DRAFT DO NOT CITE OR QUOTE

ISSUE PAPER FOR THE FIFRA SCIENTIFIC ADVISORY PANEL REVIEW

Endpoint Selection and Determination of Relative Potency in Cumulative Hazard and Dose-Response Assessment:

A Pilot Study of Organophosphorus Pesticide Chemicals

NOTICE
THIS DOCUMENT IS A PRELIMINARY DRAFT
AND HAS NOT YET BEEN RELEASED BY THE AGENCY

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington D.C. 20460

September 5, 2000

TABLE OF CONTENTS

PREFACE 5

ORGANIZATION 6

ACRONYMS 7

THE PILOT STUDY 8
- **I. Introduction** 8
  - A. Background 8
  - B. Purpose and Scope of Pilot Analysis 8
- **II. Methods** 10
  - A. Assumptions 10
  - B. Determination of Relative Potency 11
    - 1. Selection of Chemicals in the CMG 12
    - 2. Selection of Common Endpoints and a Uniform Measure of Toxicity 12
      - a. Available Toxicity Database 13
      - b. Selection of a Response Level to Determine Relative Potency 14
      - c. Selection of a Representative Study and Timepoint . . . 15
    - 3. Analysis of Dose-Response Curves for Parallel Slopes 16
    - 4. Calculation of RPFs 18
- **III. Results** 20
  - A. Relative Potency Rankings for Oral Exposure Based on Plasma ChE Inhibition 20
    - 1. Analysis of Dose-Response Relationships 23
    - 2. Comparison of Representative and Mean log ED50 for Plasma ChE Inhibition 24
    - 3. Plasma ChE Inhibition of Index Chemical 24
    - 4. Differences Between Male and Female Plasma ChEI 24
    - 6. Summary of Plasma ChEI Results for Oral Exposure 28
  - B. Relative Potency Rankings for Oral Exposure Based on RBC ChE Inhibition 29
    - 1. Analysis of Dose-Response Relationships 29
    - 2. Comparison of Representative and Mean ED50s for RBC ChE Inhibition 32
    - 3. RBC ChE Inhibition of Index Chemical 35
    - 4. Differences Between Male and Female RBC ChEI for Rat Oral Exposures 35
    - 5. Summary of RBC ChEI Results for Oral Exposure 36
  - C. Relative Potency Rankings for Brain ChE Inhibition in Rat from Oral Exposure 38
    - 1. Analysis of Dose-Response Relationships 41

2. Comparison of Representative and Mean Log $ED50_{brain}$ for Brain ChE Inhibition for Rat Oral Exposure 42
3. Brain ChE Inhibition of Index Chemical 42
4. Differences Between Male and Female Brain ChE Inhibition for Oral Exposure 42
5. Summary of Brain ChEI Results for Oral Exposure 46

D. Comparison of Relative Rankings and ED50s by Compartment and Sex for Oral Exposure 47

E. Relative Potency Rankings for Dermal Exposure 51
1. Study Selection for Dermal Studies 51
2. Establishment of Uniform Measure of Toxicity for Dermal 52

F. Relative Potency Rankings for Inhalation Exposure 54
1. Study Selection for Inhalation Studies 54
2. Establishment of Uniform Measure of Toxicity for Inhalation Studies 54

IV. Summary of Pilot Results 56

V. Issues for Cumulative Hazard and Dose-Response Analysis 58

A. Determination of Relative Potency Based on a Uniform Measure of Toxicity 58
1. Dealing with Lack of Appropriate Data 59
2. Methods used for Derivation of EDs 60
3. Selection of Index Compound 61

B. Analysis of Dose-response Curves 62
1. Assumption of Parallel Dose-Response Curves 62

VII. Charge and Questions for the Panel 63

Equations

Equation 1 15
Equation 2 18

Figures

Figure 1. Theoretical Dose-Response Data with Parallel and Non-Parallel Slopes 17
Figure 2. Comparison of Representative Log $ED50_{pl}$ with the Mean Log $ED50_{pl}$ for Male Rats 25
Figure 3. Comparison of Representative Log $ED50_{pl}$ with the Mean Log $ED50_{pl}$ for Female Rats 26
Figure 4. Scatterplot of Representative Log $ED50_{pl}$ for Male and Female Rat 27
Figure 5. Comparison of Representative Log $ED50_{rbc}$ with the Mean Log $ED50_{rbc}$ for Male Rats 33
Figure 6. Comparison of Representative Log $ED50_{rbc}$ with the Mean Log $ED50_{rbc}$ for Female Rats 34
Figure 7. Scatterplot of Male and Female Representative $LogED_{rbc}$ 37
Figure 8. Comparison of Representative Log $ED50_{brain}$ with the Mean Log $ED50_{brain}$ for Male Rats 43
Figure 9. Comparison of Representative Log $ED50_{brain}$ with the Mean Log $ED50_{brain}$ for Female Rats 44
Figure 10. Scatterplot of Male and Female Representative Log $ED50_{brain}$ 45

Tables

Table 1a. Representative Linear Regression and ED50 for Male Plasma Data 21
Table 1b. Representative Linear Regression and ED50 for Female Plasma Data 22
Table 2a. Representative Linear Regression and ED50 for Male RBC Data 30
Table 2b. Representative Linear Regression and ED50 for Female RBC Data 31
Table 3a. Representative Linear Regression and ED50 for Male Rat Brain Data 39
Table 3b. Representative Linear Regression and ED50 for Female Rat Brain Data 40
Table 5. Relative Rank of Chemicals Based on Calculated ED50s for Plasma, RBC, and Brain ChE 49
Table 6. Relative Rank of Chemicals Based on Representative ED50s for Plasma, RBC, and Brain ChE 50
Table 7. Spearman Rank Order Correlations Between Each Compartment and Sex . . 51
Table 8. Rank Order of OPs for Plasma ChE Inhibition in Subchronic Dermal Exposure Studies 53
Table 9. Rank Order of OPs for Plasma ChE Inhibition in Inhalation Exposure Studies 55

PREFACE

The Food Quality Protection Act of 1996 (FQPA) directs EPA to conduct cumulative risk assessments on pesticides that share a common mechanism of toxicity. To solicit scientific peer review on the principles and approaches for conducting cumulative risk assessments, the Agency has prepared a pilot case study involving 24 organophosphorous pesticides. EPA is now soliciting advice on this pilot study from the
FIFRA Scientific Advisory Panel (SAP), which will meet in late September 2000 to discuss the study and provide comment.

This case study demonstrates the application of the principles for conducting a cumulative hazard and dose-response assessment. The analysis follows the general approaches and steps for determining the accumulation of common hazard set forth in the *Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity (EPA, 2000a).* The case study does not address the exposure component nor risk characterization of cumulative risk assessment.

To conduct the case study, EPA evaluated 24 organophosphorous pesticides that exert their toxic effects by a common mechanism of toxicity. Described in this paper are: the selection of a common endpoint and determination of each chemical's relative potency; the strength and weaknesses of the data; and the assumptions used.

This analysis is not intended to represent a cumulative risk assessment of organophosphorus pesticides for regulatory purposes. Although this pilot analysis is based on actual data, the organophosphorus pesticides have been given code names.

ORGANIZATION

This issue paper illustrates an approach for cumulative risk assessment when evaluating the hazard and dose-response data of a group of chemicals that share a common mechanism of toxicity. Cholinesterase inhibition data on 24 organophosphorus pesticides are used to illustrate the approach taken and the issues encountered.

- Section I **(Introduction)** provides a very brief background on cumulative risk assessment under FQPA, as well as the scope and purpose of this pilot analysis.

- Section II **(Methods)** describes the methods for determining the relative potency of the chemicals, including a discussion of the assumptions used; the studies, endpoints and routes of exposure considered and the approach for establishing a uniform measure of the common toxic effect; and the statistical methods for evaluating the dose-response data.

- Section III **(Results)** presents the calculated effective doses and relative potencies and an analysis of the dose-response curves between sexes and among the different endpoints (compartments) and routes of exposure considered in the analysis.

- Section IV **(Summary of Pilot Results)** provides a brief summary of the key elements of the pilot analysis.

- Section V **(Issues for Cumulative Hazard and Dose-response Analysis)** describes the generic issues encountered in this pilot analysis that would pertain to any cumulative assessment that OPP would like the SAP to consider.

- Section VI **(Charge and Questions for the FIFRA Scientific Advisory Panel)** lists the questions that OPP is presenting to the SAP for comment.

ACRONYMS

AChE	Acetylcholinesterase enzyme
AChEI	Acetylcholinesterase inhibition
CAG	Cumulative Assessment Group
ChE	Cholinesterase
ChEI	Cholinesterase inhibiting/inhibition
CMG	Common Mechanism Group
DER	Data Evaluation Record
ED	Effective Dose
ED50	Effective Dose for 50% ChEI
EPA	Environmental Protection Agency
FIFRA	The Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
FQPA	Food Quality Protection Act of 1996
LOAEL	Lowest-Observed-Adverse-Effect-Level
MOE	Margin of Exposure
NOAEL	No-Observed-Adverse-Effect-Level
OP	Organophosphorus Pesticide
OPP	Office of Pesticide Programs
PAG	Pilot Assessment Group
PDP	Pesticide Data Program
PoC	Point of Comparison
PoD	Point of Departure
RBC	Red Blood Cell
RPF	Relative Potency Factor
SAP	FIFRA Scientific Advisory Panel
SAR	Structure-Activity Relationships
SAS	Statistical Analysis System

THE PILOT STUDY

I. Introduction

A. Background

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) were amended by the Food Quality Protection Act of 1996 (FQPA). FQPA requires EPA to perform cumulative risk assessments for pesticides with a common mechanism of toxicity.

The Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) has prepared a draft guidance document entitled *Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity* (EPA, 2000a). OPP presented this proposed guidance to the FIFRA Scientific Advisory Panel (SAP) in September and December 1999 (www.epa.gov/scipoly/sap/1999/ September/finalrpt.pdf and www.epa.gov/scipoly/sap/1999/december/report.pdf). On June 30, 2000, the OPP draft guidance document was published in the *Federal Register* for public comment (www.epa.gov/fedrgstr/EPA-PEST/2000/June/Day-30/6049.pdf).

B. Purpose and Scope of Pilot Analysis

A simple case study of three organophosphorus pesticides (OPs) by the dietary (food only) pathway was included in the June 2000 draft OPP guidance document (EPA, 2000a) to illustrate the proposed approaches, concepts, and principles under consideration in the development of cumulative risk assessment methods. After reviewing this case study, the September 1999 SAP recommended that EPA develop a more complex case study to further illustrate the details of the cumulative risk assessment process.

To this end, OPP is presenting a pilot analysis of OPs that share the common mechanism of inhibiting acetylcholinesterase (AChE) to the SAP at their September 2000 meeting. The purpose of this pilot analysis is to further illustrate the hazard and dose-response aspects of OPP's draft June 2000 cumulative guidance document. The pilot analysis includes 24 OPs and cholinesterase (ChE) data from different compartments (plasma, RBC, and brain) from oral, dermal, and inhalation studies.

This paper provides a detailed description of the methods used for determining relative potencies. In addition, an analysis of whether or not these common mechanism chemicals have parallel dose-response curves, as assumed for dose-addition, is also presented. Following the methods and results sections is a discussion of the issues encountered in selecting common endpoints, establishing uniform measures of the biological and toxicological responses, analyzing dose-response curves, and developing reliable relative potency measures. Exposure and risk assessment/characterization issues concerning cumulative risk assessment are **not** addressed in this analysis.

II. Methods

A. Assumptions

The key assumptions for the hazard and dose-response sections of a cumulative assessment are that:

- **Chemicals within an identified group act by a common mechanism of toxicity**. The selection of the OPs as a common mechanism group (CMG) based on a common mechanism of toxicity is described in Section II B. 1. OPP has previously determined that ChE-inhibiting OPs share a common mechanism of toxicity (EPA, 1999a).

- **There is an absence of interactions among the chemicals.** In other words, additivity was assumed. OPP conducted a literature review to determine if the assumption of additivity was reasonable for OP compounds (Frawley et al., 1957; Casida et al., 1963; Cohen 1984; Cohen et al., 1972; Cook et al., 1957; DuBois, 1961; Seume and O'Brien, 1960; Mei-Quey, et al., 1971). Many of these studies investigated the acute lethality of combinations of OPs (mostly as binary combinations), and not the cumulative effects on cholinesterase inhibition (ChEI) or the clinical signs of ChEI and therefore should be interpreted with caution. Most OP pair combinations produced additive effects. Thus, the assumption of additivity appears reasonable, particularly at lower exposures.

- **There is a constant proportionality among the effectiveness of the chemicals.** In other words the dose-response curves of the chemical group were parallel. If there is not a constant proportionality among the chemicals along their dose-response curves, then the rankings for relative potency would differ at different doses. An evaluation of this assumption is discussed in Section II B. 3. and Section III A. 1.

B. Determination of Relative Potency

The proposed cumulative guidance (EPA, 2000a) describes two approaches for accumulating hazard–the cumulative margin-of-exposure (MOE) method and the relative potency factor (RPF) method.

In the cumulative MOE approach, scaling is based on deriving a unitless MOE. An MOE is a chemical's point of departure (PoD) divided by the measured or estimated dose for a given route. A PoD is defined as the dose at which effects from a pesticide are first distinguishable from the background level of response. The cumulative MOE method combines individual chemical MOEs for each chemical for a given duration (e.g., all acutes or all chronics) by route.

The RPF approach expresses the potency of each chemical in relation to the potency of another member in the group that has been selected as the index chemical. An RPF was calculated for each route of interest. The exposures for each chemical expressed as exposure equivalents of the index chemical (i.e., the product of the exposure and RPF for each route). These exposure equivalents were summed to obtain an estimate of total exposure by pathway/route in terms of the index chemical. Both approaches normalize the group of chemicals to a common scale and sum the doses. This pilot analysis used the RPF method.

This pilot study was divided into the following steps, described in more detail below: (1) selection of chemicals in a CMG; (2) selection of common endpoints pertaining to the common mechanism of toxicity and a uniform measure of toxicity; (3) analysis of the dose-response data pertaining to the common mechanism of toxicity for parallel slopes; and (4) determination of relative potency for each chemical.

