CHOLINESTERASE DATA IN RISK ASSESSMENT: I. DEFINING TOXICOLOGICAL SIGNIFICANCE

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ABSTRACT

Cholinesterase (ChE) inhibition data have been utilized by regulatory agencies in risk assessments for several decades. ChE activities are most frequently measured in 3 compartments: plasma, red blood cells (RBCs) and brain. Two approaches for defining the toxicological significance of ChE inhibition, and consequently the level considered to be an adverse effect, have been used: (1) a threshold level of inhibition (usually 20%) and (2) statistical significance. One concern with using statistical significance is that treatment-related effects may be masked by large variations in the control population that are related to poorly designed or conducted studies. One tool for selecting a threshold for toxicological significance is the variation in a "normal" population. To determine the normal variation in laboratory animals, the ChE activity in control animals from studies submitted to the Department of Pesticide Regulation (DPR) for pesticide registration were evaluated for 4 types of studies: acute and subchronic neurotoxicity studies in rats, chronic toxicity studies in rats and dogs. Coefficients of variation (CVs) were calculated for each gender and time point and then averaged for each study. The CVs were then averaged for each gender to calculate a grand mean for each study type. The grand means ranged from 15-27%, 11-20% and 5-10% for plasma, RBC and whole brain ChE activity, respectively. It is apparent from this analysis that it is not appropriate to set a single threshold to cover ChE inhibition data from all 3 compartments. These CV ranges may be useful in defining different thresholds for the toxicological significance of plasma, RBC and brain ChE inhibition by organophosphates.

This abstract does not necessarily reflect DPR policy.

INTRODUCTION

BACKGROUND

Two uses of ChE data by regulatory agencies:

- To determine the risk in farm workers for developing neurological effects from organophosphate (OP) or carbamate exposure based on plasma and RBC ChE activity
- To set acceptable exposure levels for occupational, dietary, drinking water and ambient air exposure to these pesticides based on plasma, RBC and brain ChE data from animal studies.

Two primary approaches for defining biological or toxicological significance:

- Statistical significance
- Percent inhibition

Statistical Significance

Pros:

- No preconceive assumptions about variation
- Takes various sources of variation into consideration (e.g., age, gender, species, strain, methodology)

Cons:

- Treatment-related effects may be masked by large variations related to poorly designed or conducted studies
- Requires study investigators to perform statistical analysis or provide individual data

Percent Inhibition

Pros:

- Consistent interpretation of toxicological significance between studies
- Consistent interpretation of toxicological significance using either NOEL or benchmark dose (BMD) approach

Cons:

- Selection of threshold may be somewhat arbitrary

OBJECTIVE

Use the normal variation in control animals from registrant studies to help select threshold(s) for toxicological significance.

METHODS

Four different types of studies submitted to DPR by registrants were evaluated: acute neurotoxicity studies in rats, subchronic neurotoxicity studies in rats, chronic toxicity studies in rats and chronic toxicity studies in dogs.

- Coefficient of variation (CV) = standard deviation (SD) / mean x 100. The CV was calculated for each compartment (plasma, RBC and brain) at each time point. The CVs for each compartment were then averaged for all times points measured within a study to derive a study mean
- Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
- Mean range is the range of the study means.

RESULTS

Plasma ChE (Tables 1-4)

- Grand Means — 13-27%
- Study Mean Ranges — 3-41%
- Slightly higher variation in females than males
- CVs tended to increase in 2nd year of chronic studies (data not shown)
- Slightly less variation in dogs than rats

RBC ChE (Tables 1-4)

- Grand Means — 11-20%
- Study Mean Ranges — 2-50%
- In general, variation in RBCs slightly lower than in plasma
- CVs in some studies higher, possibly due to interference of hemoglobin in the Ellman assay
- Dogs had greater variation in RBC ChE than rats and gender related differences

Whole Brain ChE (Tables 1-4)

- Grand Means — 5-10%
- Study Mean Ranges — 2-33%
- Variation in dogs slightly higher than in rats based on grand means, but not mean ranges

Regional Brain ChE (Tables 5-6)

- Grand Means — 8-33%
- Study Mean Ranges — 5-50%
- Only limited data available, mostly from rat neurotoxicity studies

DISCUSSION

- The CVs for ChE activity in control animals vary depending on the tissue, gender, species and methodology. Since the normal variation in any given population depends on various factors, one threshold value may not be appropriate for all comparisons.
- Different thresholds may also be needed when comparisons are between treatment and control groups versus their own baseline values to take inter-individual variation into consideration.
- In addition, different thresholds may be needed when comparing regional brain ChE activity, which have much greater variability than in whole or half brain ChE activity.
- Although the selection of toxicologically significant thresholds can be improved by consideration of the normal variation in ChE activity, the selected thresholds will always be somewhat arbitrary. Some assumptions must be made as to whether the level selected is closer to the minimum or maximum variation that might be found in the control or baseline values (*i.e.*, whether to risk flagging some differences which are due to normal variation or risk missing some differences that are treatment-related).

