III. Appendices

B. Hazard/RPF

3. Response to SAP Comments from September 2000 Report

OPP in collaboration with ORD presented its July 31st , 2001 document entitled, "Determination of Relative Potency and Points of Departure for Cholinesterase Inhibition" to the FIFRA SAP on September 5-6, 2001. The key recommendations from the September 2001 report (http://www.epa.gov/scipoly/sap/index.htm) and OPP's responses are given below:

a. Derivation of the Adjustment Factor "B" and Modification of Decision Tree for use of "B"

The SAP Report noted that a plot of the "scaled residuals" against "predicted % inhibition" indicates that the weighting strategy used for calculating the adjustment factor "B" does not adequately reflect how the variance changes with response. The SAP was specifically concerned EPA "focused the modeling effort on achieving fidelity with observations at the high end of the range of doses tested, to the likely detriment of fitting points at the low end of the dose response relationship."

In the current analysis, all available cholinesterase datasets for the brain compartment were analyzed using a fixed horizontal y-asymptote for each chemical. The weight function was changed from one in which the variance was presumed proportional to the square of the mean to one in which the variance is proportional to the mean. The revised methodology for the determination of the horizontal y- asymptote is described in I.B and III.B.1.

b. Conduct a Formal Analysis of Residuals as a Function of Dose

Residual plots for the basic and expanded models for each chemical for the brain compartment are given in III.B.2.

c. Accuracy of the "Chi Square Approximation" for the "Goodness of Fit" Statistic

In the July 31st document, a Chi-Square Approximation was calculated for each cholinesterase dataset. This statistic was used as a measure of the goodness-of-fit for the exponential function. The concern expressed by the SAP does not apply to the current methodology. Although the OPCumRisk program was not used to determine potency of OPs in the current analysis, the program was revised to deliver a warning message to the program user indicating possible calculation inaccuracy for this statistic. The revised version of the OPCumRisk is available for download at http://www.epa.gov/scipoly/sap/index.htm and http://www.epa.gov/pesticides/cumulative/

d. Confidence Interval Calculations

The SAP report suggested that HED "reconsider the confidence interval calculations"and "perhaps try bootstrapping or some other more robust method . . . ." In the current analysis, HED has revised the calculation of the confidence intervals (See III.B.1). Bootstrapping is a very time and resource-intensive procedure. Although bootstrapping may be the preferred approach for calculating confidence intervals, due to limited availability of resources, the Agency has not conducted any bootstrapping procedures. At this time, the current method for calculating confidence intervals is adequate and satisfactory. Because it is important to evaluate the range of uncertainty around any potency or benchmark dose values used to extrapolate to human risk, the Agency will consider bootstrapping procedures in future assessments.

e. Deleting p- and t- values

The SAP Report recommended deleting the p- and t- values that are produced by the Agency's OPCumRisk program. As stated previously, the OPCumRisk program was not used in the current analysis to calculate potency or benchmark dose estimates. The requested deletions have been incorporated; the revised version of the OPCumRisk is available for download at http://www.epa.gov/scipoly/sap/index.htm and http://www.epa.gov/pesticides/cumulative/

f. Estimates of Relative Potency

The SAP Report included considerable discussion regarding whether relative potency factors should be based on ratios of the “Benchmark Dose 10's” (BMD_{10}) or on ratios of the dose-scaling factors. OPP has derived potency in the present analysis on BMD_{10} (See I.B).

g. Inhalation Dose

The SAP Report recommended that inhalation exposure be expressed in the same units as the oral doses and that the doses be adjusted for actual treatment durations. HED has calculated the inhalation doses as mg/kg/day using conversion factors that account for respiratory volume and body weight for the strain of rat used, as well as the duration of exposure in terms of hours exposed per day.

h. Use of Individual Animal Data

The SAP Report from the September 2000 SAP meeting recommended that study data on individual animals be used in calculating relative potencies. Due to the fact that all the data on organophosphates are not in an electronic format, HED has not taken this step. However, the September, 2001 Report recognizes that "individual data would not be likely to change the results using current methods." In addition, by switching from RBC to the brain compartment, some of the concern about not using individual animal data should be reduced, since the experimental designs for the brain measurements do not include a repeated measures component, unlike the RBC data.

i. Use of NOAEL's and LOAEL's For Inhalation and Dermal Routes

Several Panel members objected to EPA's use of No Observed Adverse Effect Levels ("NOAEL's") and Lowest Observed Adverse Effect Levels ("LOAEL's") for cholinesterase inhibition data by the dermal and inhalation routes of exposure instead of actual dose-response models as are used for the oral data set. HED does not intend to use dose-response modeling to determine relative potency estimates for dermal and inhalation exposure because the data are not sufficiently robust to justify the resources required.

However, it is to be noted that the current analysis uses Comparative Effect Levels (CEL's) for cholinesterase inhibition data for these two routes of exposure. The dermal and inhalation database was not suitable for dose-response analysis. Cholinesterase determinations in these studies were typically made at only one time point and several of the studies had no cholinesterase inhibition at the highest dose. For the current assessment, potencies by the dermal and inhalation routes were compared using brain cholinesterase inhibition at a dose causing a maximum of 15% brain cholinesterase inhibition.

j. Derivation of Doses from the Actual Dietary Intake Rates

The SAP Report recommends that "the doses used for evaluation of potencies at various ages within specific data sets should be derived from the actual dietary intake rates observed in the study for those ages where the consumption data are available."