1. Selection of Chemicals in the CMG

The first step in the cumulative risk assessment process was to identify a CMG. Chemicals that share a common mechanism of toxicity cause a common toxic effect by the same, or essentially same, sequence of major biochemical events (EPA, 1999a).

OP pesticides were selected as a CMG for this pilot analysis. The common toxicity of OPs is due to inhibition of the AChE by phosphorylation. When AChE is inhibited by an OP, the neurotransmitter, acetylcholine, accumulates and causes cholinergic toxicity. Effects may occur within both the central nervous system and peripheral nervous system.

There are 39 registered OPs that inhibit AChE. A subgroup of 24 OPs, called the Pilot Assessment Group (PAG) was selected from the CMG. The PAG selection was based on OPs that have been detected by the U.S. Department of Agriculture's Pesticide Data Program (PDP). The purpose of PDP is to collect data on pesticides in fresh fruit, vegetables, some processed commodities, and milk. The design of the PDP program is specific for dietary risk assessment; sampling is done at grocery store distribution points and foods are prepared before analysis as they would typically be before consumption (e.g., peeling, washing). PDP is considered an unbiased and reliable analysis of dietary intake.

2. Selection of Common Endpoints and a Uniform Measure of Toxicity

Because inhibition of AChE can occur in the central and peripheral nervous system, brain, red blood cell (RBC), and plasma ChEI were all considered. In this pilot assessment, RBC and plasma ChEI were used as surrogates for the peripheral nervous system because data on peripheral nervous system inhibition are usually not available (EPA, 2000a).

a. Available Toxicity Database

OPP reviews toxicity study reports of pesticides under the authority of FIFRA. OPP hazard assessors prepare Data Evaluation Records (DERs) that summarize and characterize information contained in submitted studies. DERs generally report mean and/or summary data and do not report individual animal data.

For this pilot analysis, DERs for pertinent studies were evaluated. Information on the laboratory animals used (species, sex, strain, age) and the methods used (measurement of ChEI, time of measurements, compartments measured) were extracted. The percent ChEI relative to control was recorded from each study. ChEI was evaluated in both sexes, in three biological compartments (plasma, RBC, and brain), for all timepoints where ChE activity was determined in each study. Oral studies were reviewed for all 24 OPs considered in this analysis. Seven of the 24 chemicals have residential uses, thus dermal and inhalation studies were also reviewed for the seven. An important focus of the analysis was on the duration of exposure given below since OP pesticides generally reach steady state by 30 days. The following types of studies were evaluated for this analysis:

- 90-day rat oral toxicity study
- Two-year chronic oral study in rats
- Carcinogenicity studies in rats
- Combined chronic toxicity/carcinogenicity studies in rats
- 90-day inhalation toxicity study in rats.

For dermal exposure, 90-day studies were generally unavailable. Twenty-one /28-day studies in rat and rabbit were evaluated.

b. Selection of a Response Level to Determine Relative Potency

A point of comparison (PoC) was selected for each chemical. A PoC is the dose at which a uniform response occurs; it is used to calculate relative potencies. Two approaches were considered for use as a PoC: (1) effective doses (EDs), or (2) no-observed-adverse-effect-levels (NOAELs).

An ED is a measured or estimated dose level associated with some designated level of response relative to a control or baseline response level. Use of an ED is the preferred method for comparing potency (EPA, 2000a). The advantage of using an ED is that the complete dose-response curve is considered. Where possible, the ED for 50% ChEI (ED50) was calculated for all timepoints from all the studies for each chemical. The ED50 values were calculated using mean ChEI data collected from DERs for each individual study for both sexes at all assay timepoints.

In cases where an ED50 could not be calculated, the NOAEL was considered as the PoC. A NOAEL is the highest dose tested in a study without any adverse health effects. NOAELs were readily available from most toxicity studies. A NOAEL is not preferred as a PoC because its value is influenced by the study design and does not present a uniform measure of response.

c. Selection of a Representative Study and Timepoint

Percent mean ChEI data were transformed into probit values using a standard probit table. Linear regression was carried out using standard spreadsheet functions to determine the best fit slope and intercept for each chemical (Equation 1). The ED50 was calculated at each assay timepoint.

$$\text{probit (\% ChEI)} = \text{slope} \times \log(\text{dose}) + \text{intercept}$$

(Equation 1)

Slopes, intercepts, and log(ED50) values were calculated for both sexes and all timepoints for all studies for each route of interest. An examination of the data was made to select a single study and timepoint with the most representative log(ED50) and slope for each chemical. This representative log(ED50) was used in the calculation of RPFs below. The following criteria were used in the selection process:

- **Steady State.** To determine if steady-state had been reached in the oral exposure studies, log(ED50) values were graphed by the dosing time independent of study type. For example, the data from a one month timepoint in a two-year feeding study would be plotted before the data from a three-month timepoint of a subchronic feeding study. An analysis for steady state could not be made for the dermal and inhalation exposure studies because only one study per chemical was available and ChE measurements were usually made only at the study termination, rather than at interim timepoints as in the oral studies.

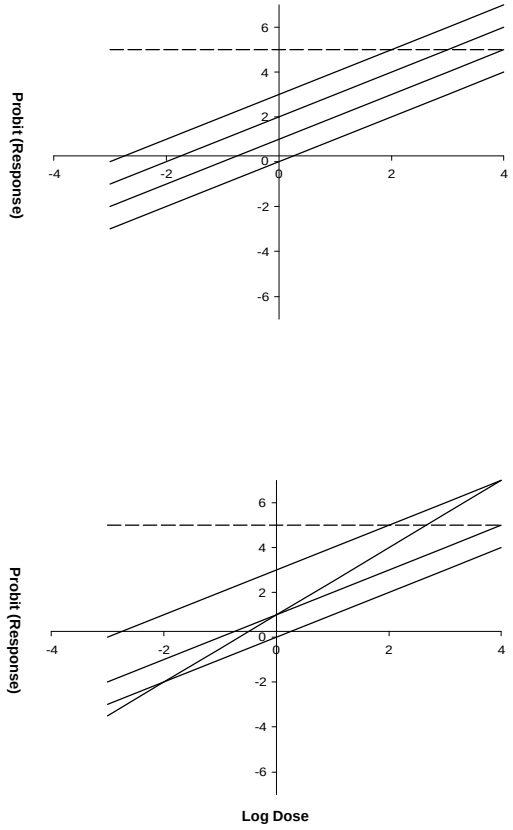
- **Dose-Response.** Slope versus assay timepoint was graphed for each study for a given compartment to better visualize the stability of the slopes with time. Goodness-of-fit (high linear correlation coefficients) and degrees of freedom were also evaluated.

Most of the 24 chemicals had good dose-response data for oral exposure resulting in reliable and consistent logED50 values for ChEI between and within studies for the oral route of exposure. However, for some chemicals, the log(ED50) values were less reliable and variable because of inadequate dose-response data or failure to achieve steady state. Although an effort was made to select the same timepoint and study for all three compartments and both sexes, the selected study and/or timepoint was not always the same.

3. Analysis of Dose-Response Curves for Parallel Slopes

To evaluate the assumptions of constant proportionality (see Section II A), an analysis of parallel slopes was performed with the linear regressions from the representative dose-response data. Using Statistical Analysis System (SAS), a class statement for the “chemical” was used in addition to an interaction term, “chemical*logdose,” representing the slopes. An F-test was performed on this interaction term. The corresponding p-value represents a group comparison of all of the slopes. In other words, $p<0.05$ for the F-test would indicate the slopes of the linear regressions were statistically different. Based on the assumption of parallel dose-response curves, the relative rank of the calculated log(ED50)s should be exactly the same as the relative order of the intercepts. In order to test this assumption of rank order, Spearman rank order correlations were performed. Figure 1 below illustrates theoretical dose-response data with parallel and non-parallel slopes.

Figure 1. Theoretical Dose-Response Data with Parallel and Non-Parallel Slopes



Note: This figure demonstrates theoretical dose-response data for four chemicals. The top graph shows four chemicals with parallel dose-response curves. The bottom graph shows one chemical whose dose-response curve is not parallel to the others. The dashed lines represent the 50% effect level.

4. Calculation of RPFs

The RPF approach to cumulative assessment expresses the potency of each chemical in relation to the potency of an index chemical. The same index compound should be used for all three routes of exposure and should have a toxicological profile pertaining to the common mechanism of toxicity consistent with the other chemical members. Therefore, the choice of an index chemical was limited to the subset of seven chemicals with residential uses. Desirable characteristics of an index chemical include well-characterized (qualitative and quantitative) dose-responses with available oral, dermal, and inhalation studies. Of the seven chemicals with residential uses, four chemicals were considered for selection as the index compound. These four chemicals had dermal and inhalation studies as well as complete databases for oral exposure (all compartments, both sexes). The index chemical, Chemical T, was selected on the basis of its complete database for oral, dermal, and inhalation routes of exposure. ED50s could be determined for the index chemical by both dermal and inhalation exposure. ED50s for several of the other chemicals could not be determined for both of these routes due to insufficient ChEI data.

The following equations were used to calculate relative potencies:

$$RPF = \frac{ED50_{\text{Index Chemical}}}{ED50_{\text{Chemical Z}}}$$

where Chemical Z is a member of the cumulative assessment group.

OR

$$RPF = \frac{NOAEL_{\text{Index Chemical}}}{NOAEL_{\text{Chemical Z}}}$$

(Equation 2)

Following the calculation of RPFs, the relative potency rankings were compared both qualitatively and quantitatively using Spearman rank order correlations.

III. Results

The following section describes results of pilot analysis for oral studies with plasma, RBC, and brain ChEI followed by analysis of dermal and inhalation studies. A total of 96 oral exposure studies were examined; the number of studies per chemical ranged from one to seven. Only one dermal study and one inhalation exposure study were available for each OP with residential uses. For the oral studies, plasma and RBC ChEI were generally reported at several timepoints while brain ChEI was usually reported only at study termination. For the dermal and inhalation studies, ChEI was determined only at the end of the study. Timepoints selected were dependent on the time to steady state and the actual time of ChEI determination.

A. Relative Potency Rankings for Oral Exposure Based on Plasma ChE Inhibition

The linear regressions for the representative study for plasma ChE inhibition in addition to the calculated log $ED50_{plasma}$ values and the plasma relative rank were given in Tables 1a and 1b for males and females, respectively. Plasma ChE inhibition data were available for all 24 chemicals for male rats and 23 of 24 chemicals for female rats. As described in the Methods section, studies were reviewed based on a set of defined criteria. The available plasma ChEI data for Chemical D in females did not meet the criteria because ChEI occurred at only one dose level. The same study and timepoint were used to evaluate both male and female plasma ChEI. The timepoints selected ranged from seven-week to 24-month timepoints.

Table 1a. Representative Linear Regression and ED50 for Male Plasma Data[a]

Chemical[b]	Endpoint	Slope	Intercept	log ED50pl[c]	ED50pl (mg/kg/day)	Male logED50pl[c] Mean	Male logED50pl Standard Deviation	RPF[d]	Relative Rank[e]
Chemical C	7 weeks	0.97	3.53	1.52	33.14	2.28	1.81	0.01	21
Chemical M	13 weeks	1.63	4.03	0.58	3.94	0.68	0.18	0.10	15
Chemical H	6 months	0.67	3.93	0.57	40.74	0.04	0.44	0.10	14
Chemical P	14 weeks	0.95	4.46	1.61	3.73	1.51	0.70	0.01	22
Chemical T	13 weeks	0.75	5.33	-0.44	0.36	-0.11	0.68	1.00	2
Chemical B	14 weeks	0.64	4.17	1.29	19.53	0.83	0.65	0.02	18
Chemical R	13 weeks	1.30	4.13	0.67	4.69	0.86	0.24	0.08	16
Chemical I	14 weeks	1.54	5.68	-0.37	0.36	-0.48	0.23	0.84	3
Chemical N	18 months	1.23	4.35	0.53	3.37	0.39	0.49	0.11	13
Chemical F	15 weeks	0.88	5.00	0.00	0.99	0.09	0.17	0.36	9
Chemical U	3 months	2.22	-1.30	2.83	685.82	3.28	0.70	0.0001	24
Chemical A	13 weeks	0.69	5.07	-0.11	0.78	0.14	0.55	0.46	7
Chemical O	8 weeks	1.10	3.46	1.76	158.79	1.46	0.94	0.01	23
Chemical J	6 months	1.03	4.88	0.08	1.56	0.13	0.13	0.30	10
Chemical W	3 months	1.32	5.23	-0.22	0.67	-0.18	0.21	0.60	5
Chemical E	7 months	0.96	5.13	-0.14	0.73	0.07	0.43	0.49	6
Chemical S	3 months	1.42	5.43	-0.28	0.50	-0.30	0.06	0.68	4
Chemical X	13 weeks	1.26	4.61	-0.07	1.96	-0.48	0.27	0.42	8
Chemical G	13 weeks	1.81	2.71	1.25	18.64	1.22	0.17	0.02	17
Chemical V	13 weeks	1.11	3.52	1.34	21.53	1.41	1.55	0.02	19
Chemical Y	6 months	1.39	4.54	0.33	2.15	0.17	0.19	0.17	12
Chemical Q	13 weeks	0.43	4.35	1.49	31.45	1.23	0.20	0.01	20
Chemical D	12 months	1.10	5.78	-0.71	0.19	0.64	1.53	1.80	1
Chemical L	24 months	0.94	4.74	0.27	1.88	0.82	0.46	0.19	11

[a]Table provides dose-response curves of representative study: probit (% male rat plasma ChEI)=log (dose) x slope + intercept
[b]Chemicals are listed in random order
[c]log ED50pl=log (effective dose) calculated to cause 50% inhibition of plasma ChE
[d]Relative Potency Factor: $RPF=ED50_{\text{Index Chemical}} \div ED50_{\text{Chemical Z}}$
[e]chemicals are ranked most potent (1) to least potent (24)

Table 1b. Representative Linear Regression and ED50 for Female Plasma Data[a]

