEI]$
t	Time

Discussion. The closed form solution was used to calculate the regeneration of enzyme activity. Model output was optimized against erythrocyte cholinesterase activities measured in rats at intervals following 0,0-diethyl S-ethylsulphonioethylmethyl phosphorothiolate (Vandekar and Heath, 1957), a compound which yields diethyl phosphorylated enzyme. Two hours was selected as the starting time for regeneration because enzyme activity began to increase after this time. The average amount of inhibited enzyme during the two hours immediately following exposure was allowed to age for two hours to establish a value for ACHEPRI.

### Intestinal absorption and gastric emptying constants

#### Derivation.

$$\frac{d \text{PSI}}{dt} = [-KA * \text{PSI}] + [KG * \text{PSST}] \quad (52)$$

$$\frac{d \text{PSST}}{dt} = -KG * \text{PSST} \quad (53)$$

Solving the system by an eigensystem procedure and substituting DOSE for PSST(0) and 0.0 for PSI(0):

$$\text{PSI}(t) = \frac{\text{DOSE} * \text{KG}}{\text{KA} - \text{KG}} \left[ e^{-\text{KG} * t} - e^{-\text{KA} * t} \right] \quad (54)$$

$$\text{Rate of absorption} = \text{KA} * \text{PSI}(t) \quad (55)$$

Discussion. The closed form solution was used to calculate parathion absorption rates following a 6 mg/kg dose. Model output was optimized against absorption rates measured in rats following a 6 mg/kg po injection of radiolabelled parathion dissolved in corn oil (Schoenig, 1975).

### Dermal absorption constant

The entire simulation model was used to calculate erythrocyte cholinesterase activity for a twelve hour dermal 10 mg/kg parathion exposure. Model output was optimized against erythrocyte cholinesterase activities measured in rats at 2, 4, 8 and 12 hours during a dermal 10 mg/kg parathion exposure (data collected as part of this investigation).

### Parathion distribution constants

The first four equations in the simulation model (parathion system state variables), which are soluble by eigensystem analysis, were used to calculate amounts of parathion in liver, plasma, fat, and muscle following a 3.1 mg/kg iv dose of parathion (the total

dose was placed into the plasma at time = 0). Model output was optimized against parathion concentrations in liver, plasma, fat and muscle measured in adult male rats following a 3.1 mg/kg i.v. injection of  $^{32}\text{P}$ -parathion (Gagne and Brodeur, 1972).

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## Appendix C

### Computer Program Listing

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C PROGRAM CHESIM
C
C PROGRAMMER-SCOTT HENNES
C DATE OCTOBER 1979
C
C DESCRIPTION-THIS PROGRAM SIMULATES PARATHION METABOLISM AND CHOLINESTERASE
C INHIBITION IN RATS. THE SYSTEM IS REPRESENTED BY A NON-LINEAR SYSTEM OF
C DIFFERENTIAL EQUATIONS. THE 6 EQUATIONS REPRESENTING PARATHION DYNAMICS
C ARE SOLVED IN CLOSED FORM (LINEAR SUBSYSTEM) BY FINDING EIGENVALUES,
C EIGENVECTORS, AND INTEGRATION CONSTANTS. THE 11 EQUATIONS REPRESENTING
C PARAOXON-CHOLINESTERASE DYNAMICS ARE NUMERICALLY INTEGRATED BY GEAR'S
C METHOD FOR STIFF SYSTEMS. THE 2 LINEAR CHOLINESTERASE REGENERATION
C SUBSYSTEMS ARE REPRESENTED BY CLOSED FORM SOLUTIONS AFTER THE INHIBITION
C PERIOD HAS ENDED.
C
C SUBROUTINES- EIGRF(IMSL-7) TO CALCULATE EIGENVALUES AND EIGENVECTORS
C LEQTF(IMSL-7) LINEAR EQUATION SOLUTION FOR INTEGRATION C'S
C DGEAR(IMSL-7) DIFFERENTIAL EQUATION SOLVER
C
C SYMBOL DESCRIPTION
C BW=BODY WEIGHT (GRAMS)
C VP1=VOLUME OF PLASMA IN A RAT (ml)
C VP=VOLUME OF PLASMA, ADJUSTED FOR PARATHION DISTRIBUTION WITHIN BLOOD (ml)
C LIV=FRACTION OF LIVER WATER WHICH IS VOLUME OF DISTRIBUTION
C VL=VOLUME OF DISTRIBUTION IN LIVER (ml)
C VF=VOLUME OF FAT (g)
C VM=VOLUME OF MUSCLE (g)
C QL=LIVER PLASMA FLOW RATE--USING VP (ml/min)
C QF=FAT PLASMA FLOW RATE--USING VP (ml/min)
C QM=MUSCLE PLASMA FLOW RATE--USING VP (ml/min)
C FSP=EQUILIBRIUM CONCENTRATION COEFFICIENT FOR PARATHION IN FAT
C FOP= " " " " PARAOXON IN FAT
C MSP= " " " " PARATHION IN MUSCLE
C PSABS=DERMAL ABSORPTION RATE CONSTANT FOR PARATHION (/min)
C PSPO=PARATHION DESULFURATION RATE CONSTANT (/min)
C PSM=PARATHION DEARYLATION RATE CONSTANT (/min)
C POML=PARAOXON LIVER METABOLISM RATE CONSTANT (/min)
C POMP= " PLASMA " " (ml/min)
C AN I AFTER A VARIABLE NAME ON INPUT INDICATES A VALUE USED TO COMPUTE THE
C VARIABLE IN THE PROGRAM
C POPP=PLASMA PROTEIN PHOSPHORYLATION RATE CONSTANT (/MICROMOL/ml)min)
C POPL=LIVER " " " "
C PKR=PHOSPHORYLATED PROTEIN REACTIVATION CONSTANT (/min)
C HCT=HEMATOCRIT--AS A FRACTION
C AACT=ACHE ACTIVITY (UNITS/ml RBC)
C ACTV=ACTIVITY PER RBC (UNITS/CELL)
C ACHEO=INITIAL TOTAL ACHE ACTIVITY (UNITS)
C NRBC=NUMBER OF RBC PER RAT
C LIFE=LIFETIME OF A RBC (min)
C AKI=BIMOLECULAR REACTION CONSTANT FOR ACHE-PARAOXON (/MICROMOL/ML)MIN)
C AKR=ACHE REACTIVATION CONSTANT (/min)
C AGE=ACHE AGING RATE CONSTANT (/min)
C ATN=TURNOVER NUMBER FOR ACHE WITH ACETYLCHOLINE (UNITS/MICROMOL)
C CACT=CHE ACTIVITY (UNITS/ml PLASMA)
C CKI=BIMOLECULAR REACTION CONSTANT FOR CHE-PARAOXON (/MICROMOL/ml)min)
C CKR=CHE REACTIVATION CONSTANT (/min)
C CAGE=CHE AGING CONSTANT (/min)
C CTN=TURNOVER NUMBER FOR CHE WITH ACETYLCHOLINE (UNITS/MICROMOL)
C CHEO=INITIAL TOTAL CHE ACTIVITY (UNITS)
C SYN=ZERO ORDER RATE OF CHE SYNTHESIS (UNITS/min)
C TYPE=THE ROUTE OF ADMINISTRATION; EITHER 'ORAL', 'SKIN', OR 'IV'