In feeding toxicity studies, laboratory rats are exposed to the test compound via the diet. Generally, the test compound is mixed in the animal feed which the laboratory animals eat. Over the course of a toxicity study, as the animals age, they will not only gain weight and but they will naturally change their rate of food consumption. The data collected for the oral route and used in both the July and December 2001 preliminary cumulative risk assessments include average compound intake (mg of active ingredient per kg per day). HED has conducted a pilot analysis in response to this recommendation to evaluate the effect of age and food consumption rate on the potency estimates. In this pilot compound intake analysis, OP potency was determined for a subset of studies [≈10% of total studies in the dose-response assessment] using compound intake measured at or around the time of cholinesterase measurements [duration-specific compound intake].

Seventy-nine oral toxicity studies were included in the dose-response assessment for the December, 2001 Cumulative Risk Assessment for OPs. Of these 79 studies, the test article was administered via the diet for 73. For each of the seven OPs selected for this analysis, the calculated compound intake (mg/kg/day) given in the study report for a weekly, biweekly, or monthly time interval closest to the time of cholinesterase measurement was extracted from the feeding toxicity studies [duration-specific compound intakes]. For example, if brain cholinesterase was measured at a one-year interim sacrifice, the compound intake for the 50-52 week reported interval was collected. The potency values obtained were compared to those in the July, 2001 analysis, which utilized average compound intake values. Potency estimates given below (Table III.B.3-4) were calculated using the OPCumRisk program with the methodology described in the July 31st document *prior* to the completion of the current methodology for the joint analysis. The pilot analysis was performed in three stages : 1) impact of age on relative potency for chronic studies only; 2) impact of age on relative potency for complete database of subchronic and chronic studies; and 3) impact of age on the points of departure on the index chemical.

Stage 1: The purpose of this pilot analysis was to investigate the impact of age on food consumption and body weight, and ultimately OP potency. In order to maximize the age-related differences in body weight and food consumption, chronic studies were analyzed first. Seven chronic feeding studies were selected randomly and analyzed as described above. Relative potency of each was calculated using the methamidophos chronic study. Results given in Table III.B.3-1.

In the chronic study analysis (Table III.B.3-1) comparing the RPFs calculated using the slope scale factor (m) and also the BMD_{10}s for ChE data using the average and duration-specific compound intakes, *the RBC and brain data for both sexes display comparable potency values*. For tribufos a 5-fold difference between the average and duration-specific intake assessments for male brain CHeI was observed. This difference is an artifact of the decision tree for the determination B (horizontal asymptote) and not from differences in potency between the average and duration specific intakes. Two timepoints (364 and 721 days) are available for the male brain ChE data in MRID 42335101. In the duration specific analysis, the 364 day time point did not converge and was therefore not included in the potency estimates.

Table III.B.3-1a. Results of Dietary Intake Comparison [actual *vs* average] Using Chronic Studies

CHEMICAL	MRID	COMPARTMENT	SEX	Dietary Intake Calculation	Relative Potency using 'm'	Lower 95% CL	Upper 95% CL	BMD_{10}	BMDL	Relative Potency using BMD_{10}
BENSULIDE	44161101	BRAIN	F	average	0.005	0.004	0.006	14.11	12.40	0.005
				biweekly	0.004	0.004	0.005	14.04	12.17	0.004
DIAZINON	41942002	BRAIN	F	average	0.034	0.031	0.038	1.85	1.78	0.038
				biweekly	0.031	0.028	0.035	1.85	1.80	0.034
DICROTOPHOS	44527802	BRAIN	F	average	1.77	1.41	2.22	0.041	0.035	1.74
				biweekly	1.89	1.51	2.38	0.035	0.030	1.79
METHAMIDOPHOS	00148452	BRAIN	F	average	1.00	1.00	1.00	0.071	0.063	1.00
				biweekly	1.00	1.00	1.00	0.063	0.058	1.00
PHOSALONE	44801002	BRAIN	F	average	0.015	0.013	0.018	4.13	3.70	0.017
				biweekly	0.024	0.020	0.029	2.40	2.14	0.026
PHOSMET	41916401	BRAIN	F	average	0.023	0.010	0.053	4.41	3.74	0.016
				biweekly	0.021	0.016	0.027	2.76	2.33	0.023
TRIBUFOS	42335101	BRAIN	F	average	0.018	0.007	0.048	3.26	1.88	0.022
				biweekly	0.017	0.007	0.045	3.14	1.83	0.020
BENSULIDE	44161101	BRAIN	M	average	0.002	0.002	0.003	24.69	19.37	0.003
				biweekly	0.002	0.001	0.003	24.93	19.54	0.002
DIAZINON	41942002	BRAIN	M	average	0.011	0.003	0.041	3.38	1.83	0.018
				biweekly	0.011	0.003	0.035	3.31	1.83	0.016
DICROTOPHOS	44527802	BRAIN	M	average	2.06	1.70	2.38	0.028	0.026	2.23
				biweekly	2.32	2.03	2.67	0.022	0.020	2.45
METHAMIDOPHOS	00148452	BRAIN	M	average	1.00	1.00	1.00	0.062	0.057	1.00
				biweekly	1.00	1.00	1.00	0.055	0.049	1.00
PHOSALONE	44801002	BRAIN	M	average	0.021	0.018	0.025	2.58	2.37	0.024
				biweekly	0.038	0.033	0.044	1.29	1.18	0.042
PHOSMET	41916401	BRAIN	M	average	0.011	0.008	0.015	5.35	4.33	0.012
				biweekly	0.013	0.009	0.018	3.71	2.98	0.015
TRIBUFOS	42335101	BRAIN	M	average	0.020	0.017	0.022	4.22	2.51	0.015
				biweekly	0.004	0.001	0.020	15.64	6.19	0.003