Chemical[b]	Endpoint	Slope	Intercept	log ED50pl[c]	ED50pl (mg/kg/day)	Female logED50pl Mean	Female logED50pl Standard Deviation	RPF[d]	Relative Rank[e]
Chemical C	7 weeks	0.62	4.45	0.89	7.77	1.26	0.27	0.005	18
Chemical M	13 weeks	2.46	3.87	0.46	2.86	0.35	0.09	0.012	16
Chemical H	6 months	1.06	4.57	0.41	2.54	-0.11	0.09	0.014	14
Chemical P	14 weeks	1.13	4.96	0.03	1.08	0.43	0.05	0.033	11
Chemical T	13 weeks	0.79	6.16	-1.45	0.04	-1.40	0.58	1.00	1
Chemical B	14 weeks	1.54	3.08	1.24	17.58	1.24	0.17	0.002	21
Chemical R	13 weeks	2.25	3.50	0.67	4.64	0.92	0.37	0.008	17
Chemical I	14 weeks	1.84	7.02	-1.09	0.08	-0.93	0.17	0.437	2
Chemical N	18 months	1.00	4.90	0.10	1.26	0.14	0.10	0.028	13
Chemical F	15 weeks	1.08	5.75	-0.70	0.20	-0.71	0.10	0.178	3
Chemical U	3 months	1.72	0.66	2.52	318.95	2.68	0.32	0.0001	23
Chemical A	13 weeks	1.56	5.25	-0.16	0.70	-0.21	0.38	0.051	8
Chemical O	8 weeks	1.31	2.73	1.74	54.55	1.88	1.44	0.001	22
Chemical J	6 months	1.32	4.98	0.02	1.04	0.04	0.10	0.034	10
Chemical W	3 months	1.96	5.83	-0.42	0.38	-0.59	0.13	0.093	4
Chemical E	7 months	1.58	5.37	-0.24	0.58	-0.13	0.14	0.062	6
Chemical S	3 months	1.63	5.43	-0.26	0.55	-0.21	0.08	0.065	5
Chemical X	13 weeks	2.44	0.54	-0.07	0.27	-0.72	0.10	0.042	9
Chemical G	13 weeks	1.48	3.61	0.94	8.75	1.00	0.10	0.004	19
Chemical V	13 weeks	0.66	4.21	1.20	15.85	0.99	0.21	0.002	20
Chemical Y	6 months	1.53	4.92	0.05	1.13	-0.12	0.14	0.032	12
Chemical Q	13 weeks	0.90	5.18	-0.20	0.64	0.18	0.29	0.056	7
Chemical D[f]									
Chemical L	24 months	1.25	4.47	0.42	2.64	0.44	0.12	0.013	15

[a]Table provides dose-response curves of the representative study: probit (% female rat plasma ChEI)=log (dose) x slope + intercept
[b]Chemicals are listed in random order
[c]log ED50pl=log (effective dose) calculated to cause 50% inhibition of plasma ChE
[d]Relative Potency Factor: $RPF=ED50_{Index\ Chemical} \div ED50_{Chemical\ Z}$
[e]chemicals are ranked most potent (1) to least potent (24)
[f] No quality data available

1. Analysis of Dose-Response Relationships

As indicated above, a representative study for each chemical was selected based on several criteria including occurrence of steady state as well as a dose-response relationship that was consistent with other studies. Table 1a presents the linear regressions from these studies in addition to the respective $ED50_{plasma}$, RPF, and relative rank. For males, the slopes for these selected studies ranged from 0.43 to 2.22. The linear regressions for the female plasma ChEI data are given in Table 1b. The slopes for these studies ranged from 0.62 to 2.46.

Additivity assumes for chemicals that act by a common mechanism that there were no interactions among these chemicals and that dose-response curves for them were parallel. An analysis of both the slope and the intercepts has been performed to evaluate this assumption. For males, the statistical slope analysis indicates that for the OP group comparison, slopes of the dose-response curves were not statistically different ($p = 0.10$). Conversely, for female plasma ChEI, the statistical slope analysis indicates that for the selected studies, at least one OP has a different slope ($p = 0.002$) from the rest of the group. Further analysis indicated that compared to the median slope of 1.32 for Chemical J, three chemicals (Chemicals C, M and X) exhibited statistically different slopes.

Based on the assumption of the parallel dose-response curves, the relative rank of the intercept values should theoretically be the same as the relative rank of the calculated $ED50_{plasma}$. To test this, Spearman rank order correlations were performed between the relative rank and the intercept. The rank order correlation between the calculated $ED50_{plasma}$ and the intercept for the representative plasma male ChEI data was 0.95 ($p<0.0001$). Although the three slopes were statistically different from the median slope, Spearman rank order correlations were performed between the intercepts and the $ED50_{plasma}$ for the female plasma ChEI data. This correlation was 0.86 ($p<0.0001$) for females indicating that although the slopes for three chemicals may be statistically different, overall, the dose-response regressions intersect little.

2. Comparison of Representative and Mean log ED50 for Plasma ChE Inhibition

For males, the representative log $ED50_{plasma}$ was equal to or almost equal to the mean log $ED50_{plasma}$ in 18 of 24 chemicals (Figure 2). For females, the representative log $ED50_{plasma}$ was equal to or almost equal to the mean in 21 of 23 chemicals (Figure 3). None of the log $ED50_{plasma}$ values were outside of one standard deviation for either sex. For about half the OPs, the available data for male plasma ChEI were highly variable. Plus or minus one standard deviation, the mean $ED50_{plasma}$ values varies by at least 10-fold. The available database for female plasma ChEI appears less variable overall than the respective male data.

3. Plasma ChE Inhibition of Index Chemical

Chemical T was selected as the index chemical based on the availability of data for all three routes of exposure (oral, dermal, and inhalation). Based on the $ED50_{plasma}$ for male plasma ChEI, the index compound ranks as the second most potent chemical for the oral route of exposure. Based on the $ED50_{plasma}$ for female plasma ChEI, it ranks as the most potent chemical for plasma ChEI.

4. Differences Between Male and Female Plasma ChEI

As shown in Tables 1a and 1b, the representative $logED50_{plasma}$ values ranged widely– from -0.71 to 2.83 for Chemical D and Chemical U in males and from -1.45 to 2.52 for Chemical T and Chemical U in females, respectively. RPFs ranged over five orders of magnitude–from 0.0005 to 1.80 in males and from 0.0001 to 1.00 in females. The representative $logED50s_{plasma}$ for females were lower than for males for six of 23 chemicals. For 15 of 23 chemicals, the female RPFs were 10-fold lower than the male plasma RPFs. This difference was caused by a one log unit (i.e., 10-fold) difference between the female and male logED50. For 17 of 23 chemicals, the male $ED50_{plasma}$ was almost equal or equal to the female $ED50_{plasma}$. For the remaining six chemicals, the $ED50_{plasma}$ (mg/kg/day) for females was at least three-fold higher than the respective male data. Figure 4 shows a scatterplot comparing the log $ED50_{plasma}$ values for each sex.

Figure 2. Comparison of Representative Log $ED50_{pl}$ with the Mean Log $ED50_{pl}$ for Male Rats

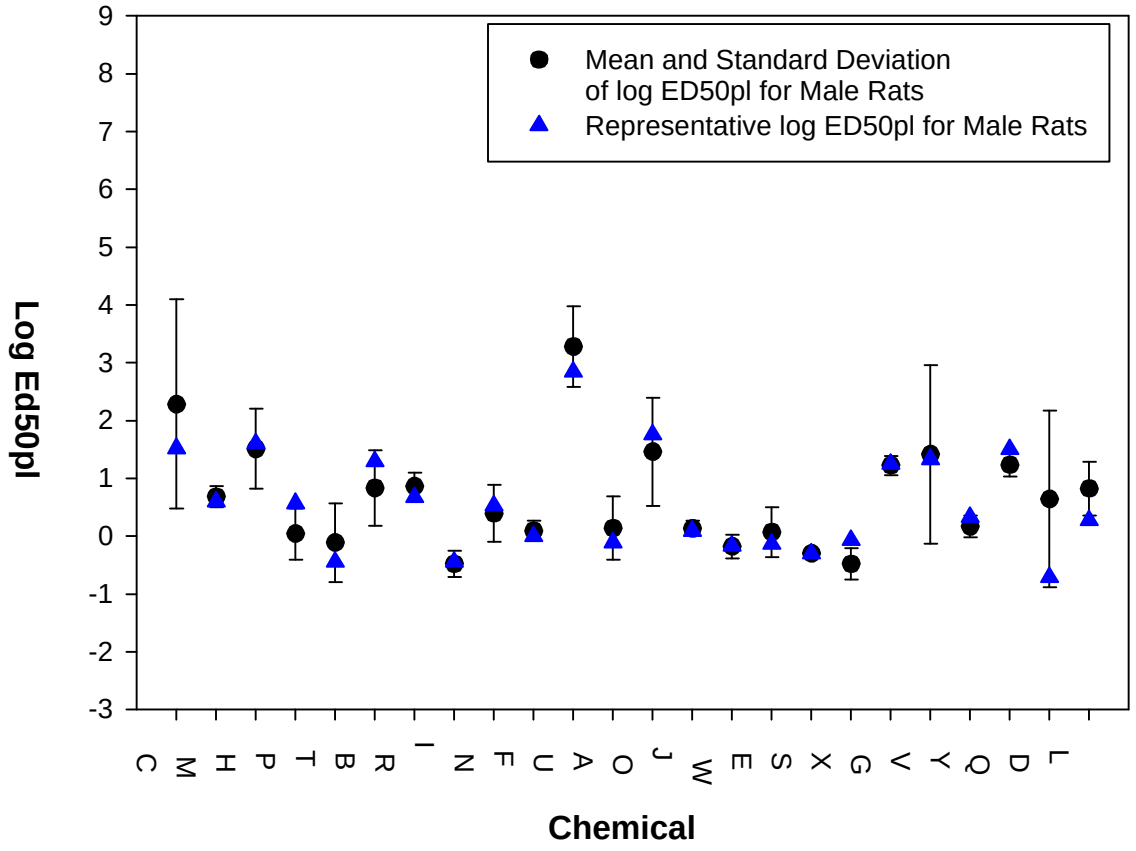


Figure 3. Comparison of Representative Log $ED50_{pl}$ with the Mean Log $ED50_{pl}$ for Female Rats

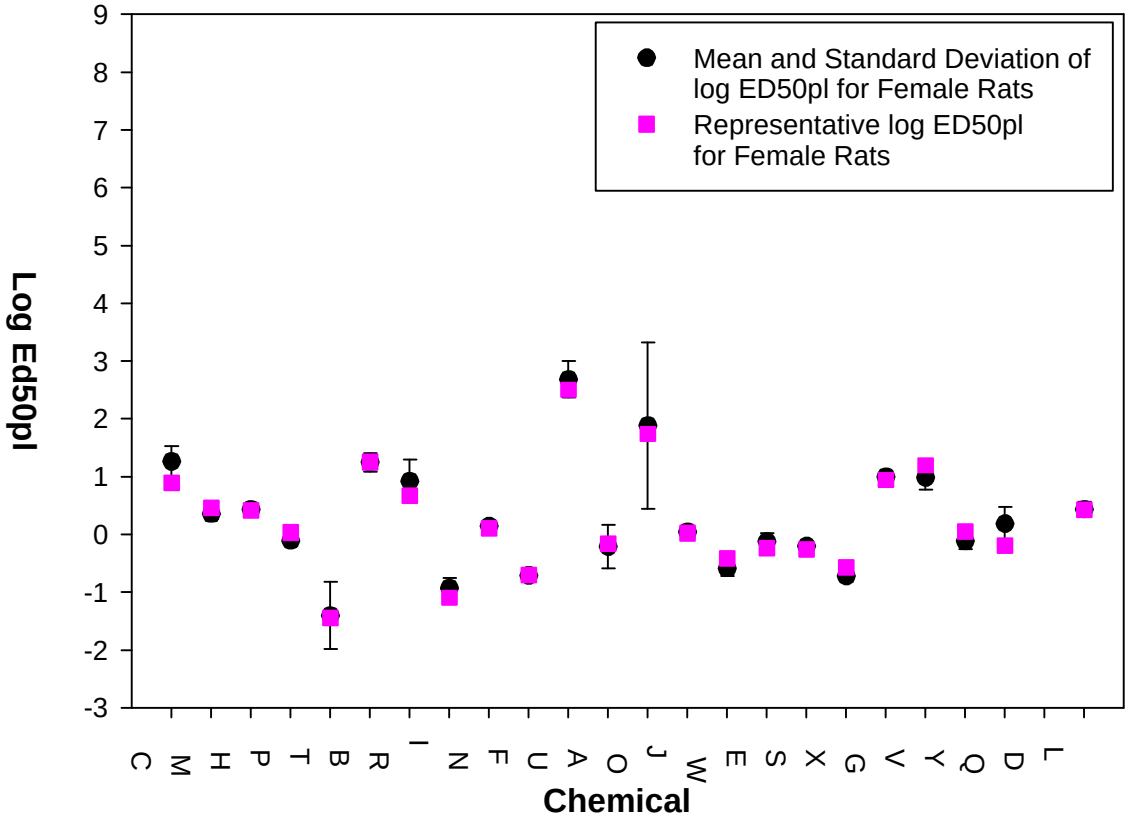
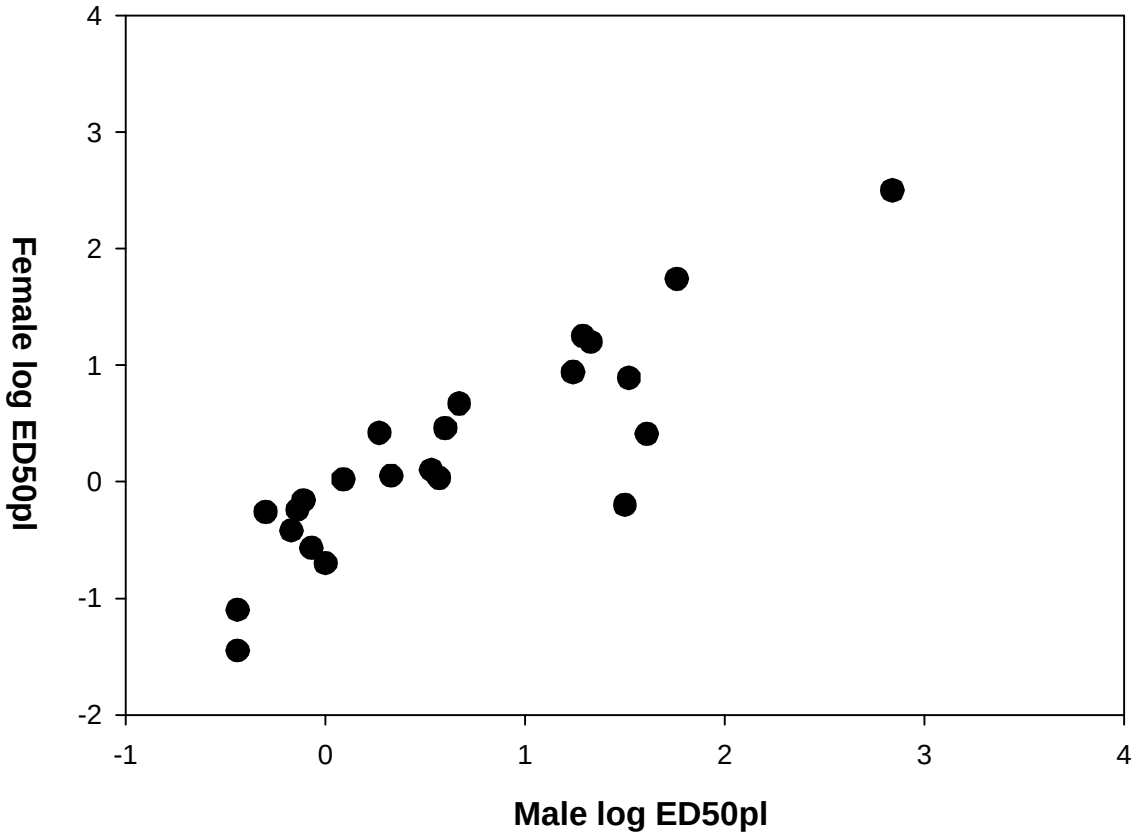