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801 FORMAT(A4)
803 FORMAT(2F2.1,F5.4,F2.1,F6.5,6F2.1)
804 FORMAT(I1)
805 FORMAT(4I1)
806 FORMAT(2I1)
808 FORMAT(5(E8.4),/5(5(E8.4)/),/5(E8.4))
900 FORMAT('0 BODY WEIGHT = ',F6.1/'0 LIV = ',F6.3/'0 FSP = ',F7.4/
1'0 FOP = ',F6.3/'0 MSP = ',F6.3/'0 PSPOI = ',
2F6.3/'0 PSMI = ',F6.3/'0 POMLI = ',F6.3/'0 POMPI = ',F6.3/
3'0 POPP = ',F5.1/'0 POPL = ',F5.1/'0 PKR = ',F8.5/'0 HCT = ',F6.3/
4'0 AACT = ',F6.3/'0 LIFE = ',F7.1/'0 AKI = ',E11.4/
5'0 AKR = 'E10.3/'0 AGE = ',F8.5/'0 ATN = ',E9.2/'0 CACT = ',F6.3)
901 FORMAT('0 CKI = ',F5.1/'0 CKR = ',F8.5/'0 CAGE = ',F8.5/
1'0 CTN = '1PE9.2/'0 SFP = 'E15.5/'0 SMP = 'E15.5/'0 OFP = 'E15.5/)
902 FORMAT('0 PARATHION SYSTEM INITIAL STATES'/'0',6(F5.2,5X)/)
903 FORMAT('0 PARADOXON SYSTEM INITIAL INPUTS'/'0',2(F4.1,5X)/
1'0 Y(3) = ',F7.4/'0',F4.1/'0 Y(5) = ',F8.5/'0',6(F4.1,5X)///)
904 FORMAT('0 SWICH1 = ',I1,10X,'SWICH2 = ',I1///)
905 FORMAT('0 THE COEFFICIENT MATRIX A FOR THE PS SYSTEM'
1' OF LINEAR DIFFERENTIAL EQUATIONS',///0',6(6(1PE10.3,5X)///)
906 FORMAT('0 THE EIGENVALUES OF MATRIX A:',///0',
16(1PE10.3,E8.1,3X)///)
907 FORMAT('0 THE EIGENVECTORS--COLUMN J CORRESPONDS TO EIGENVAUE J',
1///0',6(6(1PE10.3,E8.1,3X)///)///)
908 FORMAT('0 L = ',2I,5X,'IF L IS GREATER THAN 0, THERE ARE '
1' IMAGINARY OR MULTIPLE EIGENVALUES AND THE RUN WILL TERMINATE'///)
909 FORMAT('0 THE INTEGRATION CONSTANT VECTOR '6(1PE11.4,5X)/'0 IER = '
115/'0 IDGT = ',I1/'0 M = ',I1/)
910 FORMAT('0 NUMBER OF EQUATIONS TO BE SOLVED = ',I2/'0 INITIAL TIME'
1' = ',F4.1/'0 INITIAL STEP SIZE = ',F6.3/'0 TIME AT WHICH FIRST'
2' SOLUTION IS DEISRED = ',F6.3/'0 RELATIVE ERROR BOUND = ',E9.2/
3'0 METHOD = ',I1/'0 ITERATION METHOD = ',I1/'0 INDEX = ',I2/
4'0 NUMBER OF CALLS TO DGEAR DESIRED = ',I4/
5'0 MAXIMUM NUMBER OF ITERATIONS DESIRED = ',I6///)
911 FORMAT('1 THE EIGENVAUES ARE:',///0',5(E11.4,5X)///
1'0 THE EIGENVECTORS--COLUMN J CORRESPONDS TO EIGENVALUE J',
2///0',5(5(E11.4,5X)///)///0 INTEGRATION CONSTANTS:/'
3'0',5(E11.4,5X))
912 FORMAT(' ',F8.3,I6,' PS ',6(1PE10.3,2X))
913 FORMAT(' ',F8.3,7(1PE10.3,2X),' PD ')
914 FORMAT(' ',F8.3,2X,1PE10.3,' CHE '.4(E10.3,2X))
915 FORMAT('0 THE INTEGRATION SUBROUTINE REACHED A TERMINAL ERROR AT'
1' T = ',F8.3/'0 IER = ',I3/'0 TOL = ',F8.5/'0 N = ',I3/
2'0 XEND = ',F9.4/'0 H = ',F10.5/'0 METH = ',I3/'0 MITER = 'I3/
3'0 INDEX = ',I3/'0 ITERATIONS = ',I5)
916 FORMAT('0 PARAMETER VALUES'/'0 VP1 = ',F6.2' ML',/'0 VP = ',F6.2
1' ML',/'0 VL = ',F6.2' ML',/'0 VF = ',F6.2' GRAMS',/'0 VM = ',F6.2
2' GRAMS',/'0 QL = ',F6.2' ML/MIN',/'0 QF = ',F6.2' ML/MIN',/'0 QM '
3' = 'F6.2' ML/MIN',/'0 PSPO = '1PE15.5' /MIN',/'0 PSM = 'E15.5' /MIN'
4'0 POML = 'E15.5' /MIN',/'0 POMP = 'E15.5' ML/MIN',/'0 ACTV = ',
5E11.4' UNITS/CELL',/'0 ACHEO = ',E11.4' UNITS')
919 FORMAT('0 NRBC = '1PE11.4' CELLS',/'0 CHEO = ',E11.4' UNITS',/
1'0 SYN = ',E11.4' UNITS/MIN',/'0 LIVER PROTEIN = ',E11.4
2' MICROMOL',/'0 PLASMA PROTEIN = ',E11.4' MICROMOL'///)
917 FORMAT(' T = ',F8.3,5X,' IER = ',I3' ITERATIONS = ',I5)
920 FORMAT('0 IER FOR EIGRF = ',I3/'0 PERFORMANCE INDEX = ',F10.5///)
921 FORMAT('0 THE PARADOXON SYSTEM CONSTANTS'/)
922 FORMAT('0',4(4(1PE15.5,5X)///)
923 FORMAT('0 KA = ',1PE15.5,10X,'KG = ',E15.5/'0 THIS IS AN ORAL'
1'DOSE')
924 FORMAT('0 PSABS1 = ',1PE15.5,10X,'PSABS2 = ',E15.5/'0 THIS IS'
2' A DERMAL DOSE')
925 FORMAT('0 THE IV DOSE IS = ',1PE15.5)
926 FORMAT('0 THE ORAL DOSE IS = ',1PE15.5)
927 FORMAT('0 THE 1 COMPARTMENT DERMAL DOSE IS = ', 1PE15.5)
928 FORMAT('0 THE 2 COMPARTMENT DERMAL DOSE IS = ', 1PE15.5)