Table III.B.3-1b. Results of Dietary Intake Comparison [actual *vs* average] Using Chronic Studies

CHEMICAL	MRID	COMPARTMENT	SEX	Dietary Intake Calculation	Relative Potency using 'm'	Lower 95% CL	Upper 95% CL	BMD_{10}	BMDL	Relative Potency using BMD_{10}
BENSULIDE	44161101	RBC	F	average	0.012	0.005	0.025	5.53	3.69	0.012
				biweekly	0.011	0.005	0.024	5.35	3.55	0.012
DIAZINON	41942002	RBC	F	average	0.12	0.037	0.38	0.28	0.17	0.24
				biweekly	0.11	0.036	0.33	0.29	0.18	0.21
DICROTOPHOS	44527802	RBC	F	average	2.77	1.88	4.08	0.039	0.030	1.71
				biweekly	2.89	1.95	4.29	0.035	0.027	1.78
METHAMIDOPHOS	00148452	RBC	F	average	1.00	1.00	1.00	0.067	0.063	1.00
				biweekly	1.00	1.00	1.00	0.062	0.058	1.00
PHOSALONE	44801002	RBC	F	average	0.068	0.027	0.17	0.71	0.48	0.094
				biweekly	0.076	0.035	0.17	0.64	0.44	0.097
PHOSMET	41916401	RBC	F	average	0.080	0.058	0.11	0.84	0.75	0.080
				biweekly	0.083	0.065	0.11	0.70	0.57	0.089
TRIBUFOS	42335101	RBC	F	average	0.095	0.048	0.19	0.61	0.48	0.11
				biweekly	0.089	0.045	0.18	0.60	0.46	0.10
BENSULIDE	44161101	RBC	M	average	0.013	0.006	0.026	7.56	6.34	0.008
				biweekly	0.013	0.006	0.027	7.55	6.33	0.007
DIAZINON	41942002	RBC	M	average	0.040	0.013	0.13	2.36	1.92	0.025
				biweekly	0.042	0.013	0.13	2.09	1.57	0.025
DICROTOPHOS	44527802	RBC	M	average	1.33	1.10	1.61	0.039	0.035	1.51
				biweekly	1.55	1.26	1.91	0.033	0.030	1.60
METHAMIDOPHOS	00148452	RBC	M	average	1.00	1.00	1.00	0.059	0.056	1.00
				biweekly	1.00	1.00	1.00	0.053	0.047	1.00
PHOSALONE	44801002	RBC	M	average	0.053	0.021	0.13	0.96	0.56	0.062
				biweekly	0.067	0.032	0.14	1.49	1.31	0.035
PHOSMET	41916401	RBC	M	average	0.079	0.055	0.11	0.81	0.72	0.073
				biweekly	0.10	0.077	0.14	0.58	0.53	0.091
TRIBUFOS	42335101	RBC	M	average	0.14	0.090	0.21	0.49	0.40	0.12
				biweekly	0.10	0.050	0.21	0.57	0.42	0.094

Stage 2: Out of the seven OPs analyzed in Stage 1, the entire oral databases; i.e., both chronic and subchronic studies, of three randomly selected OPs were analyzed as in Stage 1. Relative potency was calculated using all available methamidophos studies (Table III.B.3-2).

In the pilot analysis of the complete oral database for three OPs (diazinon, dimethoate, and phosalone; Table III.B.3-2) comparing the RPFs calculated with slope scale factors and BMD_{10}s for ChE data using the average and duration-specific compound intakes, *the RBC and brain data for both sexes display comparable potency values*. For phosalone RBC male *only*, a 7-fold difference between the average and duration-specific intake assessments was observed.

Graphs of potency vs. time are shown in Figures III.B.3-1,2 for the analyzes of average chemical intake and for duration specific chemical intake. The patterns observed in the graphs for the average intake analyzes are similar to those of the duration specific intakes.