Figure 4. Scatterplot of Representative Log $ED50_{pl}$ for Male and Female Rat



6. Summary of Plasma ChEI Results for Oral Exposure

- Plasma ChEI data were available for all 24 OPs for males and for 23 of 24 chemicals for females.

- Based on the following evidence, the assumption of parallel lines was supported for the male plasma ChEI data for the representative studies:

  - Slopes were not statistically different (p=0.10)

  - Correlation between the intercepts and the relative rank was strong (0.95)

- There was moderate support for parallel dose-response curves for the female plasma ChEI data for the representative studies:

  - Slopes of dose-response lines were statistically-different from the median slope for three of 23 chemicals.

  - Correlation between the intercepts and the relative rank was strong (0.86).

- Comparing the entire database, in general the male plasma ChEI data were more variable than the female plasma ChEI data. For 15 of 23 chemicals, the female RPFs were 10-fold lower than the male plasma RPFs. This difference was caused by a one log unit (i.e., 10-fold) difference between the female and male logED50. For six of 23 chemicals, the representative $ED50_{plasma}$ (mg/kg/day) for females was six- to 20-fold lower for plasma ChEI than the respective male data.

B. Relative Potency Rankings for Oral Exposure Based on RBC ChE Inhibition

The linear regressions for the representative study for RBC ChE inhibition in addition to the representative $logED50_{rbc}$ values and the RBC relative rank are given in Tables 2a and 2b below. RBC ChE inhibition data were available for 23 of 24 chemicals for male rats and 22 of 24 chemicals for female rats. Based on the defined criteria for evaluating studies, Chemical Y RBC ChEI data of sufficient quality were not available for both sexes; the data showed minimal RBC ChEI at the highest dose with poor dose-response characteristics. Also, Chemical P RBC ChEI data of sufficient quality were not available for females (no dose-response observed at dose levels tested). The same study and endpoint were used to evaluate both male and female RBC ChEI. Timepoints selected ranged from four-weeks to 12-months.

1. Analysis of Dose-Response Relationships

As indicated above, a representative study for each chemical was selected based on several criteria including occurrence of steady state and consistency of the dose-response relationship. Tables 2a and 2b show the linear regressions from these representative studies, representative $ED50_{rbc}$, relative rank, and RPFs for both male and female rats.

Table 2a. Representative Linear Regression and ED50 for Male RBC Data[a]

Chemical[b]	Endpoint	Slope	Intercept	Representative log ED50rbc[c]	Male logED50rbc[c] Mean	Male logED50rbc Standard Deviation	ED50rbc (mg/kg/day)	RPF[d]	Relative Rank[e]
Chemical C	7 weeks	0.63	3.79	1.90	2.09	0.52	79.43	2.04	19
Chemical M	13 weeks	2.11	4.84	0.07	0.80	0.52	1.17	138.04	7
Chemical H	6 months	0.41	3.36	3.44	4.05	1.76	2754.23	0.06	23
Chemical P	14 weeks	0.30	3.96	1.85	1.85	.	70.79	2.29	17
Chemical T	3 months	0.68	3.50	2.21	1.23	1.61	162.18	1.00	21
Chemical B	14 weeks	0.55	4.17	1.49	1.24	0.37	30.90	5.25	16
Chemical R	13 weeks	1.40	4.70	0.21	0.37	0.23	1.62	100.00	9
Chemical I	14 weeks	1.32	5.88	-0.67	-0.75	0.06	0.21	758.58	2
Chemical N	3 months	2.31	4.45	0.24	0.18	0.28	1.74	93.33	11
Chemical F	15 weeks	1.70	5.28	-0.16	0.01	0.15	0.69	234.42	6
Chemical U	13 weeks	0.96	2.41	2.72	2.65	0.23	524.81	0.31	22
Chemical A	13 weeks	1.88	5.77	-0.41	-0.12	0.39	0.39	416.87	5
Chemical O	4 weeks	1.88	4.72	0.15	0.95	0.84	1.41	114.82	8
Chemical J	3 months	0.65	3.70	2.01	2.63	1.15	102.33	1.58	20
Chemical W	6 weeks	0.60	3.89	1.86	1.12	1.64	72.44	2.24	18
Chemical E	7 months	0.80	5.52	-0.65	0.04	0.52	0.22	724.44	4
Chemical S	13 weeks	0.68	4.85	0.22	0.80	0.74	1.66	97.72	10
Chemical X	8 weeks	2.88	6.93	-0.67	-0.60	0.10	0.21	758.58	3
Chemical G	13 weeks	1.08	3.98	0.94	1.23	0.81	8.71	18.62	13
Chemical V	13 weeks	1.83	3.72	0.70	0.93	0.25	5.01	32.36	12
Chemical Y [f]									
Chemical Q	10 weeks	1.16	3.47	1.32	1.76	0.63	20.89	7.76	15
Chemical D	8 weeks	2.43	7.93	-1.21	-1.13	0.07	0.06	2630.27	1
Chemical L	1 year	1.14	3.89	0.97	1.01	0.23	9.33	17.38	14

[a]Table provides dose-response curves of representative study: probit (% male rat RBC ChEI)=log (dose) x slope + intercept
[b]Chemicals are listed in random order
[c]log ED50rbc=log (effective dose) calculated to cause 50% inhibition of RBC ChE
[d]Relative Potency Factor: RPF $=ED50_{\text{Index Chemical}} \div ED50_{\text{Chemical Z}}$
[e]chemicals are ranked most potent (1) to least potent (24)
[f] No quality data available

Table 2b. Representative Linear Regression and ED50 for Female RBC Data[a]

Chemical[b]	Endpoint	Slope	Intercept	Representative log $ED50_{rbc}$[c]	Female log$ED50_{rbc}$ Mean	Female log$ED50_{rbc}$ Standard Deviation	$ED50_{rbc}$ (mg/kg/day)	RPF[d]	Relative Rank[e]
Chemical C	7 weeks	0.34	4.65	1.01	2.01	0.56	10.3	12.02	15
Chemical M	13 weeks	2.36	4.62	0.16	1.00	0.64	1.44	85.11	12
Chemical H	6 months	0.24	4.23	3.23	4.03	0.89	1685.60	0.07	22
Chemical P[f]		.							
Chemical T	3 months	0.54	3.87	2.09	1.46	0.89	121.68	1.00	20
Chemical B	14 weeks	0.59	4.19	1.36	2.68	0.92	23.16	5.37	18
Chemical R	13 weeks	1.06	4.87	0.12	0.42	0.32	1.32	93.33	10
Chemical I	14 weeks	1.49	6.2	-0.81	-0.90	0.11	0.15	794.33	3
Chemical N	3 months	4.67	4.54	0.10	-0.05	0.21	1.25	97.72	9
Chemical F	15 weeks	3.76	4.53	0.12	0.10	0.07	1.33	93.33	11
Chemical U	13 weeks	0.82	2.84	2.63	2.71	0.21	427.14	0.29	21
Chemical A	13 weeks	1.65	5.55	-0.33	-0.10	0.43	0.47	263.03	5
Chemical O	4 weeks	1.70	4.93	0.04	0.81	1.18	1.10	112.20	8
Chemical J	3 months	1.09	3.29	1.57	2.39	0.80	37.33	3.31	19
Chemical W	6 weeks	1.12	5.86	-0.77	-0.75	0.03	0.17	724.44	4
Chemical E	7 months	1.36	5.39	-0.28	-0.09	0.73	0.52	234.42	6
Chemical S	13 weeks	1.25	5.08	-0.06	0.66	0.93	0.87	141.25	7
Chemical X	8 weeks	3.34	8.15	-0.94	-0.75	0.11	0.11	1071.52	2
Chemical G	13 weeks	0.66	4.27	1.11	1.07	0.52	12.80	9.55	16
Chemical V	13 weeks	1.78	4.23	0.43	1.03	0.57	2.71	45.71	13
Chemical Y[f]					.	.			
Chemical Q	10 weeks	0.94	3.77	1.31	1.35	0.14	20.31	6.03	17
Chemical D	8 weeks	6.86	12.25	-1.06	-1.11	0.07	0.09	1412.54	1
Chemical L	1 year	0.63	4.4	0.96	1.02	0.46	9.19	13.49	14

[a]Table provides dose-response curves of representative study: probit (% female rat RBC ChEI)=log (dose) x slope + intercept
[b]Chemicals are listed in random order
[c]log ED50rbc=log (effective dose) calculated to cause 50% inhibition of RBC ChE
[d]Relative Potency Factor: $RPF=ED50_{Index\ Chemical} \div ED50_{Chemical\ Z}$
[e]chemicals are ranked most potent (1) to least potent (24); [f]No quality data available

For male rats, the slopes for these representative studies ranged from 0.30 to 2.88. The group slope analysis for male RBC ChEI data indicated that the slopes were (p=0.002). Compared to the median slope of 1.14 for Chemical L, three chemicals exhibited statistically different slopes (Chemicals H, P, and B). The slopes for RBC ChEI in females ranged widely from 0.24 to 4.67. A significant outlier outside of this large range was the slope value of 6.86. Slope analysis for the representative studies for female RBC ChEI indicated that the slopes were different ($p<0.001$). Compared to the median slope of 1.25 for Chemical S, five chemicals (Chemicals C, H, N, F, and X) exhibited statistically different slopes.

Additional evidence of parallel dose-response regressions was the correlation between the order of the representative $ED50_{rbc}$ values and the intercept. The Spearman rank order correlation was 0.90 and 0.95 for males and females, respectively ($p<0.0001$). This strong correlation indicates that although slopes for RBC ChEI may not be similar for all the OPs, the dose-response curves intersect very little.

2. Comparison of Representative and Mean ED50s for RBC ChE Inhibition

Tables 2a and 2b show the mean and standard deviations for $logED50_{rbc}$ values from the RBC ChEI data for OP. The male and female RBC data were highly variable. The standard deviations for approximately half (11 of 23) of the chemicals were >0.5 log units. In other words, for about half of the chemicals, within plus or minus one standard deviation, the mean varied by at least 10-fold.

The representative log $ED50_{rbc}$ for males was equal to or almost equal to the respective mean in 15 of 24 chemicals (Figure 5). None of the representative log $ED50_{rbc}$ values were outside of one standard deviation. For females, the log $ED50_{rbc}$ was equal to or almost equal to the respective mean in 12 of 22 chemicals (Figure 6). The representative log $ED50_{rbc}$ for two chemicals was below one standard deviation (i.e., more potent than the mean).