Table 1. Coefficient of Variation in Cholinesterase Activity for Control Animals in Acute Neurotoxicity Studies in Rats[a]

MALES	Plasma	RBC	Brain
Grand Mean[b]	15% (13)	17% (13)	9% (7)
Mean Range[c]	8-31%	3-43%	3-26%
FEMALES	**Plasma**	**RBC**	**Brain**
Grand Mean	27% (13)	15% (13)	9% (7)
Mean Range	8-36%	6-40%	3-25%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

Table 2. Coefficient of Variation in Cholinesterase Activity for Control Animals in Subchronic Neurotoxicity Studies in Rats[a]

MALES	Plasma	RBC	Brain
Grand Mean[b]	15% (16)	15% (16)	5% (9)
Mean Range[c]	6-27%	4-28%	2-9%
FEMALES	**Plasma**	**RBC**	**Brain**
Grand Mean	22% (16)	15% (16)	5% (9)
Mean Range	9-36%	5-44%	3-9%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

Table 3. Coefficient of Variation in Cholinesterase Activity for Control Animals in Chronic Toxicity Studies in Rats[a]

MALES	Plasma	RBC	Brain
Grand Mean[b]	21% (18)	11% (18)	9% (18)
Mean Range[c]	10-41%	3-30%	3-33%
FEMALES	**Plasma**	**RBC**	**Brain**
Grand Mean	18% (18)	12% (18)	9% (18)
Mean Range	9-32%	2-35%	2-33%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

Table 4. Coefficient of Variation in Cholinesterase Activity for Control Animals in Chronic Toxicity Studies in Dogs[a]

MALES	Plasma	RBC	Brain
Grand Mean[b]	13% (17)	16% (17)	9% (13)
Mean Range[c]	3-20%	3-37%	3-18%
FEMALES	**Plasma**	**RBC**	**Brain**
Grand Mean	17% (17)	20% (17)	10% (13)
Mean Range	8-32%	3-50%	3-20%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

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Table 5. Coefficient of Variation in Regional Brain Cholinesterase Activity for Control Animals in Acute Neurotoxicity Studies in Rats[a]

MALES	Olfactory	Cerebellum	Hippo-campus	Cortex	Brainstem	Midbrain	Striatum
Grand Mean	14% (4)	10% (5)	12% (4)	9% (4)	8% (3)	9% (3)	33% (2)
Mean Range	10-19%	7-15%	9-19%	7-15%	5-12%	5-14%	16-50%
FEMALES	**Olfactory**	**Cerebellum**	**Hippo-campus**	**Cortex**	**Brainstem**	**Midbrain**	**Striatum**
Grand Mean	15% (4)	9% (5)	11% (4)	11% (4)	9% (3)	11% (3)	24% (2)
Mean Range	8-20%	7-11%	6-19%	6-23%	8-11%	6-20%	9-38%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

Table 6. Coefficient of Variation in Regional Brain Cholinesterase Activity for Control Animals in Subchronic Neurotoxicity Studies in Rats[a]

MALES	Olfactory	Cerebellum	Hippo-campus	Cortex	Brainstem	Midbrain	Striatum
Grand Mean	13% (4)	11% (7)	14% (6)	16% (6)	10% (4)	10% (3)	22% (3)
Mean Range	8-16%	6-22%	6-27%	8-26%	7-14%	7-11%	14-37%
FEMALES	**Olfactory**	**Cerebellum**	**Hippo-campus**	**Cortex**	**Brainstem**	**Midbrain**	**Striatum**
Grand Mean	16% (4)	12% (7)	21% (6)	19% (6)	13% (4)	13% (3)	14% (3)
Mean Range	9-25%	9-16%	7-50%	9-43%	9-15%	12-14%	9-21%

a Coefficient of variation (CV) = standard deviation (SD) / mean x 100.
b Grand mean is the mean of the study means. The number in parentheses is the number of study means included.
c Mean range is the range of the study means.

SUMMARY

The normal variation in ChE activity was analyzed in registrant submitted studies to help identify thresholds for use in risk assessments.

- The grand means of the CVs for plasma ChE activity ranged from 15-27%, being slightly less variable in dogs than rats.
- The grand means of the CVs for RBC ChE activity were between 11-20% with slightly higher variability in dogs than rats.
- The grand means of the CVs for whole brain ChE activity in rats and dogs ranged from 5-10%.
- The grand means of the CVs for regional brain ChE activity were higher than for whole brain ChE activity, ranging from 8-33%.

These data indicate that the normal variation in ChE activity is different between plasma, RBC and brain and, therefore, it may be useful to use different thresholds when evaluating ChE data from these compartments. They also suggest that different thresholds may be needed when evaluating regional versus whole brain ChE data.