Table III.B.3-2. Results of Dietary Intake [actual *vs* average] Using All Available Studies

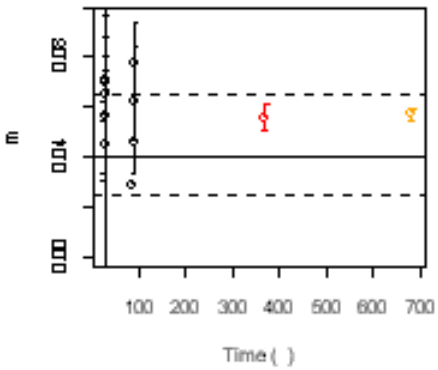
CHEMICAL	MRID	COMPARTMENT	SEX	Dietary Intake Calculation	Relative Potency using 'm'	Lower 95% CL	Upper 95% CL	BMD_{10}	BMDL	Relative Potency using BMD_{10}
DIAZINON	43543901 43543902 40815003 41942002	BRAIN	F	average	0.031	0.018	0.053	2.48	1.78	0.036
				biweekly	0.033	0.019	0.058	2.08	1.51	0.038
DIMETHOATE	43128201 164177	BRAIN	F	average	0.531	0.41	0.69	0.25	0.23	0.36
				biweekly	0.58	0.45	0.75	0.20	0.18	0.40
METHAMIDOPHOS	41867201 00148452 43197901	BRAIN	F	average	1.00	1.00	1.00	0.09	0.08	1.00
				biweekly	1.00	1.00	1.00	0.08	0.07	1.00
PHOSALONE	44852504 44801002	BRAIN	F	average	0.019	0.014	0.025	5.05	3.83	0.018
				biweekly	0.021	0.010	0.040	3.37	2.24	0.024
DIAZINON	43543901 43543902 40815003 41942002	BRAIN	M	average	0.005	0.002	0.012	24.77	24.15	0.003
				biweekly	0.005	0.002	0.010	18.28	17.83	0.004
DIMETHOATE	43128201 164177	BRAIN	M	average	0.71	0.53	0.94	0.10	0.08	0.80
				biweekly	0.83	0.60	1.15	0.08	0.06	0.88
METHAMIDOPHOS	41867201 148452 43197901	BRAIN	M	average	1.00	1.00	1.00	0.08	0.07	1.00
				biweekly	1.00	1.00	1.00	0.07	0.06	1.00
PHOSALONE	44852504 44801002	BRAIN	M	average	0.019	0.011	0.032	3.49	2.49	0.023
				biweekly	0.028	0.012	0.063	1.96	1.22	0.036
DIAZINON	43543901 43543902 40815003 41942002	RBC	F	average	0.38	0.22	0.65	0.24	0.22	0.38
				biweekly	0.41	0.27	0.62	0.18	0.17	0.44
DIMETHOATE	43128201 164177	RBC	F	average	0.32	0.14	0.73	0.29	0.14	0.31
				biweekly	0.27	0.14	0.53	0.33	0.16	0.24

CHEMICAL	MRID	COMPARTMENT	SEX	Dietary Intake Calculation	Relative Potency using 'm'	Lower 95% CL	Upper 95% CL	BMD_{10}	BMDL	Relative Potency using BMD_{10}
METHAMIDOPHOS	41867201 148452 43197901	RBC	F	average	1.00	1.00	1.00	0.09	0.07	1.00
				biweekly	1.00	1.00	1.00	0.08	0.06	1.00
PHOSALONE	44852504 44801002	RBC	F	average	0.044	0.015	0.13	1.45	0.77	0.062
				biweekly	0.048	0.017	0.14	1.31	0.68	0.061
DIAZINON	43543901 43543902 40815003 41942002	RBC	M	average	0.12	0.024	0.63	0.40	0.22	0.18
				biweekly	0.14	0.027	0.68	0.34	0.18	0.18
DIMETHOATE	43128201 164177	RBC	M	average	0.27	0.15	0.48	0.36	0.20	0.19
				biweekly	0.25	0.13	0.47	0.40	0.22	0.15
METHAMIDOPHOS	41867201 148452 43197901	RBC	M	average	1.00	1.00	1.00	0.07	0.05	1.00
				biweekly	1.00	1.00	1.00	0.06	0.05	1.00
PHOSALONE	44852504 44801002	RBC	M	average	0.054	0.022	0.13	18.07	9.81	0.004
				biweekly	0.072	0.032	0.16	2.72	1.40	0.023

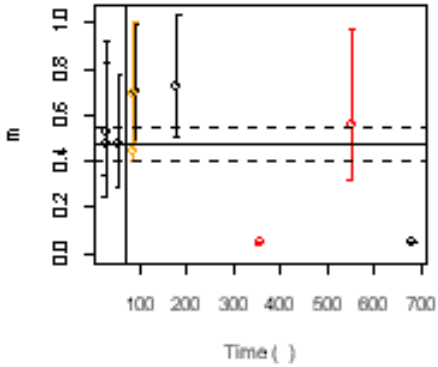
Figure III.B.3-1a. Plots of potency versus time for brain cholinesterase measured in rats exposed to diazinon