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REAL LIFE,LIV,MSP,NRBC,ORAL,IV,KA1,KG2
INTEGER SWICH1,TIME,SWICH2
COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
COMMON/CHEC/C1,C2,C01,CNH,C16
COMMON/DGER/AKI,AKR,CKI,CKR,PKR,PSL
COMMON/ACHEC/C50,ACHEPI,CA1,CA2,CA3,ACHEI,C15
COMMON/EVAL/VL,VF,VM,QL,QF,QM,PSM
COMMON/PARAMS/POPL,POPP,ATN,CTN,FOP,LIFE,AAGE,CAGE
COMMON/GEAR/DUMMY(48),SDUMMY(4),IDUMMY(38)
COMMON/FCNS/B(6),WR(6),NUM,A(6,6),SWICH2,XEND
DIMENSION Y(11),PS(6),E(6),D(12),TIMES(13)
DIMENSION WKAREA(6),IWK(11),WK(242)
COMPLEX Z(6,6),W(6),ZN
EXTERNAL FCN,FCNJ
DATA ORAL/'ORAL'/,SKIN/'SKIN'/,IV/'IV'/

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OPEN(UNIT=1,NAME='INPUT.DAT',TYPE='OLD',ACCESS='SEQUENTIAL',
IREADONLY,FORM='FORMATTED',CARRIAGECONTROL='LIST')
C PARAMETER INPUT VALUES
READ(1,*)BW,LIV,FSP,FOP,MSP,PSPOI,PSMI,POMLI,POMPI,
IPOPP,POPL,PKR
READ(1,*)HCT,AACT,LIFE,AKI,AKR,SFP,SMP,OFF
READ(1,*)ATN,CACT,CKI,CKR,CAGE,CTN,AAGE,CAT
READ(1,*)KA1,KG2,NOUT
READ(1,801)TYPE
WRITE(NOUT,900)BW,LIV,FSP,FOP,MSP,PSPOI,PSMI,POMLI,POMPI,
IPOPP,POPL,PKR,HCT,AACT,LIFE,AKI,AKR,AAGE,ATN,CACT
WRITE(NOUT,901)CKI,CKR,CAGE,CTN,SFP,SMP,OFF
IF(TYPE.EQ.ORAL) WRITE(NOUT,923)KA1,KG2
IF(TYPE.EQ.SKIN) WRITE(NOUT,924)KA1,KG2
C PARATHION SYSTEM INITIAL STATES INPUT, IN MG/KG
READ(1,*)(PS(I),I=1,6)
WRITE(NOUT,902)(PS(I),I=1,6)
PS(2)=PS(2)*BW/291.27
PS(5)=PS(5)*BW/291.27
PS(6)=PS(6)*BW/291.27
IF(TYPE.EQ.IV) WRITE(NOUT,925)PS(2)
IF(TYPE.EQ.ORAL) WRITE(NOUT,926)PS(6)
IF(TYPE.EQ.SKIN.AND.PS(5).NE.0.0) WRITE(NOUT,927)PS(5)
IF(TYPE.EQ.SKIN.AND.PS(6).NE.0.0) WRITE(NOUT,928)PS(6)
C PARAOXON SYSTEM INITIAL INPUTS
READ(1,803)(Y(I),I=1,11)
WRITE(NOUT,903)(Y(I),I=1,11)
C EVALUATE PARAMETERS
CALL PARAM(BW,LIV,PSPOI,PSMI,POMLI,POMPI,AACT,HCT,CACT,
IVP1,POML,POMP,ACTV,ACHED,NRBC,CHEO,Y,AKI,AKR,CKR,CKI,OFF)
WRITE(NOUT,916)VP,VL,VF,VM,QL,QF,QM,PSPO,PSM,POML,POMP,ACTV,
ACHED
WRITE(NOUT,919)NRBC,CHEO,SYN,Y(3),Y(5)
C PROGRAM SWITCHES; IF PS SYSTEM IS TO BE SOLVED,SWICH1=1; IF THE SOLVED
C SYSTEM IS TO BE INPUT, SWICH1=0; IF PS(5)=0.0, AT 12 HOURS, SWICH2=1
READ(1,*)SWICH1,SWICH2
WRITE(NOUT,904)SWICH1,SWICH2
IF(SWICH1.EQ.0)GO TO 1100
C EVALUATE PS SYSTEM PARAMETERS AND FORM COEFFICIENT MATRIX A
IF(TYPE.EQ.ORAL) CALL PSSYS3(FSP,MSP,SFP,SMP,KA1,KG2,D,A)
IF(TYPE.EQ.SKIN) CALL PSSYS(FSP,MSP,SFP,SMP,KA1,KG2,D,A)
WRITE(NOUT,905)((A(I,J),J=1,6),I=1,6)
WRITE(NOUT,921)
WRITE(NOUT,922)(C(I),I=1,16)
C COMPUTE EIGENVALUES AND EIGENVECTORS FOR COEFFICIENT MATRIX 'A'
READ(1,805)IA,IZ,N,IJOB
NUM=N
CALL EIGRF(A,N,IA,IJOB,W,Z,IZ,WK,IER)
WRITE(NOUT,906)(W(I),I=1,N)
C NORMALIZE EIGENVECTORS
DO 5 J=1,N
B(J)=PS(J)
ZN=Z(1,J)
DO 5 I=1,N
Z(I,J)=Z(I,J)/ZN
A(I,J)=REAL(Z(I,J))
5 CONTINUE
C WRITE THE EIGENVALUE MATRIX
WRITE(NOUT,907)((Z(I,J),J=1,6),I=1,N)
WRITE(NOUT,920)IER,WK(1)

```