Figure 5. Comparison of Representative Log $ED50_{rbc}$ with the Mean Log $ED50_{rbc}$ for Male Rats

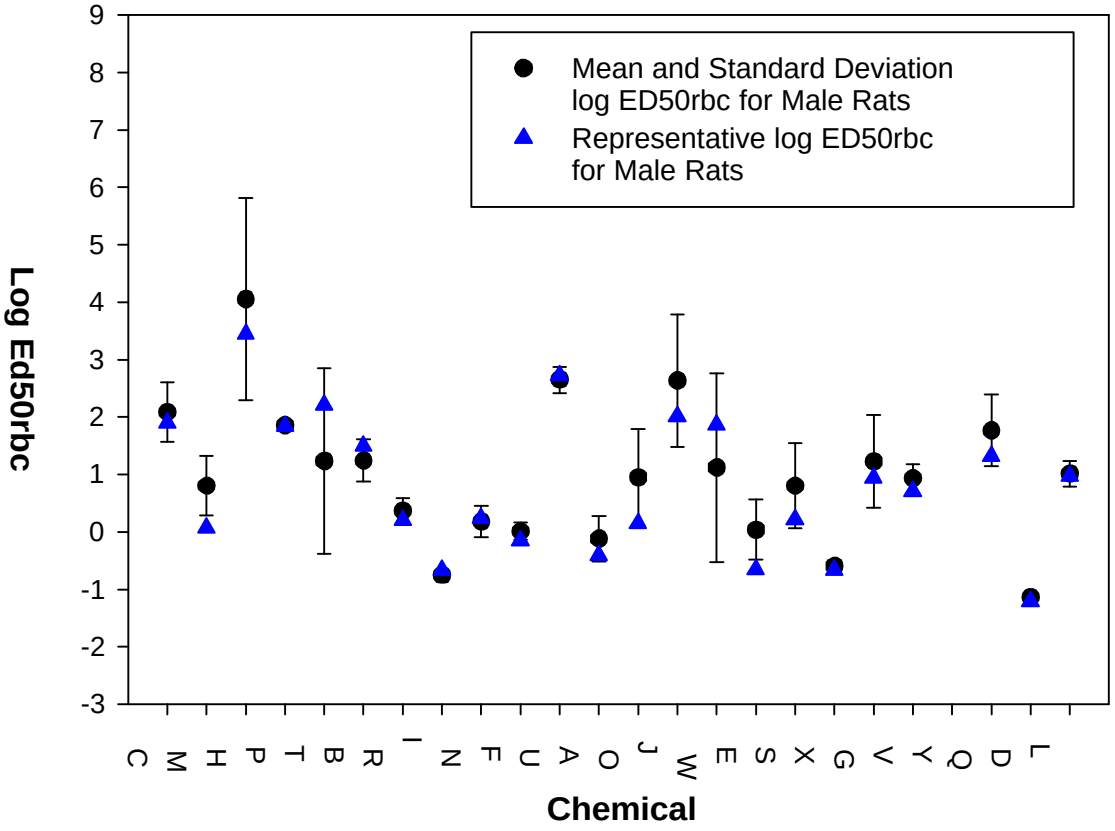
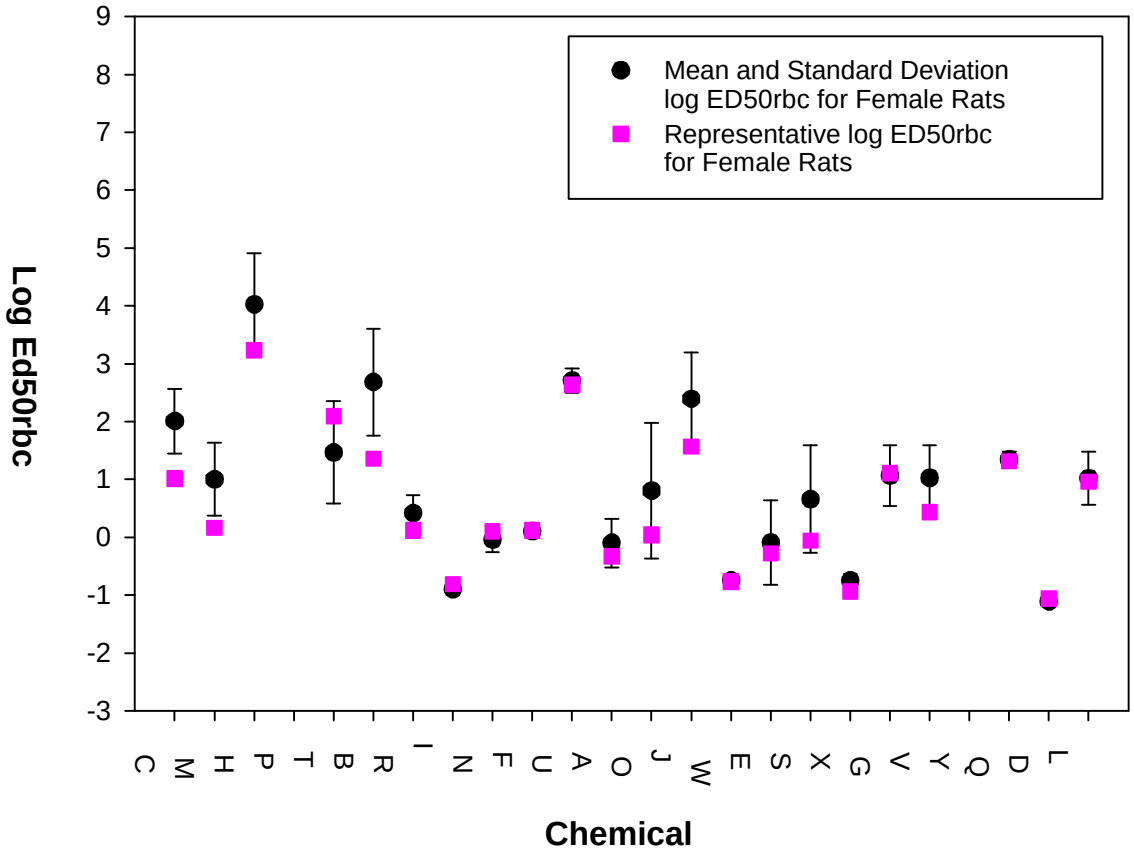


Figure 6. Comparison of Representative Log $ED50_{rbc}$ with the Mean Log $ED50_{rbc}$ for Female Rats



3. RBC ChE Inhibition of Index Chemical

For the index chemical (Chemical T), the relative rank, representative slope, representative log $ED50_{rbc}$, mean log $ED50_{rbc}$, and overall variability were similar for male and female RBC ChEI in rats. The representative slopes were 0.68 and 0.54 for males and females, respectively. The representative log $ED50_{rbc}$ values were 2.21 and 2.09 for males and females, respectively. The mean log $ED50_{rbc}$ values were 1.23 +/- 1.61 and 1.46 +/- 0.89 for males and females, respectively. These representative log $ED50_{rbc}$ values corresponded to relative rankings of 21 of 23 and 20 of 22, respectively. It was notable that Chemical T was among the most potent OPs in the group for plasma ChEI, but for RBC ChEI, it was among the least potent chemicals.

4. Differences Between Male and Female RBC ChEI for Rat Oral Exposures

As shown in Tables 2a and 2b, the representative log $ED50_{rbc}$ values ranged widely from -1.21 to 3.44 (mean=0.81 +/- 1.23) in males and from -1.06 to 3.28 (mean=0.55 +/-1.16) in females. RPFs ranged over five orders of magnitude, from 0.31 to 2630 in males and from 0.07 to 1413 in females. With the exception of Chemical W, the potencies of the representative $ED50_{rbc}$ and RPFs for male and female rats were similar based on RBC ChEI. Figure 7 shows a scatterplot comparing the log $ED50_{rbc}$ (mg/kg/day) values for each sex.

5. Summary of RBC ChEI Results for Oral Exposure

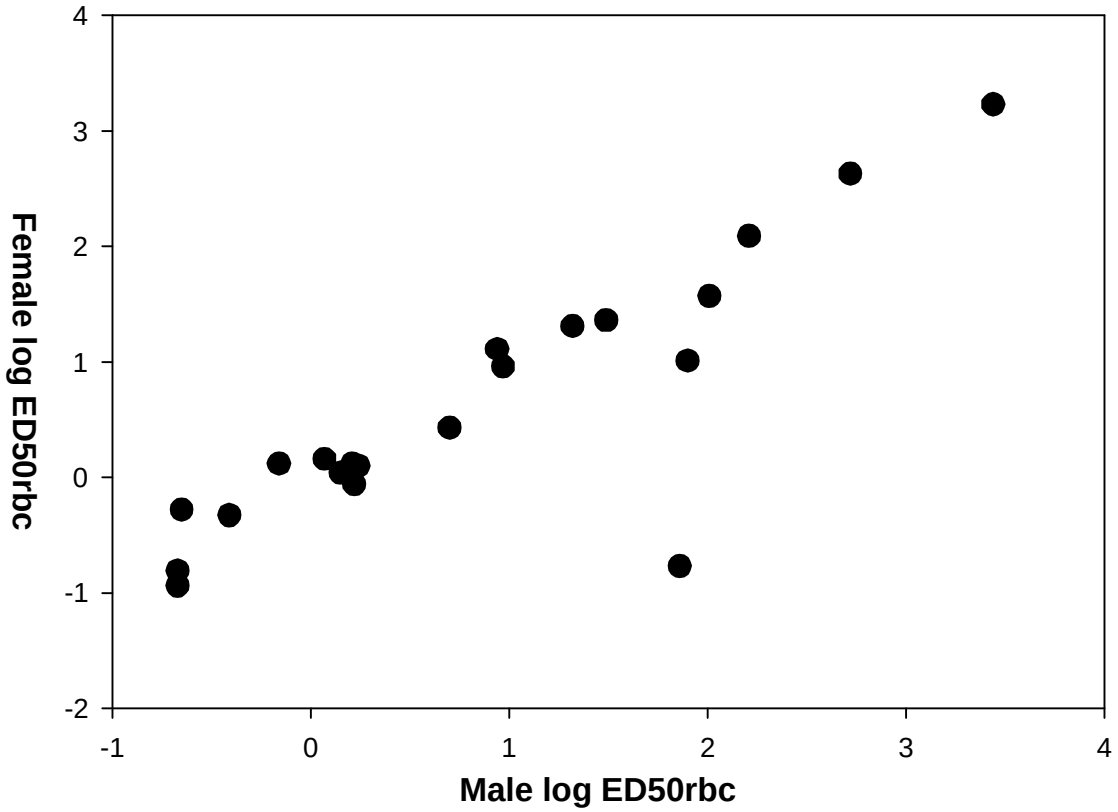
- RBC ChE inhibition data were available for 23 chemicals for male rats but only 22 chemicals for female rats.

- Based on the following evidence, the assumption of parallel lines was moderately supported for the male RBC ChEI data for the representative studies:

  - Three chemicals out of 23 exhibited statistically-different slopes from the median slope.

  - Correlation between the intercepts and the relative rank was very strong (0.90).

Figure 7. Scatterplot of Male and Female Representative LogED$_{rbc}$



- Based on the following evidence, the assumption of parallel lines was only weakly supported for the female RBC ChEI data for the representative studies:
  - The slope representing the dose-response curve for Chemical D was a significant outlier.
  - Slopes of dose-response lines were statistically-different for five chemicals.
  - Correlation between the intercepts and the relative rank was very strong (0.95).
- Male and female RBC ChEI data were highly variable. The standard deviations for approximately half of the chemicals were >0.5 log units. In other words, for about half of the group, the mean varied by at least 10-fold. With exception of Chemical W, the RPFs and potencies of the representative and $\log ED50_{rbc}$ for male and female rats were similar based on RBC ChEI.

C. Relative Potency Rankings for Brain ChE Inhibition in Rat from Oral Exposure

The linear regressions for the representative study for brain ChE inhibition in addition to the calculated log $ED50_{brain}$ values and the brain relative rank are given in Tables 3a and 3b. Brain ChE inhibition data were available for 22 of 24 chemicals for male rats and 21 of 24 chemicals for female rats. For Chemical J, brain ChEI was saturated at the two highest doses in the only available study; no meaningful dose-response was observed. For Chemical F, brain ChEI did not exceed 12% at doses tested and showed no (males) or poor (females) dose-response characteristics; in this same study, marked inhibition of both plasma (69% males; 93% females) and RBC (91% males; 96% females) ChE activity was observed. With the exception of one chemical, both the study and endpoint selected for male and female brain ChEI were the same. Selected timepoints ranged from 13-week to 24-months.

Table 3a. Representative Linear Regression and ED50 for Male Rat Brain Data[a]

Chemical[b]	Endpoint	Slope	Intercept	Representative Log ED50br[c]	Representative ED50br (mg/kg/day)	Male LogED50br[c] Mean	Male LogED50br Standard Deviation	RPF[d]	Relative Rank[e]
Chemical C	13 weeks	0.89	4.51	0.55	3.55	0.78	0.12	8.91	12
Chemical M	13 weeks	2.66	3.7	0.49	3.09	0.49	.	10.23	11
Chemical H	13 weeks	0.9	3.15	2.06	114.82	2.06	.	0.28	20
Chemical P	1 year	1.15	4.21	0.69	4.90	0.69	.	6.46	13
Chemical T	1 year	2.57	1.14	1.50	31.62	1.53	0.29	1.00	17
Chemical B	13 weeks	1.17	3.04	1.68	47.86	1.68	.	0.66	19
Chemical R	2 years	1.34	4.37	0.47	2.95	0.47	.	10.72	10
Chemical I	13 weeks	1.97	5.66	-0.33	0.47	-0.55	0.31	67.61	2
Chemical N	3 months	1.22	3.37	1.33	21.38	1.10	0.18	1.48	16
Chemical F[f]									
Chemical U	13 weeks	1.26	0.94	3.22	1659.59	4.36	1.43	0.02	22
Chemical A	13 weeks	1.43	5.19	-0.13	0.74	-0.16	0.25	42.66	6
Chemical O	13 weeks	1.36	4.51	0.36	2.29	0.42	0.15	13.80	9
Chemical J[f]									
Chemical W	1 year	2.22	5.41	-0.18	0.66	-0.18	.	47.86	5
Chemical E	2 years	1.46	5.49	-0.33	0.47	-0.18	0.12	67.61	3
Chemical S	13 weeks	1.95	5.21	-0.11	0.78	0.05	0.14	40.74	7
Chemical X	13 weeks	2.85	5.9	-0.31	0.49	-0.31	.	64.57	4
Chemical G	13 weeks	1.52	2.63	1.56	36.31	1.24	0.23	0.87	18
Chemical V	13 weeks	1.97	3.45	0.79	6.17	1.29	0.69	5.13	14
Chemical Y	2 years	1.13	4.65	0.31	2.04	0.30		15.49	8
Chemical Q	3 months	1.06	2.72	2.15	141.25	2.15		0.22	21
Chemical D	13 weeks	2.79	6.38	-0.49	0.32	-0.49	0.01	97.72	1
Chemical L	2 years	2.66	1.98	1.13	13.49	1.13		2.34	15

[a]Table provides dose-response curves of representative study: probit(% male rat brain ChEI)=log (dose) x slope + intercept; [b]Chemicals are listed in random order

[c]log $ED50_{brain}$=log (effective dose) calculated to cause 50% inhibition of brain ChE; [d]Relative Potency Factor: $RPF=ED50_{Index\ Chemical} \div ED50_{Chemical\ z}$

[e]chemicals are ranked most potent (1) to least potent (24); [f]No quality data available

Table 3b. Representative Linear Regression and ED50 for Female Rat Brain Data[a]