Average Dose

Female

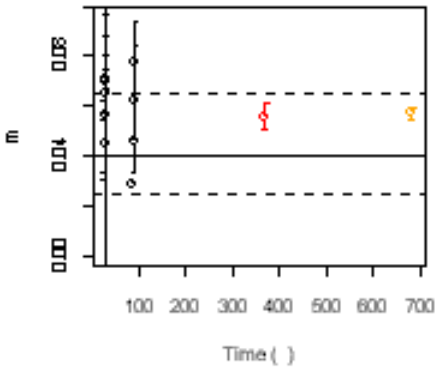


Male



Duration Specific Dose

Female



Male

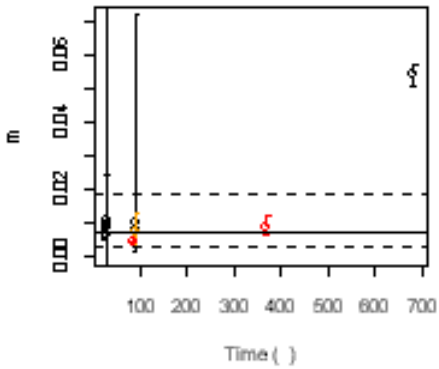
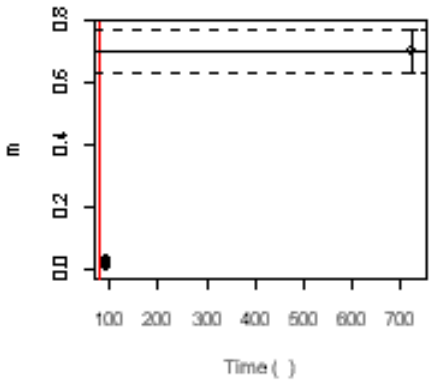


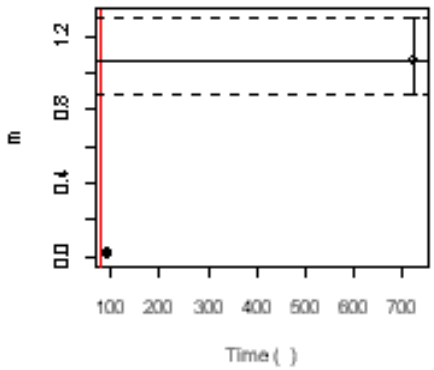
Figure III.B.3-1b. Plots of potency versus time for brain cholinesterase measured in rats exposed to dimethoate

Average Dose

Female

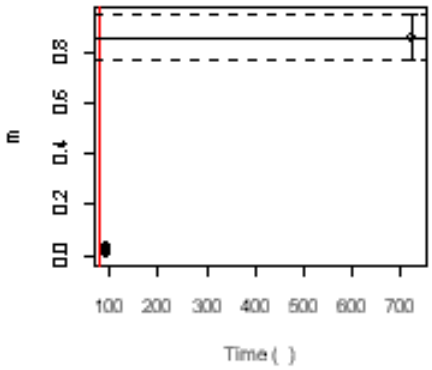


Male



Duration Specific Dose

Female



Male

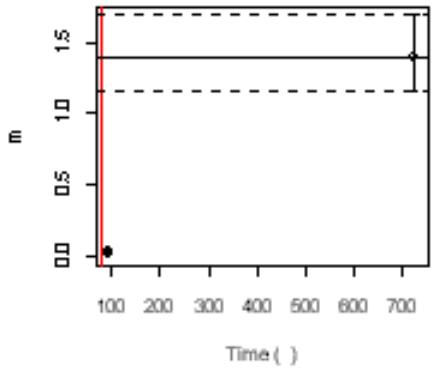
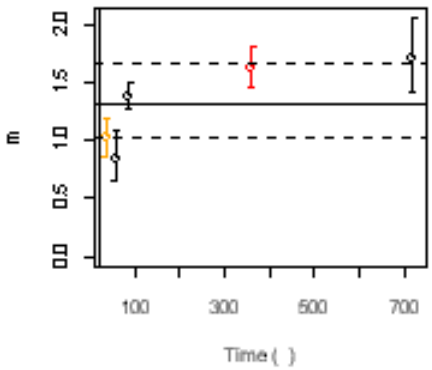


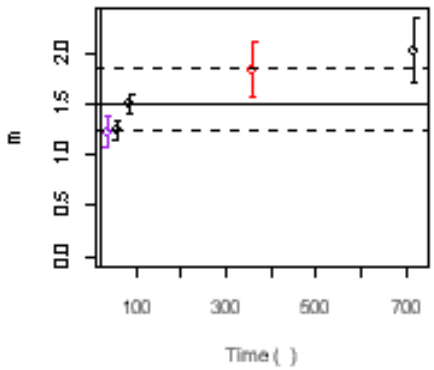
Figure III.B.3-1c. Plots of potency versus time for brain cholinesterase measured in rats exposed to methamidophos