```

C CHECK FOR IMAGINARY OR MULTIPLE EIGENVALUES, STOP PROGRAM IF ANY ARE FOUND
C PUT REAL PART OF EIGENVALUE INTO WR
  CALL RTCHEK(W, NUM, L, WR)
  IF (L.EQ.0) GO TO 1000
  WRITE(NOUT, 908) L
  GO TO 1400
C SOLVE FOR INTEGRATION CONSTANTS (X) BY EVALUATING AX=B (B=PS INITIAL STATES)
1000 IER=0
  READ(1, 806) M, IDGT
  CALL LEQT1F(A, M, N, IA, B, IDGT, WKAREA, IER)
  WRITE(NOUT, 909) (B(I), I=1, 6), IER, IDGT, M
  IF (IER.GT.127) GO TO 1400
  GO TO 1200
C INPUT EIGENVALUES, EIGENVECTORS, AND INTEGRATION CONSTANTS FOR SOLVED
C PS SYSTEM
1100 READ(1, 808) (WR(I), I=1, 5), ((Z(I, J), J=1, 5), I=1, 5), (B(I), I=1, 5)
  WRITE(NOUT, 911) (WR(I), I=1, 5), ((Z(I, J), J=1, 5), I=1, 5), (B(I), I=1, 5)
1200 DO 15 I=1, 35
15 WK(I)=0.0
C INPUT PARAMETERS FOR CONTROLLING THE INTEGRATION SUBROUTINE
  READ(1, *) N, X, H, XEND, TOL, TOL2, METH, MITER, INDEX, IQUIT, TIME,
  READ(1, *) (TIMES(I), I=1, 8)
  READ(1, *) (TIMES(I), I=9, 13)
  WRITE(NOUT, 910) N, X, H, XEND, TOL, METH, MITER, INDEX, TIME, IQUIT
  CLOSE(UNIT=1)
  OPEN(UNIT=2, NAME='OUTPUT.DAT', TYPE='NEW', ACCESS='SEQUENTIAL',
  IFORM='FORMATTED', CARRIAGECONTROL='LIST')
C PUT EIGENVECTORS INTO MATRIX A
  DO 20 I=1, NUM
  DO 20 J=1, NUM
20 A(I, J)=REAL(Z(I, J))
C FUNCTION EVALUATION LOOP
  DO 55 K=1, TIME
  XEND2=XEND
  IF (SWICH2.EQ.1.AND.XEND.GT.720.0) XEND2=XEND-720.0
C EVALUATE C*E**K*LAMBDA*TIME, X=TIME; COMPUTE PS STATE VARIABLES
  DO 25 I=1, NUM
  PS(I)=0.0
25 E(I)=B(I)*EXP(WR(I)*XEND2)
  DO 30 I=1, NUM
  DO 30 J=1, NUM
30 PS(I)=E(J)*A(I, J)+PS(I)
C EVALUATE PD SYSTEM; IF INTEGRATION SUCCEEDS, WRITE RESULTS TO AN OUTPUT
C FILE; IF INTEGRATION FAILS, STOP THE PROGRAM
  CALL DGEAR(N, FCN, FCNJ, X, H, Y, XEND, TOL, METH, MITER, INDEX, IWK, WK, IER)
  IF (IER.GT.128) GO TO 1250
  IF (IER.GE.33.AND.IER.LE.67) WRITE(NOUT, 917) X, IER, IDUMMY(7)
  WRITE(2, 912) X, IDUMMY(7), (PS(I), I=1, NUM)
  WRITE(2, 913) X, (Y(I), I=1, 7)
  WRITE(2, 914) X, PSL, (Y(I), I=8, N)
  IF (IDUMMY(7).GT.IQUIT) GO TO 1300
C CHANGE PS(5) TO 0.0 IF SWICH2=1 AND XEND=12; COMPUTE XEND
  IF (SWICH2.NE.1.OR.XEND.NE.720.0) GO TO 54
  INDEX=-1
  TOL=TOL2
  DO 31 I=1, 4
31 B(I)=PS(I)
  B(5)=0.0
  NOW=4
  CALL LEQT1F(A, M, NOW, IA, B, IDGT, WKAREA, IER)
  DO 33 I=1, 5
  DO 33 J=1, 5
33 A(I, J)=REAL(Z(I, J))
  WRITE(NOUT, 909) (B(I), I=1, NUM)
54 CALL ENDTIM(XEND, TIMES)
  ICOUNT=K
55 CONTINUE
  GO TO 1300
C DIAGNOSTICS FOR IER.GT.120
1250 WRITE(NOUT, 915) X, IER, TOL, N, XEND, H, METH, MITER, INDEX, IDUMMY(7)
  WRITE(NOUT, 912) X, (PS(I), I=1, NUM)
  WRITE(NOUT, 913) X, (Y(I), I=1, 7)
  WRITE(NOUT, 914) X, (Y(I), I=8, N)
1300 CLOSE(UNIT=2)
  WRITE(NOUT, *) ICOUNT
1400 STOP
  END

```

```

SUBROUTINE PARAM(BW,LIV,PSPOI,PSMI,POMLI,POMPI,AACT,HCT,CACT,
1VP1,POML,POMP,ACTV,ACHEO,NRBC,CHEO,Y,AKI,AKR,CKR,CKI,OFF)
REAL LIFE,LIV,NRBC
COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
COMMON/EVAL/VL,VF,VM,QL,QF,QM,PSM
COMMON/PARAMS/POPL,POPP,ATN,CTN,FOP,LIFE,AAGE,CAGE
DIMENSION Y(11)
C PARAMETER EVALUATIONS
VP1=.0413*BW
VP=VP1/0.78
VL=LIV*0.75*34*((BW/1000)**0.87)
VF=0.10*BW
VM=0.455*BW
QL=(2820*((BW/1000)**0.87*0.6))/(60*1.05*0.78)
QF=(0.6*124*((BW/1000)**0.84))/(1.05*60*0.78)
QM=(0.6*1484*((BW/1000)**0.85))/(1.05*60*0.78)
PSPO=PSPOI*(QL/VL)
PSM=PSMI*(QL/VL)
POML=POMLI*(QL/VL)
POMP=POMPI*VP1
ACTV=HCT*AACT/8.9E+09
ACHEO=AACT*(VP1*(HCT/(1-HCT)))
NRBC=8.9E+09*(VP1/(1-0.46))
CHEO=CACT*VP1
SYN=CAT*CHEO
C INITIAL CONDITIONS
Y(3)=Y(3)*VL
Y(5)=Y(5)*VP1
Y(8)=ACHEO
Y(10)=CHEO
C EVALUATE CONSTANTS FOR PARADOXON SYSTEM
C(1)=QL/VL+POML
C(2)=POPL/VL
C(3)=QL/VP
C(4)=QL/VL
C(5)=C(3)+POMP/VP+(QF/VP)*OFF
C(6)=POPP/VP
C(7)=AKI/ATN
C(8)=0.0
C(9)=CKI/CTN
C(10)=0.0
C(11)=(QF/VP)*OFF
C(12)=(QF*FOP)/VF
C(13)=(NRBC*ACTV)/LIFE
C(14)=C(13)/ACHEO
C(15)=AKR+AAGE+C(14)
C(16)=CKR+CAGE+CAT
RETURN
END

```