Chemical[b]	Endpoint	Slope	Intercept	Representative Log ED50br[c]	Representative ED50br (mg/kg/day)	Female LogED50br Mean	Log ED50br Standard Deviation	RPF[d]	Relative Rank[e]
Chemical C	13 weeks	0.90	4.35	0.72	5.25	0.85	0.14	3.47	13
Chemical M	13 weeks	2.53	4.05	0.37	2.34	0.43	0.06	7.76	9
Chemical H	13 weeks	1.04	2.75	2.15	141.25	2.15	.	0.13	20
Chemical P	1 year	1.55	4.46	0.35	2.24	0.35	.	8.13	8
Chemical T	1 year	1.40	3.23	1.26	18.20	1.10	0.24	1.00	17
Chemical B	13 weeks	1.73	2.94	1.19	15.49	1.19	.	1.17	16
Chemical R	2 years	1.23	4.51	0.4	2.51	0.40	.	7.24	10
Chemical I	13 weeks	2.18	6.43	-0.65	0.22	-0.74	0.13	81.28	1
Chemical N	3 months	5.19	2.01	0.58	3.80	0.55	0.04	4.79	12
Chemical F[f]		.					.		
Chemical U	13 weeks	1.81	-0.22	2.88	758.58	4.04	0.93	0.02	21
Chemical A	13 weeks	1.46	5.16	-0.11	0.78	-0.03	0.57	23.44	6
Chemical O	13 weeks	2.02	4.61	0.19	1.55	0.33	0.17	11.75	7
Chemical J[f]		.				.	.		
Chemical W	1 year	1.35	5.41	-0.3	0.50	-0.30	.	36.31	3
Chemical E	2 years	1.38	5.39	-0.28	0.52	-0.18	0.26	34.67	5
Chemical S	13 weeks	0.77	5.23	-0.3	0.50	0.08	0.33	36.31	4
Chemical X	13 weeks	3.91	6.79	-0.46	0.35	-0.46	.	52.48	2
Chemical G	13 weeks	2.80	0.84	1.49	30.90	1.43	0.06	0.59	18
Chemical V	13 weeks	1.59	3.72	0.81	6.46	1.08	0.34	2.82	14
Chemical Y	2 years	1.52	4.19	0.53	3.39	0.42	.	5.37	11
Chemical Q	3 months	0.71	3.85	1.61	40.74	1.32	0.33	0.45	19
Chemical D[f]						.	.		
Chemical L	2 years	2.45	2.24	1.13	13.49	1.20	0.10	1.35	15

[a]Table provides dose-response curves of representative study: probit(% female rat brain ChEI)=log (dose) x slope + intercept; [b]Chemicals are listed in random order

[c]log $ED50_{brain}$=log (effective dose) calculated to cause 50% inhibition of brain ChE; [d]Relative Potency Factor: RPF=$ED50_{Index\ Chemical} \div ED50_{Chemical\ X}$

[e]chemicals are ranked most potent (1) to least potent (24); [f]No quality data available

1. Analysis of Dose-Response Relationships

As indicated above, a representative study for each chemical was selected based on several criteria including occurrence of steady state and the dose-response relationship. Tables 3a and 3b show the linear regressions from these representative studies in addition to the respective log $ED50_{brain}$ and relative rank for male and female rats. For male rats, the slopes for these representative studies ranged from 0.89 to 2.85. Among male data, the statistical slope analysis indicated that the slopes were not statistically different ($p=0.13$). Supporting this slope analysis for male brain ChEI data was the Spearman rank correlation of 0.96 ($p<0.0001$) between the intercepts and respective representative log $ED50_{brain}$.

For female rats, the slopes of the representative studies ranged from 0.71 to 3.91. Within the female brain ChEI data, the slope from the dose-response relationship for Chemical N was a significant outlier (5.19). As expected from the relatively large range of slopes (3.11), the statistical slope analysis indicates that for the female brain ChEI linear regressions, the slopes were statistically different ($p=0.01$ including Chemical N, and $p=0.02$ excluding Chemical N). Compared to the median slope of 1.55, further analysis indicates that slopes of two chemicals (Chemicals X and L) were significantly different. Although the slopes were statistically different, Spearman rank order correlations were performed between the intercepts and the log $ED50_{brain}$ for the female brain ChEI data. This correlation was 0.92 ($p<0.0001$) for females indicating that although the slopes may be statistically different, the dose-response relationships intersect very little.

2. Comparison of Representative and Mean Log $ED50_{brain}$ for Brain ChE Inhibition for Rat Oral Exposure

For 10 of 22 and seven of 21 chemicals for male and females, respectively, only one study or endpoint was available. For these chemicals, the "mean" was the same value as the representative slope and log $ED50_{brain}$. For the remaining chemicals, the standard deviations were <0.35 log units for all but three chemicals (Figures 8 and 9).

3. Brain ChE Inhibition of Index Chemical

For the index chemical, Chemical T, the relative rank (17 of 22 and 17 of 21) and representative log $ED50_{brain}$ (1.26 and 1.50) among representative studies were comparable for the males and females. The representative slopes differed slightly, 2.57 and 1.40, for males and females. It should be noted that RBC and brain were similar for Chemical T.

4. Differences Between Male and Female Brain ChE Inhibition for Oral Exposure

As shown in Tables 3a and 3b, the representative log $ED50_{brain}$ values ranged widely from -0.49 to 3.22 in males and from -0.65 to 2.88 in females. RPFs ranged over five orders of magnitude, from 0.02 to 98 in males and from 0.02 to 81 in females. RPFs and representative $logED50_{brain}$ values were comparable for all but three chemicals. The $ED50_{brain}$ (mg/kg/day) for females was three- to seven-fold lower than the respective male data for three chemicals. Overall, there was a good relationship between the $ED50_{brain}$ for the OPs. Figure 10 shows a scatterplot comparing the log $ED50_{brain}$ values for each sex.

Figure 8. Comparison of Representative Log $ED50_{brain}$ with the Mean Log $ED50_{brain}$ for Male Rats

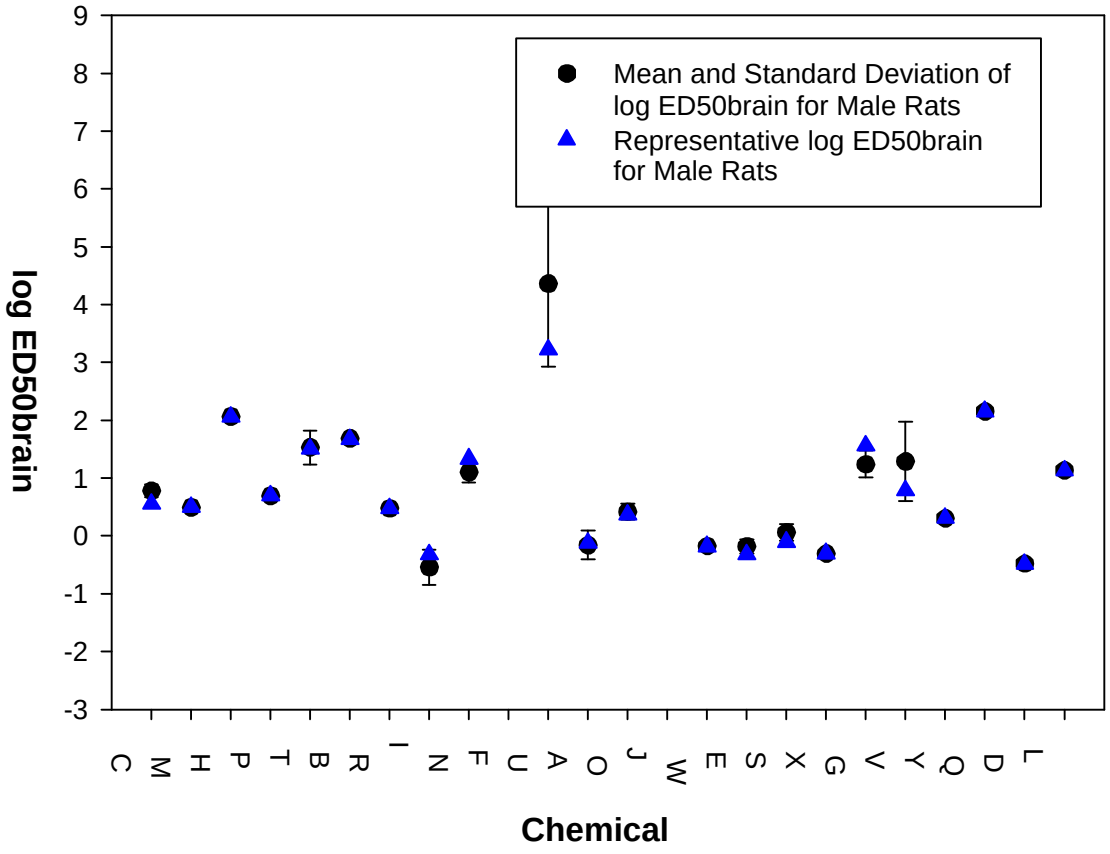


Figure 9. Comparison of Representative Log $ED50_{brain}$ with the Mean Log $ED50_{brain}$ for Female Rats

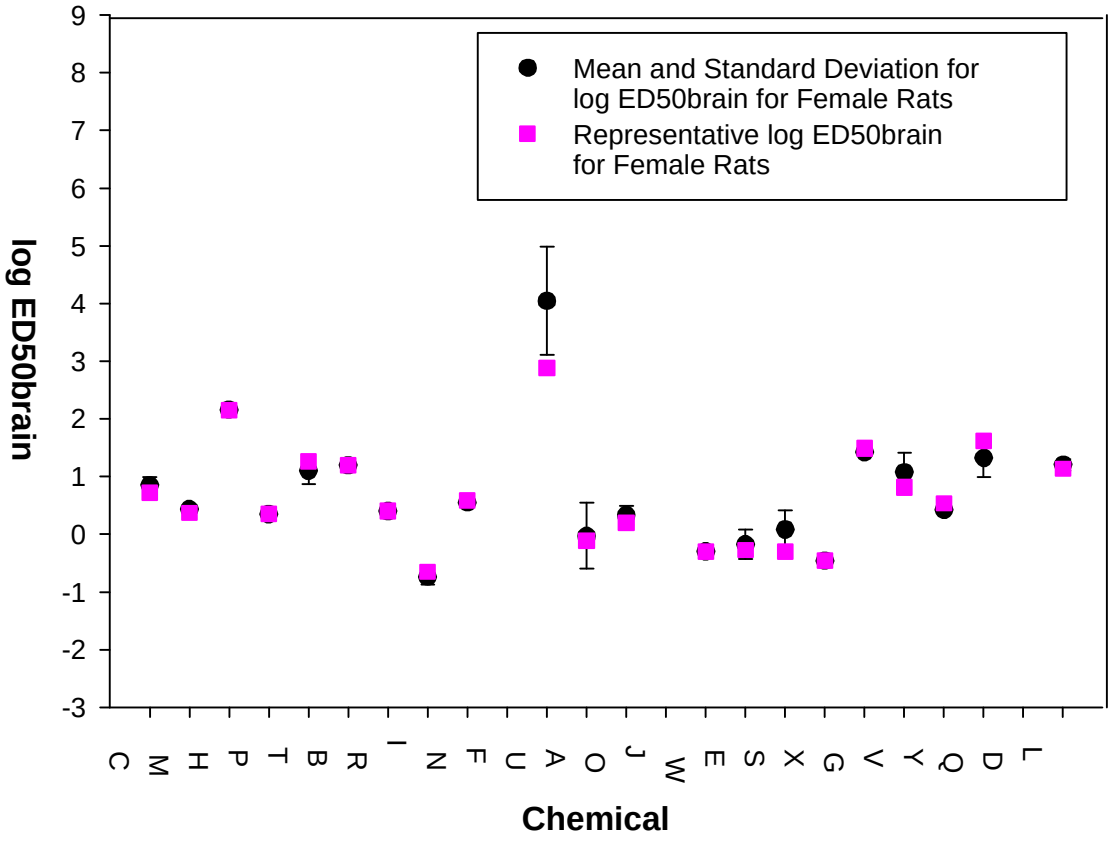
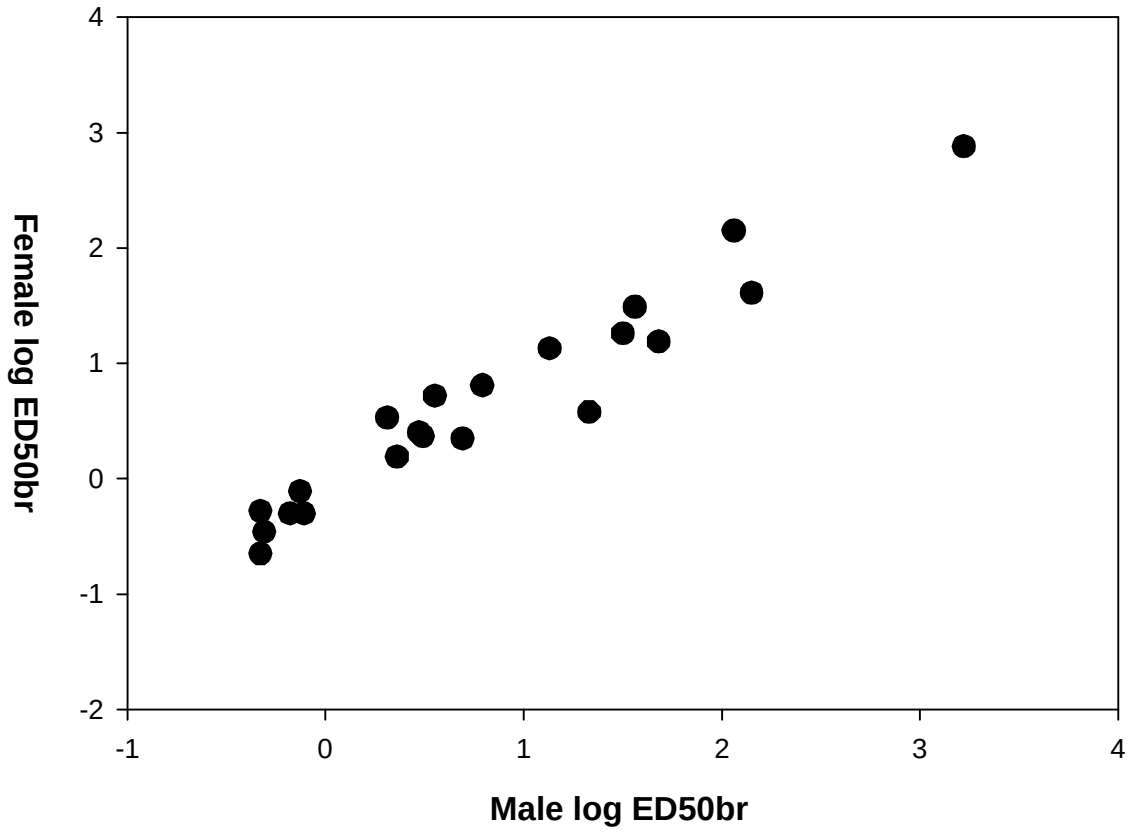


Figure 10. Scatterplot of Male and Female Representative Log $ED50_{brain}$



5. Summary of Brain ChEI Results for Oral Exposure

- Brain ChEI data were available for 22 of 24 OPs for males and for 21of 24 chemicals for females.