Average Dose

Female

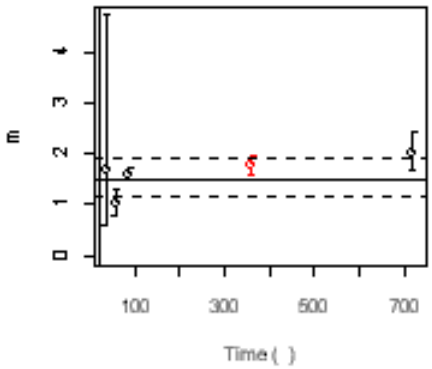


Male



Duration Specific Dose

Female



Male

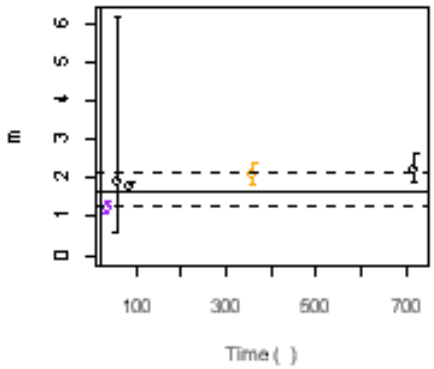
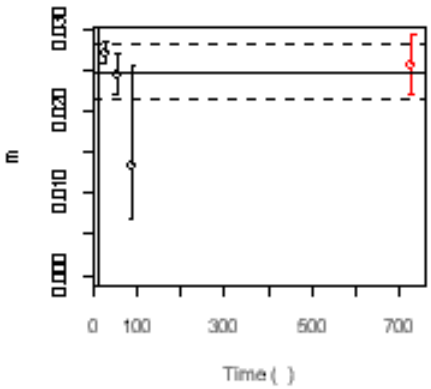


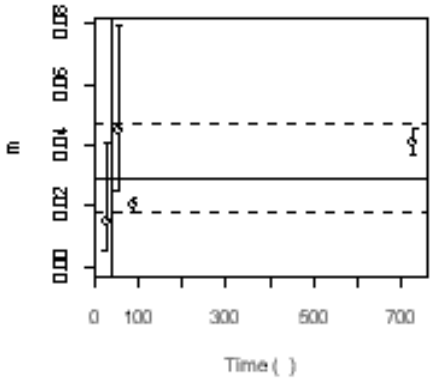
Figure III.B.3-1d. Plots of potency versus time for brain cholinesterase measured in rats exposed to phosalone

Average Dose

Female

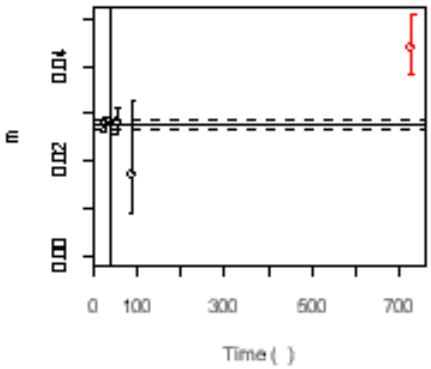


Male



Duration Specific Dose

Female



Male

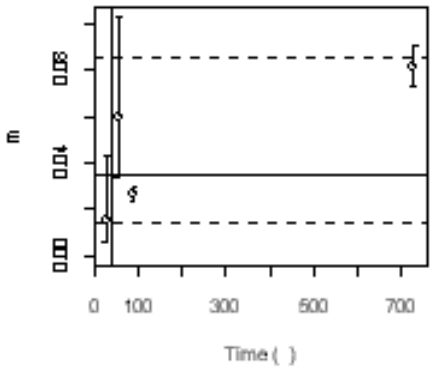
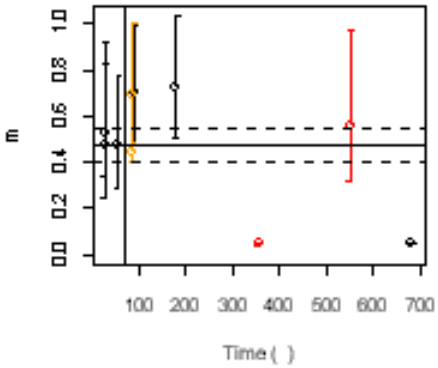


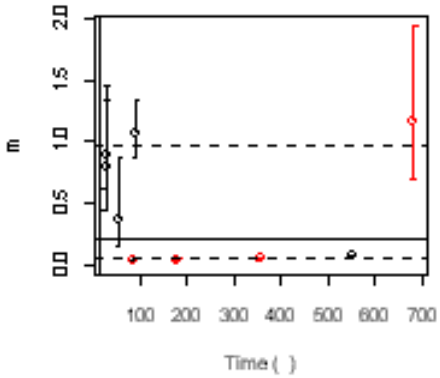
Figure III.B.3-2a. Plots of potency versus time for RBC cholinesterase measured in rats exposed to diazinon