```

SUBROUTINE RTCHEK(W,NUM,L,WR)
C CHECK FOR MULTIPLICITY OF EIGENVALUES
COMPLEX W(NUM)
DIMENSION WR(NUM)
L=0
DO 20 I=1,(NUM-1)
DO 20 J=I+1,NUM
IF(W(I).EQ.W(J))L=L+1
20 CONTINUE
C CHECK FOR IMAGINARY ROOTS
DO 25 I=1,NUM
IF(AIMAG(W(I)).NE.0)L=L+1
WR(I)=REAL(W(I))
25 CONTINUE
RETURN
END

```

```

      SUBROUTINE FCN(N,X,Y,YPRIME)
C THE DIFFERENTIAL EQUATIONS FOR THE PARADOXON SYSTEM
COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
COMMON/DGER/AKI,AKR,CKI,CKR,PKR,PSL
COMMON/FCNS/B(6),WR(6),NUM,A(6,6),SWICH2,XEND
REAL Y(N),YPRIME(N)
INTEGER SWICH2
PSL=0.0
X2=X
IF(SWICH2.EQ.1.AND.XEND.GT.720.0) X2=X-720.0
DO 205 I=1,NUM
PSL=PSL+B(I)*EXP(WR(I)*X2)*A(1,I)
DO 10 I=1,11
10 IF(Y(I).LT.0.0) Y(I)=0.0
C100=(AKI/VP)*Y(2)
C101=(CKI/VP)*Y(2)
YPRIME(1)=PSPO*PSL-(C(1)+C(2)*Y(3))*Y(1)+C(3)*Y(2)
YPRIME(2)=C(4)*Y(1)-(C(5)+C(6)*Y(5)+C(7)*Y(8)
1+(C(9)*Y(10))*Y(2)+C(12)*Y(7)
YPRIME(3)=-C(2)*Y(1)*Y(3)+PKR*Y(4)
YPRIME(4)=-YPRIME(3)
YPRIME(5)=-C(6)*Y(2)*Y(5)+PKR*Y(6)
YPRIME(6)=-YPRIME(5)
YPRIME(7)=C(11)*Y(2)-C(12)*Y(7)
YPRIME(8)=C(13)-(C100+C(14))*Y(8)+AKR*Y(9)
YPRIME(9)=C100*Y(8)-C(15)*Y(9)
YPRIME(10)=SYN-(CAT+C101)*Y(10)+CKR*Y(11)
YPRIME(11)=C101*Y(10)-C(16)*Y(11)
RETURN
END

```

```

      SUBROUTINE FCNJ(N,X,Y,PD)
C JACOBIAN MATRIX OF PARTIAL DERIVATIVES
COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
COMMON/DGER/AKI,AKR,CKI,CKR,PKR,PSL
REAL Y(N),PD(N,N)
DO 10 I=1,11
10 IF(Y(I).LT.0.0) Y(I)=0.0
C100=(AKI/VP)*Y(2)
C101=(CKI/VP)*Y(2)
PD(1,1)=-C(1)+C(2)*Y(3)
PD(1,2)=C(3)
PD(1,3)=-C(2)*Y(1)
PD(2,1)=C(4)
PD(2,2)=-C(5)-C(6)*Y(5)-C(7)*Y(8)-C(9)*Y(10)
PD(2,5)=-C(6)*Y(2)
PD(2,7)=C(12)
PD(2,8)=-C(7)*Y(2)
PD(2,10)=-Y(2)*C(9)
PD(3,1)=-C(2)*Y(3)
PD(3,3)=-C(2)*Y(1)
PD(3,4)=PKR
PD(4,1)=-PD(3,1)
PD(4,3)=-PD(3,3)
PD(4,4)=-PKR
PD(5,2)=-C(6)*Y(5)
PD(5,5)=-C(6)*Y(2)
PD(5,6)=PKR
PD(6,2)=-PD(5,2)
PD(6,5)=-PD(5,5)
PD(6,6)=-PKR
PD(7,2)=C(11)
PD(7,7)=-C(12)
PD(8,2)=-AKI/VP*Y(8)
PD(8,8)=-C(100)+C(14)
PD(8,9)=AKR
PD(9,2)=-PD(8,2)
PD(9,8)=C100
PD(9,9)=-C(15)
PD(10,2)=-C(101)*Y(10)
PD(10,10)=-C(101)
PD(10,11)=CKR
PD(11,2)=-PD(10,2)
PD(11,10)=C101
PD(11,11)=-C(16)
RETURN
END

```