- Based on the following factors, the assumption of parallel lines was supported for the male brain ChEI data for the representative studies:

  - Slopes were not statistically different (p=0.13)

  - Correlation between the intercepts and the relative rank was strong (0.96)

- Based on the following factors, the assumption of parallel lines was weakly supported for the female brain ChEI data for the representative studies:

  - The slope representing the dose-response curve for Chemical N was a significant outlier.

  - Slopes of dose-response lines were statistically different for two of 21 chemicals.

  - Correlation between the intercepts and the relative rank was strong (0.92).

- For 10 of 22 and seven of 21 chemicals for male and females, respectively, only one study or endpoint was available. For these chemicals, the "mean" was the same value as the representative slope and log $ED50_{brain}$ value. For the remaining chemicals, the standard deviations were <0.35 log units except for three chemicals. Based on the RPF and representative $logED50_{brain}$ values, male and female rats were comparable for all but three chemicals based on brain ChEI.

D. Comparison of Relative Rankings and ED50s by Compartment and Sex for Oral Exposure

This section presents a comparative discussion of trends among different compartment and sexes.

The RPFs calculated for female plasma ChEI were at least 10-fold lower than the male plasma RPFs for 15 of 23 chemicals. This difference was caused by a more potent response for plasma ChEI with the index chemical in female rats compared to male rats. Similar potency was observed for the index chemical for male and female rat RBC and brain ChEI. With the exception of Chemical T and W for RBC ChEI and Chemicals U and Q for brain ChEI, the RPFs for male and female rats were comparable between sexes and compartments.

As shown in Table 4, ED50s were similar across compartment and sex for about one-half of the OPs. ED50s for remaining chemicals ranged by at least 10-fold across compartment or sex.

Tables 5 and 6 present the relative rank order for each OP. Table 7 shows a matrix of relative rank order correlations for each compartment and sex. Male to female rank order was highly correlated within compartment (0.80, 0.83, and 0.95, for plasma, RBC, and brain ChEI, respectively). The relative ranking correlations of the female plasma ChEI data compared to other compartments or sex data exhibited the poorest correlation coefficients (0.39-0.80).

Table 4. Comparison of Representative ED50s for Plasma, RBC, and Brain ChE[a]

Chemical	Male Plasma	Female Plasma	Male RBC	Female RBC	Male Brain	Female Brain
Chemical C	33.14	7.77	79.43	10.30	3.55	5.25
Chemical M	3.94	2.86	1.17	1.44	3.09	2.34
Chemical H	40.74	2.54	2754.23	1685.60	114.82	141.25
Chemical P	3.73	1.08	70.79	1.00	4.90	2.24
Chemical T	0.36	0.04	162.18	121.68	31.62	18.20
Chemical B	19.53	17.58	30.90	23.16	47.86	15.49
Chemical R	4.69	4.64	1.62	1.32	2.95	2.51
Chemical I	0.36	0.08	0.21	0.15	0.47	0.22
Chemical N	3.37	1.26	1.74	1.25	21.38	3.80
Chemical F[f]	0.99	0.20	0.69	1.33	1.00	1.00
Chemical U	685.82	318.95	524.81	427.14	1659.59	758.58
Chemical A	0.78	0.70	0.39	0.47	0.74	0.78
Chemical O	158.79	54.55	1.41	1.10	2.29	1.55
Chemical J[f]	1.56	1.04	102.33	37.33	1.00	1.00
Chemical W	0.67	0.38	72.44	0.17	0.66	0.50
Chemical E	0.73	0.58	0.22	0.52	0.47	0.52
Chemical S	0.50	0.55	1.66	0.87	0.78	0.50
Chemical X	1.96	0.27	0.21	0.11	0.49	0.35
Chemical G	18.64	8.75	8.71	12.80	36.31	30.90
Chemical V	21.53	15.85	5.01	2.71	6.17	6.46
Chemical Y	2.15	1.13	1.00	1.00	2.04	3.39
Chemical Q	31.45	0.64	20.89	20.31	141.25	40.74
Chemical D[f]	0.19		0.06	0.09	0.32	
Chemical L	1.88	2.64	9.33	9.19	13.49	13.49

[a]Table shows ED50 for different compartments in representative studies
[b]Qualtity data were not available for calculating ED50s in all compartments

Table 5. Relative Rank of Chemicals Based on Calculated ED50s for Plasma, RBC, and Brain ChE[a]

Relative Rank	Male Plasma	Female Plasma	Male RBC	Female RBC	Male Brain	Female Brain
1	Chemical D	Chemical T	Chemical D	Chemical D	Chemical D	Chemical I
2	Chemical T	Chemical I	Chemical I	Chemical X	Chemical I	Chemical X
3	Chemical I	Chemical F	Chemical X	Chemical I	Chemical E	Chemical W
4	Chemical S	Chemical W	Chemical E	Chemical W	Chemical X	Chemical S
5	Chemical W	Chemical S	Chemical A	Chemical A	Chemical W	Chemical E
6	Chemical E	Chemical E	Chemical F	Chemical E	Chemical A	Chemical A
7	Chemical A	Chemical Q	Chemical M	Chemical S	Chemical S	Chemical O
8	Chemical X	Chemical A	Chemical O	Chemical O	Chemical Y	Chemical P
9	Chemical F	Chemical X	Chemical R	Chemical N	Chemical O	Chemical M
10	Chemical J	Chemical J	Chemical S	Chemical R	Chemical R	Chemical R
11	Chemical L	Chemical P	Chemical N	Chemical F	Chemical M	Chemical Y
12	Chemical Y	Chemical Y	Chemical V	Chemical M	Chemical C	Chemical N
13	Chemical N	Chemical N	Chemical G	Chemical V	Chemical P	Chemical C
14	Chemical H	Chemical H	Chemical L	Chemical L	Chemical V	Chemical V
15	Chemical M	Chemical L	Chemical Q	Chemical C	Chemical L	Chemical L
16	Chemical R	Chemical M	Chemical B	Chemical G	Chemical N	Chemical B
17	Chemical G	Chemical R	Chemical P	Chemical Q	Chemical T	Chemical T
18	Chemical B	Chemical C	Chemical W	Chemical B	Chemical G	Chemical G
19	Chemical V	Chemical G	Chemical C	Chemical J	Chemical B	Chemical Q
20	Chemical Q	Chemical V	Chemical J	Chemical T	Chemical H	Chemical H
21	Chemical C	Chemical B	Chemical T	Chemical U	Chemical Q	Chemical U
22	Chemical P	Chemical O	Chemical U	Chemical H	Chemical U	
23	Chemical O	Chemical U	Chemical H			
24	Chemical U					

[a]Table provides relative rank of OPs based on representative study for all three compartments and both sexes

Table 6. Relative Rank of Chemicals Based on Representative ED50s for Plasma, RBC, and Brain ChE[a]

Chemical [b]	Male Plasma ChEI	Female Plasma ChEI	Male RBC ChEI	Female RBC ChEI	Male Brain ChEI	Female Brain ChEI
Chemical C	21	18	19	15	12	13
Chemical M	15	16	7	12	11	9
Chemical H	14	14	23	22	20	20
Chemical P	22	11	17		13	8
Chemical T	2	1	21	20	17	17
Chemical B	18	21	16	18	19	16
Chemical R	16	17	9	10	10	10
Chemical I	3	2	2	3	2	1
Chemical N	13	13	11	9	16	12
Chemical F	9	3	6	11		
Chemical U	24	23	22	21	22	21
Chemical A	7	8	5	5	6	6
Chemical O	23	22	8	8	9	7
Chemical J	10	10	20	19		
Chemical W	5	4	18	4	5	3
Chemical E	6	6	4	6	3	5
Chemical S	4	5	10	7	7	4
Chemical X	8	9	3	2	4	2
Chemical G	17	19	13	16	18	18
Chemical V	19	20	12	13	14	14
Chemical Y	12	12			8	11
Chemical Q	20	7	15	17	21	19
Chemical D	1		1	1	1	
Chemical L	11	15	14	14	15	15

[a]Table gives relative rank for each chemical for all three compartments and both sexes
[b]Chemicals are listed in random order

Table 7. Spearman Rank Order Correlations Between Each Compartment and Sex[a]

	Plasma Female	RBC Male	RBC Female	Brain Male	Brain Female
Male Plasma	0.80 p < 0.0001	0.56 p=0.007	0.71 p=0.0004	0.73 p=0.0002	0.64 p=0.002
Female Plasma		0.39 p=0.08	0.55 p=0.01	0.61 p=0.004	0.65 p=0.002
RBC Males			0.83 p < 0.0001	0.76 p < 0.0001	0.69 p=0.001
RBC Females				0.95 p < 0.0001	0.97 p < 0.0001
Brain Males					0.95 p < 0.0001

[a]The rank order of each compartment and sex were compared. The Spearman rank order correlations and p-values for each comparison are given.

E. Relative Potency Rankings for Dermal Exposure

1. Study Selection for Dermal Studies

Only one dermal study was available per chemical. Three studies used rats and three used rabbits. Six of seven dermal studies were of three weeks duration. One dermal study was of six days exposure only.

2. Establishment of Uniform Measure of Toxicity for Dermal Studies

Chemicals were not ranked on the basis of brain or RBC ChEI. Brain or RBC ChEI was of insufficient quality for deriving an ED50 or was not determined in several studies. ED50 values for plasma ChEI were calculated in male rats for two chemicals. An ED50 for plasma ChEI was derived for females for one chemical due to lack of plasma ChEI at highest dose in males. An ED50 for plasma ChEI could not be calculated for three chemicals. NOAEL values for plasma ChEI were reported in each study. Given the lack of a common basis for ranking chemicals by ED50, NOAELs were used to determine relative potency.

ED50 values and NOAELs based on plasma ChEI from the dermal studies are shown in Table 8. An analysis of steady state was not performed because ChEI determination was limited to study termination in all studies. A statistical analysis of slopes was not conducted for dermal exposure due to the limited number of studies.

Table 8. Rank Order of OPs for Plasma ChE Inhibition in Subchronic Dermal Exposure Studies

Chemical	ChE assay time	Slope	Intercept	log ED50	ED50 mg/kg/day	NOAEL[d] mg/kg/day
Chemical 3[a]	week 3					300
Chemical 7[a]	week 3					15
Chemical 4	week 3	2.56	-2.58	2.93	854	5
Chemical 5[a]	week 3					5
Chemical 1	week 3	0.47	4.44	1.19	15.4	< 1[b]
Chemical 2	day 6	1.92	4.00	0.52	3.32	0.4
Chemical 6[c]					NA	NA

[a]An ED50 could not be calculated because of insufficient data points
[b]Plasma ChE inhibition occurred at the lowest dose in the study
[c]A dermal exposure study was not available
[d]chemicals listed by rank order (least to most potent) based on plasma ChEI NOAEL
Note: Chemical codes do not correspond to codes used in oral and inhalation studies.

F. Relative Potency Rankings for Inhalation Exposure

1. Study Selection for Inhalation Studies

Only one inhalation study was available for each OP with residential uses. Three studies were whole-body inhalation exposures and three studies were nose-only exposures. Whole-body exposure studies may expose the animal to the chemical by the oral route as well as by inhalation.

ChE determinations were made after three to four weeks exposure for two chemicals, at 38 and 90 days for one chemical, after 90 days of exposure to two chemicals, and after two years exposure to one chemical. Exposure was for six hours per day for six of seven chemicals. The remaining study was for 23 hours per day.

2. Establishment of Uniform Measure of Toxicity for Inhalation Studies

ED50 values for plasma ChEI in males were determined for five of seven chemicals. An ED50 could not be determined for one chemical because plasma ChEI only occurred in the high dose group. Compounds were not ranked on the basis of brain or RBC ChEI. Brain and RBC ChEI were either insufficient for deriving an ED50 or were not determined in several studies.

An ED50 value for plasma ChEI could not be calculated for one chemical. NOAEL values for plasma ChEI were reported in the studies. Given the lack of a common basis for ranking chemicals by ED50, comparison of NOAELs was used to determine relative potency.

ED50 values, NOAELs, and LOAELs based on plasma ChEI are shown in Table 9. An analysis of steady state was not performed since endpoint selection was limited to the time of ChE determination. A statistical analysis of slopes was not conducted for inhalation exposure because the available studies were conducted under different conditions.

Table 9. Rank Order of OPs for Plasma ChE Inhibition in Inhalation Exposure Studies

Chemical	ChE Assay Time	Slope	Intercept	Log ED50pl	ED50 mg/kg/day	NOAEL[b] mg/kg/day
Chemical a	week 13	0.87	3.82	1.35	5,709	115
Chemical g	day 29	0.95	5.52	-0.55	78.3	0.3
Chemical c	week 13	0.80	5.05	-0.056	0.9	0.1
Chemical e	year 2	1.05	4.58	0.40	2.3	0.05
Chemical d[a]	day 38					0.04
Chemical f	day 21	0.48	4.94	0.12	0.3	< 0.026
Chemical b					NA	NA

[a]An ED50 could not be calculated because of insufficient data points
[b]Chemicals listed by rank order (least to most potent) based on plasma ChEI NOAEL
Note: Chemical codes do not correspond to codes used in oral and dermal studies.