Average Dose

Female

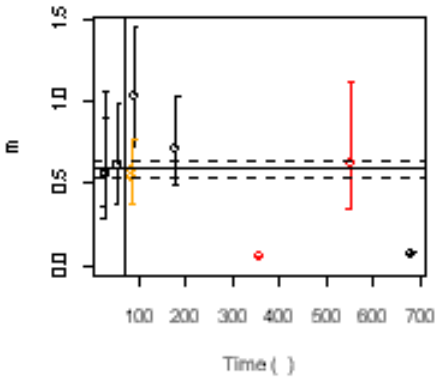


Male



Duration Specific Dose

Female



Male

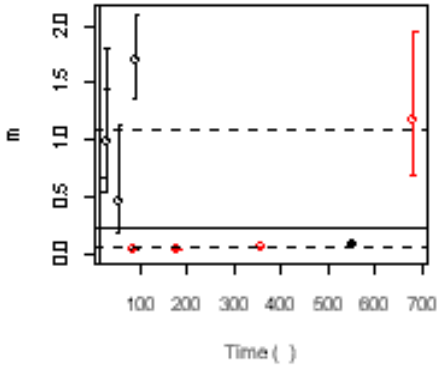
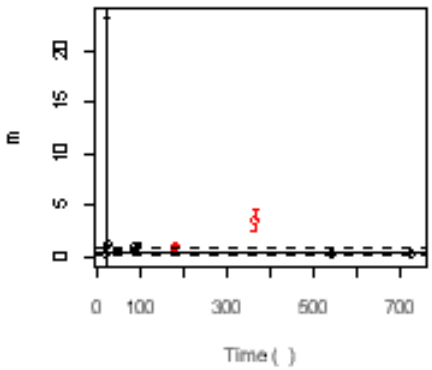


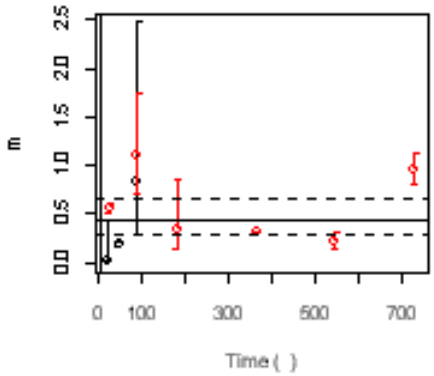
Figure III.B.3-2b. Plots of potency versus time for RBC cholinesterase measured in rats exposed to dimethoate

Average Dose

Female

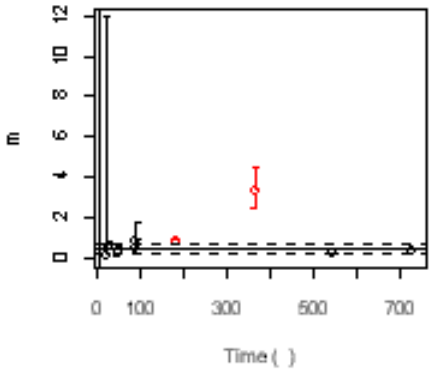


Male



Duration Specific Dose

Female



Male

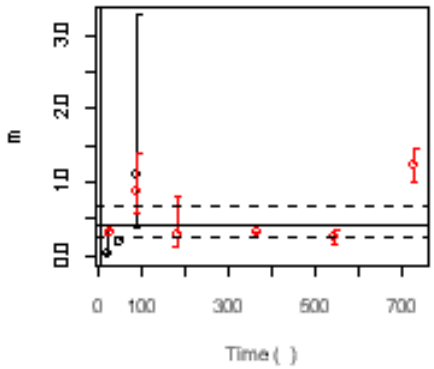
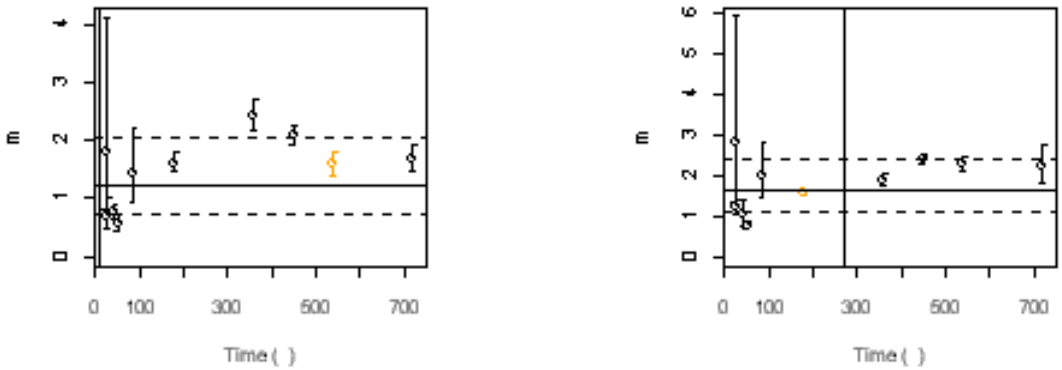


Figure III.B.3-2c. Plots of potency versus time for RBC cholinesterase measured in rats exposed to methamidophos