```

C THIS SUBROUTINE IS USED TO SET UP THE COEFFICIENT MATRIX FOR THE PARATHION
C SYSTEM WHEN AN ORAL DOSE IS GIVEN
  SUBROUTINE PSSYS3(FSP,MSP,SFP,SMP,KA,KG,D,A)
    REAL MSP,KA,KG
    COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
    COMMON/EVAL/VL,VF,VM,QL,QF,QM,PSM
    DIMENSION D(12),A(6,6)
C PS SYSTEM PARAMETERS
  D(1)=QL/VL
  D(2)=PSPO+PSM+D(1)
  D(3)=QL/VP
  D(4)=QF/VP
  D(5)=QF/VF
  D(6)=QM/VP
  D(7)=QM/VM
  D(8)=D(5)*FSP
  D(9)=D(7)*MSP
  D(10)=D(4)*SFP
  D(11)=D(6)*SMP
  D(12)=D(3)+D(10)+D(11)
C COEFFICIENT MATRIX A FOR PS SYSTEM (X'=AX)
  A(1,1)=-D(2)
  A(1,2)=D(3)
  A(1,5)=KA
  A(2,1)=D(1)
  A(2,2)=-D(12)
  A(2,3)=D(8)
  A(2,4)=D(9)
  A(3,2)=D(10)
  A(3,3)=-D(8)
  A(4,2)=D(11)
  A(4,4)=-D(9)
  A(5,5)=-KA
  A(5,6)=KG
  A(6,6)=-KG
  RETURN
  END

```