IV. Summary of Pilot Results

A major objective in the hazard and dose-response components of cumulative risk assessment is to select a toxicity endpoint(s) pertaining to the common mechanism of toxicity and a level of response for each chemical member so that relative potency can be established. This pilot analysis examined 24 OPs which operate via the common mechanism of acetylcholinesterase inhibition in the nervous system.

This pilot analysis illustrated that it was possible to attain a reasonable uniform measure of ChEI for most of the 24 OPs in all three compartments (plasma, RBC, and brain) from rat oral studies using ED50s as a point of comparison. However, for the inhalation and dermal routes of exposure, the dose-response information was more limited (only one study available per chemical) and of a lesser quality. Thus, NOAELS had to be considered for determination of relative potency.

An integrative analysis across the three compartments and between the two sexes revealed that the RBC and brain ChEI values were the most concordant with respect to relative ranking and potency. For brain ChEI, the representative $ED50_{brain}$ values and RPFs were comparable for all but three chemicals between the males and females (3-fold to 7-fold less potent in males). For RBC ChEI, male and female rats exhibited very comparable $ED50_{rbc}$ and RPFs to OPs in the group except one chemical whose male mean ED50 was 40-fold less potent. Differences in relative ranking were mostly found for the plasma ChEI values for both sexes compared to RBC and brain ChEI values. For example, potential sex differences up to 38-fold for representative ED50pl were observed for one-fourth of the chemicals based on plasma ChEI.

The assumption of parallel dose-response curves is a major principle in cumulative assessment. Evidence of parallel dose-response slopes was found for male plasma and brain ChEI (i.e., Statistical analysis indicated that the slopes were not statistically different). There was moderate evidence for parallel dose-response curves for male RBC ChEI and female plasma ChEI data. Weaker evidence for parallel dose-response curves was observed for female RBC and female brain ChEI data.

In the relative potency factor approach, an index compound must be selected. In this pilot analysis, Chemical T was considered as the index chemical because it was the only chemical with dietary and residential exposure which had a

complete database. Oral (all compartments, both sexes), dermal, and inhalation studies were available for Chemical T. Chemical T was less potent in RBC and brain ChEI compared to plasma ChEI. In oral studies, Chemical T ranked as one of the most potent chemicals based on plasma ChEI. The index chemical ranked as the most potent chemical for inhalation exposure based on plasma ChEI and was intermediate in potency by the dermal route based on NOAEL for plasma ChEI.

In summary, when selecting an index chemical for OPs, it will be important to select one that has high quality data and stable relative potency ranking among compartments and sexes endpoint(s). When selecting an endpoint(s) for the chemical group as the basis for a cumulative risk assessment, it will be important to select one with evidence or support of parallel dose-response relationships. Based on this criteria, there are pro's and con's for each compartment and sex considered.

V. Issues for Cumulative Hazard and Dose-Response Analysis

The purpose of this pilot cumulative hazard and dose-response analysis is to examine the methods described in the proposed cumulative guidance (EPA, 2000a). The methods for RPF calculation and cumulative hazard characterization need to be sufficiently rigorous for objective and consistent assessments, but also flexible enough to handle diverse mechanisms of toxicity and datasets. The presentation of methods in this pilot analysis is not meant to be a presentation of the final methods that may be used to calculate RPFs or to determine parallel dose-response curves. This pilot was designed as a part of the evolution of cumulative risk assessment guidance and procedures. Over the course of this pilot, generic issues were encountered that could effect any cumulative assessment. These issues are presented below, as well as alternatives in dealing with them.

A. Determination of Relative Potency Based on a Uniform Measure of Toxicity

A key objective in cumulative risk assessment is the determination of relative toxic potency for each chemical in the CMG. It is important that each chemical's relative potency be based on a uniform measure of the common response. The June 2000 draft guidance document indicates the relative potency preferably should be based on data for the same study design, effect, species, and sex. There may be several biological or toxicological responses associated with the common mechanism of toxicity among a group of chemicals. These responses may range from a common effect measured in different biological compartments (e.g., ChEI in plasma, RBC, and brain) to a cascading of events associated with a mechanism of toxicity (e.g., hormonal changes that lead to organ weight and pathological changes). The availability and quality of data may vary among endpoints for the exposure routes of interest. Different relative potency rankings between endpoints, species, and sexes also may be observed. Thus, it will be necessary to determine which endpoints are the most appropriate to consider in the determination of relative potency.

1. Dealing with Lack of Appropriate Data

As encountered in this pilot, there will be situations for some chemicals in which data were missing or of poor quality for the critical measurements in certain tissues, species, sex , or a certain route of exposure. If critical data were lacking to provide a uniform basis for determination of relative potency (e.g., ED50s), there are several alternative approaches that could be considered:

- **Use Surrogate Data.** The use of surrogate data in cumulative risk assessment is a reasonable approach given that the chemicals of interest are linked by a common mechanism of toxicity. Thus, a weight-of-the-evidence approach could be taken to derive an RPF for a chemical that lacks critical data. This weight of the evidence approach would consider all available endpoint and metabolism data related to the common mechanism on all members of the Cumulative assessment group, as well as structure-activity relationships (SAR). Based on this analysis, an ED would be derived from data from a surrogate chemical(s).

  This weight of the evidence approach could also be taken where some data were available on a chemical but not for the sex or species being used to derive relative potencies. For example, male derived data were the basis of the uniform measure of response but were lacking for Chemical Z. Available female data could be used for Chemical Z (and adjusted if necessary based on the differential sex response of structurally related chemicals).

- **Use NOAELs.** Rather than defaulting to the use of NOAELs for all chemical members, if poor dose-response data were found for a chemical or a few members, their NOAEL could be substituted for their PoC. A limitation of using NOAELs with EDs is the exaggeration of relative potency compared to calculation of ED levels (e.g., ED50s).

2. Methods used for Derivation of EDs

In this pilot, the relative potency of each OP was calculated based on one compartment measured at one timepoint from one study in one sex. ED50s were selected to represent a common PoC and were derived using a log-probit analysis of dose-response data.

There are different mathematical approaches that could be used to derive a common PoC (e.g., the curve fitting models described in software used for benchmark dose analysis). The probit analysis presented within this paper was based on mean ChEI values rather than individual animal data. Although the use of individual animal data are preferred over mean values, mean values were used here for illustrating the major issues encountered in cumulative assessment.

Another option to strengthen the dose-response analysis for derivation of EDs is to compile data from different studies. Data could be compiled in several different ways, for example: (1) across timepoints within a single study using a single compartment; (2) among different studies at a common timepoint or across timepoints for one compartment or measurement type; (3) among compartments. Because more data points would be available by compiling data from different studies, dose-response curves and calculated ED50's would be less variable and potentially more representative. Criteria would need to be established for the compilation of data.

3. Selection of Index Compound

It is important to have a single index compound for the RPF approach. An ideal index compound would be the chemical member of the group that is best studied and has the largest acceptable database, including good dose response data for all the health endpoints and routes of interest. It should have a toxicological profile pertaining to the common mechanism of toxicity consistent with other chemical members. The index chemical, Chemical T, was selected from the subset of chemicals with residential exposure. This index chemical was selected on the basis of its complete database for oral exposure (all compartments, both sexes) as well as well-characterized dose-response relationships in the oral, dermal, and inhalation studies. The index chemical may not represent an ideal index chemical because there are major differences in sensitivity between plasma and RBC ChEI with this chemical. It should be noted that the RBC and Brain ChEI responses were similar for this chemical.

Of the six potential candidates for an index chemical, none of these chemicals met all of the criteria for selecting an ideal index compound. These other candidates exhibited similar responses for plasma and RBC ChEI, but lacked good dose-response data for the three routes of interest. From this analysis, the judgement of which index compound to select involves tradeoff among the various criteria that must be considered.

B. Analysis of Dose-response Curves

1. Assumption of Parallel Dose-Response Curves

A statistical analysis of parallel slopes was performed in this pilot to evaluate overall variability and detect outliers in the data. Because mean values were used to generate the dose-response relationships, the linear regressions contained only two to four data points. As discussed above the utilization of individual animal data and/or the compilation of data from multiple timepoints and studies would improve the quality of the dose-response curves, and therefore analysis of parallel slopes. Improved dose-response curves would allow a more rigorous and quantitative analysis, particularly the comparison of EDs with the distance between intercepts. Even with the limitations of this analysis, the results obtained in this pilot indicate that the oral toxicity data are adequate for dose-response analysis. The null-hypothesis (i.e., the slopes were not different) cannot prove parallelism, but the statistical test for parallel lines performed in this pilot does provide some support for the assumption of proportionality of dose-response.

In this pilot analysis, there was weak evidence for parallel dose-response curves for certain chemicals. This was due to observed steep dose responses compared to the other chemicals. Some of the chemicals produced pronounced differential responses between the two sexes or among compartments. Assuming these slopes were true outliers, this situation raises the issue of pharmacokinetic differences based on sex and compartment for specific chemicals. Therefore, an important issue is the inclusion of these outliers in the cumulative assessment.

VII. Charge and Questions for the Panel

Based on a review of the pilot analysis on 24 OPs contained within this paper, and given the issues discussed in Section V of this paper, OPP seeks comment and advice from the SAP on the following questions:

Question 1 For most compartment and sex groupings, there were one or more chemicals for which multiple studies could be used to calculate an ED 50. (See Figures 2, 3, 5, 6, 8, and 9.) Please comment on the criteria OPP used to select the "representative study" from among the available studies available for a specific chemical to calculate the ED50 for that chemical, compartment, and sex.

Question 2 There will be situations for some chemicals in which data are lacking for the critical measurements, in a certain species or sex, or for a certain route of exposure. The lack of data may be because critical measurement(s) simply were not measured or because data are considered to be of poor quality.

Q2.1 We would like the panel's view on the use of surrogate data as a substitute for the lack of appropriate data. To what extent should surrogate information be used to determine a chemical's relative potency?

Q2.2 How should situations be handled where an ED_{50} can be determined for many of the chemical members but cannot be determined for a few members? We would like the Panel's view on the use of NOAELs as substitutions for ED_{50}s for points of comparison.

Question 3 We would like the Panel's view on the relative importance of the factors discussed in the paper for selecting an index compound.

Question 4 We would like the panel to comment on the log dose-probit analysis used to extrapolate the ED_{50}s for the chemicals evaluated in this pilot.

Question 5 For this group of chemicals, OPP has sufficient data to calculate relative potency factors by the oral route for six different compartment/sex groups. Relative potency factors could be calculated by use of a single compartment/sex or by compiling data across compartment/sex groups.

Q5.1 If the Panel favors a single compartment/sex, please comment on the criteria that should guide the choice of a compartment/sex group.

Q5.2 It is proposed in this pilot analysis, that data could be compiled across different studies to provide more confidence in the determination of relative potency. In establishing an effective dose (e.g., ED_{50}), to what extent should one compile data for each chemical of interest within or across different measures and/or studies? What are important criteria to consider when compiling data?

Question 6 Dose addition is considered an appropriate default approach to cumulative risk assessment. The mathematical definition of dose-addition requires a constant proportionality between the effectiveness of the chemicals being considered. It is anticipated that extensive dose-response data will not be available for many chemicals. Please comment on the approach taken to evaluate parallel dose-response curves. Please comment on how rigorous an analysis is needed to evaluate the assumption of parallel dose-response curves.

Question 7 How does one handle a response for a chemical that displays a different slope (i.e. an outlier)? Examples were demonstrated in this pilot analysis where one or a few chemicals of the common mechanism group exhibited pronounced species, sex, or compartment differences from the majority of chemicals.

REFERENCES

Casida, J.E., Baron, R.L., Eto, M., and Engel, J.L. (1963). Potentiation and Neurotoxicity Induced by Certain Organophosphates. *Biochem. Pharmacol.,* **12:**73-83.

Cohen, S.D. (1984). Mechanisms of Toxicological Interactions Involving Organophosphate Insecticides. *Fundam. Appl. Toxicol.,* **4:**315-324.

Cohen, S.D., Callaghan, J.E., and Murphy, S.D. (1972). Investigation of Multiple Mechanisms for Potentiation of Malaoxon's Anticholinesterase Action by Triorthotolyl Phosphate. *Proc. Soc. Exp. Biol. Med.,* **141:**906-910.

Cook, J.W., Blake, J.R., and Williams, M.W. (1957). The Enzymatic Hydrolysis of Malathion and its Inhibition by EPN and Other Organic Phosphorus Compounds. *J. Assoc. of Official Agricultural Chemists.* **40:**664-665.

DuBois, K.P. (1961). Potentiation of the Toxicity of Organophosphorus Compounds. *Adv. Pest Control Res. ,* **4:**117-151.

EPA, 1999a. "Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity;" January 29, 1999. (64 FR 5796)

EPA, 2000a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity;" draft document. June 22, 2000. (65 FR 40644)

Frawley, J.P., Fuyat, H.N., Hagan, E.C., Blake, J.R., and Fitzhugh, O.G. (1957). Marked Potentiation in Mammalian Toxicity From Simultaneous Administration of Two Anticholinesterase Compounds. *J. Pharmacol Exp. Therap.* **121:**96-106.

Mei-Quey, S., Kinoshita, F.K., Frawley, J.P., and DuBois, K.P. (1971). Comparative Inhibition of Aliesterases and Cholinesterase in Rats Fed Eighteen Organophosphorus Insecticides. *Toxicol. Appl. Pharmacol.,* **20:**241-249.

Moser VC, Chanda SM, Mortensen SR, and S. Padilla 1998. Age- and Gender-Related Differences in Sensitivity to Chlorpyrifos in the Rat Reflect Developmental Profiles of Esterases Activities. Toxicological Sciences 46:211-222.

Seume, F.W., Casida, J.E., and O'Brien, R.D. (1960). Effects of Parathion and Malathion Separately and Jointly Upon Rat Esterases in Vivo. *J. Agric. Food Chem.,* **8:**43-47.

Jokanovic M, Kosanovic M, and M. Maksimovic. 1996. Interaction of Organophosphorus Compounds with Carboxylestesrases in the Rat. Arch. Toxicol 70:444-450.

Walker CH 1993. The Classification of Esterases which Hydrolyze Organophosphates: Recent Developments. Chem-Biol. Interact. 87:17-24.