Average Dose

Female **Male**



Duration Specific Dose

Female **Male**

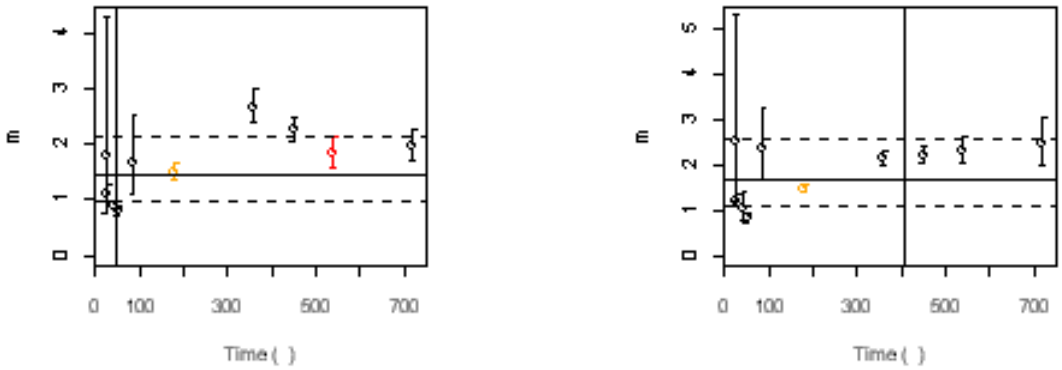
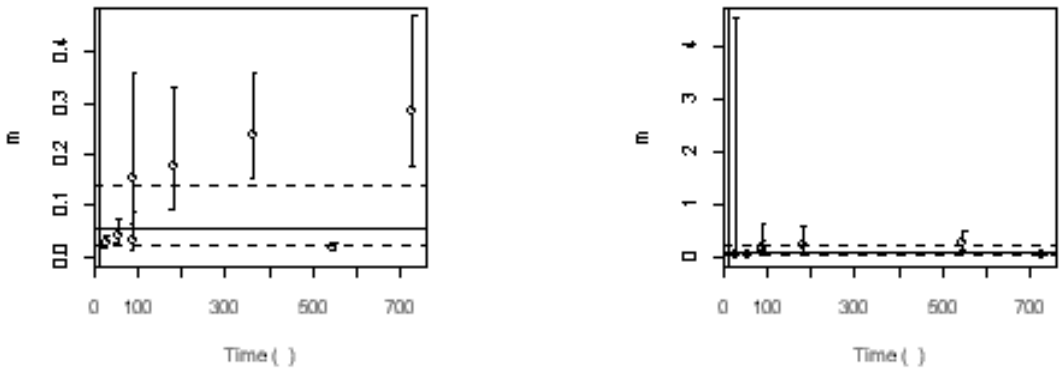


Figure III.B.3-2d. Plots of potency versus time for RBC cholinesterase measured in rats exposed to phosalone

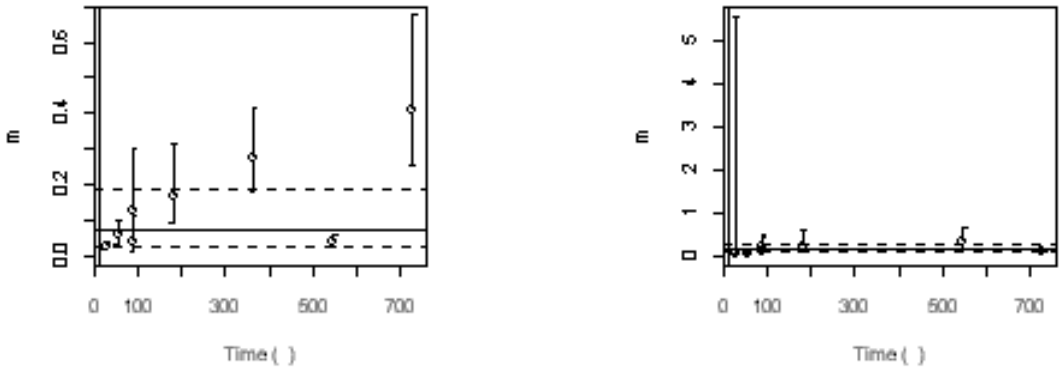
Average Dose

Female **Male**



Duration Specific Dose

Female **Male**



Stage 3: Compare the BMD_{10} 's and BMDL's of the index chemical calculated from the average compound intakes and the duration-specific compound intakes (Table III.B.3-3).

As shown in Table III.B.3-3, BMD_{10} and BMDL calculated using the average compound intake from July analysis are similar to but slightly smaller those calculated with the July methods with duration-specific compound intakes. BMD_{10} and BMDL calculated using the average compound intake from July analysis are similar those calculated with the December methods with duration-specific compound intakes.

Table III.B.3-3. Comparison of Average Intake *vs* Duration-Specific Intake BMD_{10}s and BMDLs

Compartment Sex	JULY				DECEMBER		
	Average Intake		Duration-Specific Intake		Compartment Sex	BMD_{10}	BMDL
	BMD_{10}	BMDL	BMD_{10}	BMDL			
FEMALE RBC	0.09	0.07	0.08	0.06	FEMALE brain	0.08	0.07
FEMALE brain	0.09	0.08	0.08	0.07			
MALE RBC	0.07	0.05	0.06	0.05	MALE brain	0.07	0.06
MALE brain	0.08	0.07	0.07	0.06			

Conclusions: The pilot analysis of compound intakes using duration specific values showed that relative potency estimates calculated from slope-scaling factors and BMD_{10}s are similar to those calculated using the average study compound intake. Based on this analysis, it is reasonable for OPP to continue using the average compound intake for its potency estimates. Concerning the PODs for the index chemical, although the values are very similar, the PODs calculated from duration-specific intake values result in slightly smaller BMD_{10}s.