```

SUBROUTINE PSSYS(FSP,MSP,SFP,SMP,PSABS1,PSABS2,D,A)
  REAL MSP
  COMMON/ALL/SYN,VP,CAT,PSPO,C(16)
  COMMON/EVAL/VL,VF,VM,QL,QF,QM,PSM
  DIMENSION D(12),A(6,6)
C PS SYSTEM PARAMETERS
  D(1)=QL/VL
  D(2)=PSPO+PSM+D(1)
  D(3)=QL/VP
  D(4)=QF/VP
  D(5)=QF/VF
  D(6)=QM/VP
  D(7)=QM/VM
  D(8)=D(5)*FSP
  D(9)=D(7)*MSP
  D(10)=D(4)*SFP
  D(11)=D(6)*SMP
  D(12)=D(3)+D(10)+D(11)
C COEFFICIENT MATRIX A FOR PS SYSTEM (X'=AX)
  A(1,1)=-D(2)
  A(1,2)=D(3)
  A(2,1)=D(1)
  A(2,2)=-D(12)
  A(2,3)=D(8)
  A(2,4)=D(9)
  A(2,5)=PSABS2
  A(3,2)=D(10)
  A(3,3)=-D(8)
  A(4,2)=D(11)
  A(4,4)=-D(9)
  A(5,5)=-PSABS2
  A(5,6)=PSABS1
  A(6,6)=-PSABS1
  RETURN
  END

```



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C THIS SUBROUTINE COMPUTES XEND FOR SUBROUTINE DGEAR USED IN CHESIM.TSK
C TIMES(1-8) ARE THE ENDING TIMES IN DESCENDING ORDER WHICH ARE USED TO
C DECIDE HOW MUCH TO INCREASE XEND
C TIMES(9-13) ARE THE AMOUNTS BY WHICH XEND IS INCREASED
C UNUSED TIMES SHOULD BE FILLED IN WITH ZEROES
      SUBROUTINE ENDTIM(XEND,TIMES)
      DIMENSION TIMES(13)
      IF(XEND.GE.TIMES(8)) XEND=XEND+TIMES(9)
      IF(XEND.GE.TIMES(6).AND.XEND.LE.TIMES(7)) XEND=XEND+TIMES(10)
      IF(XEND.GE.TIMES(4).AND.XEND.LE.TIMES(5)) XEND=XEND+TIMES(11)
      IF(XEND.GE.TIMES(2).AND.XEND.LE.TIMES(3)) XEND=XEND+TIMES(12)
      IF(XEND.LE.TIMES(1)) XEND=XEND+TIMES(13)
      RETURN
      END

C THIS PROGRAM MAKES A PRINTER PLOT OF THE OUTPUT FROM CHESIM IN OUTPUT.DAT
C THE CONTROLLING PARAMETERS ARE INPUT FROM PLOT.DAT
C UGETIO AND USPLT SUBROUTINES FROM IMSL-7 ARE USED
C ILOG=1: PLOT LOG TIME
C N=NUMBER OF TIME POINTS TO BE READ AND PRINTED
C NPLOT=NUMBER OF TIME POINTS TO BE PLOTTED
C ISWICH=0: PLOT ALL THREE GRAPHS
C ISWICH=1: SKIP THE CHE INHIBITION DATA
C ISWICH=2: SKIP THE PS AND PO DATA
      DIMENSION IMAG4(5151), ITITLE(144), ICHAR(10), X(100), Y(100,4),
      IRANGE(4)
      DATA ICHAR(1) / ' '
      OPEN(UNIT=1, NAME='PLOT.DAT', TYPE='OLD', ACCESS='SEQUENTIAL',
      IREADONLY, FORM='FORMATTED', CARRIAGECONTROL='LIST')
      FORMAT(72A1)
800  FORMAT(/F8.3, 37X, E10.3, 12X, E12.3/26X, E10.3, 12X, E12.3)
801  FORMAT(F8.3, 11X, E10.3, 3E12.3//)
802  FORMAT(/F8.3, 1X, E10.3, E12.3, 50X, E10.3/)
803  FORMAT('0 TIME', 12X'ACHE', 17X'CHE', 16X'ESTLP', 16X'ESTPP')
900  FORMAT('0', F6.1, 1PE20.5, 3(E20.5))
901  FORMAT('1 TIME', 14X'PSL', 17X'PSP', 17X'PSF', 16X'PSMU')
902  FORMAT('1 TIME', 14X'POL', 17X'POP', 17X'POF')
903  FORMAT('0', F6.1, 1PE20.5, 2(E20.5))
C THE FOLLOWING INPUT PARAMETERS WILL CHANGE WHEN THE RAT WEIGHT CHANGES
      READ(1,*) VL, VP, VF, VM, ACHEO, CHEO, ESTLO, ESTPO
      ACHE=100.0/ACHEO
      CHE=100.0/CHEO
      ESTL=100.0/ESTLO
      ESTP=100.0/ESTPO
      VLC=1.0/VL
      VPC=1.0/VP
      VFC=1.0/VF
      VMC=1.0/VM
      READ(1,*) ISWICH, ILOG, ISTART, NIN, NOUT
      READ(1,800) (ITITLE(I), I=1,72)
      READ(1,800) (ITITLE(I), I=73,144)
      READ(1,*) INC, N, NPLOT, M, IOPT, IY, (RANGE(I), I=1,4)
      X(1)=0.0
      DO 3 I=1,4
3     Y(1,I)=100.0
      OPEN(UNIT=2, NAME='OUTPUT.DAT', TYPE='OLD', ACCESS='SEQUENTIAL',
      IREADONLY, FORM='FORMATTED', CARRIAGECONTROL='LIST')
      CALL UGETIO(3, NIN, NOUT)
      IF(ISWICH.EQ.1) GO TO 200
      DO 10, I=ISTART, N
C READ ESTLP, ESTPP, ACHE, CHE
      READ(2,801) X(I), Y(I,3), Y(I,4), Y(I,1), Y(I,2)
      Y(I,1)=Y(I,1)*ACHE
      Y(I,2)=Y(I,2)*CHE
      Y(I,3)=100.0-Y(I,3)*ESTL
      Y(I,4)=100.0-Y(I,4)*ESTP
      IF(ILOG.EQ.1) X(I)=ALOG10(X(I))
10     CONTINUE

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WRITE(NOUT,900)
DO 100 I=1,N
100  WRITE(NOUT,901)X(I),Y(I,1),Y(I,2),Y(I,3),Y(I,4)
      CALL USPLT(X,Y,IY,NPLOT,M,INC,ITITLE,RANGE,ICHAR,IOPT,IMAG4,IER)
      IF(ISWICH.EQ.2) GO TO 210
      REWIND 2
200  READ(1,800)(ITITLE(I),I=1,72)
      READ(1,800)(ITITLE(I),I=73,144)
      READ(1,*)M,(RANGE(I),I=1,4)
      DO 4 I=1,4
4     Y(I,I)=0.0
      DO 15 I=ISTART,N
C   READ PSL,PSP,PSF,PSMU
      READ(2,802)X(I),Y(I,1),Y(I,2),Y(I,3),Y(I,4)
      Y(I,1)=Y(I,1)*VLC
      Y(I,2)=Y(I,2)*VPC
      Y(I,3)=Y(I,3)*VFC
      Y(I,4)=Y(I,4)*VMC
      IF(ILOG.EQ.1)X(I)=ALOG10(X(I))
15    CONTINUE
      WRITE(NOUT,902)
      DO 110 I=1,N
110   WRITE(NOUT,901)X(I),Y(I,1),Y(I,2),Y(I,3),Y(I,4)
      CALL USPLT(X,Y,IY,NPLOT,M,INC,ITITLE,RANGE,ICHAR,IOPT,IMAG4,IER)
      REWIND 2
      READ(1,800)(ITITLE(I),I=1,72)
      READ(1,800)(ITITLE(I),I=73,144)
      READ(1,*)M,(RANGE(I),I=1,4)
      DO 20 I=ISTART,N
C   READ POL,POP,POF
      READ(2,803)X(I),Y(I,1),Y(I,2),Y(I,3)
      Y(I,1)=Y(I,1)*VLC
      Y(I,2)=Y(I,2)*VPC
      Y(I,3)=Y(I,3)*VFC
      IF(ILOG.EQ.1)X(I)=ALOG10(X(I))
20    CONTINUE
      WRITE(NOUT,904)
      DO 120 I=1,N
120   WRITE(NOUT,905)X(I),Y(I,1),Y(I,2),Y(I,3)
      CALL USPLT(X,Y,IY,NPLOT,M,INC,ITITLE,RANGE,ICHAR,IOPT,IMAG4,IER)
210  CLOSE(UNIT=1)
      CLOSE(UNIT=2)
      END

```