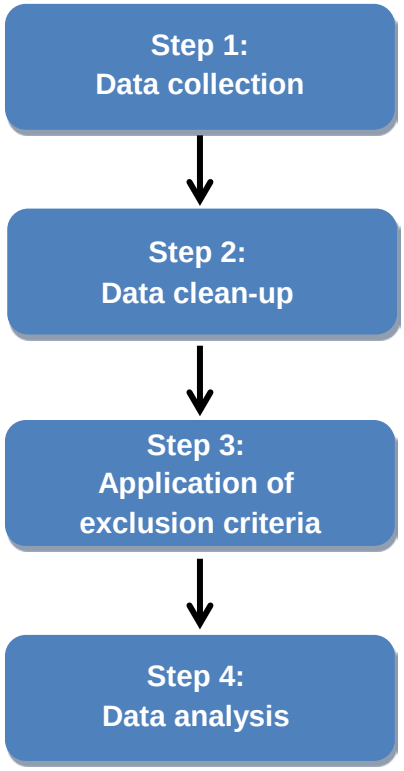
Appendix C: ELECTRONIC CHOLINESTERASE DATA ANALYSIS

HSC §105206, enacted in 2011, stipulated several changes in California's medical supervision program. The law requires certified laboratories that analyze the ChE activity in blood samples of employees who regularly handle OP/CB pesticides, to report specific information pertaining to the test result, the employee, his or her employer, his or her physician, and the laboratory to DPR. DPR shares this information with OEHHA and CDPH.

DPR worked with these laboratories to streamline the blood ChE reporting process. OEHHA analyzed the test results in order to evaluate the effectiveness of the Program and the utility of laboratory-based reporting, and to develop recommendations regarding continuation of the Program beyond the end of 2016. Figure C1 illustrates the four steps taken in data acquisition and analysis.

Figure C1: *Workflow for ChE Data Acquisition and Analysis*



Step 1: Data collection

Six laboratories are approved by CDPH for the analysis of ChE activity levels in blood specimens for occupational health surveillance, and each sends test results to DPR in Excel format on a monthly basis (at minimum). Figure C2 summarizes the number of records of ChE test results transmitted each year from 2011 through 2014 by the six laboratories.

One of the major challenges to effectively interpreting the ChE monitoring data was missing and incorrect data reported by the laboratories. DPR manually performed a first round of data clean-up, correcting misspelled names, reconciling variations in the spelling of names when all other fields were the same, and reconciling birth dates for paired samples (e.g., 01/01/1991 for the RBC ChE test and 07/01/1991 for the plasma ChE test for the same person). DPR also asked for clarification from the reporting laboratory when an entered date was not logical (i.e. 01/01/1900) or when test results from a single laboratory exceeded the normal range. DPR has been working with the laboratories to provide consistent, complete and accurate reporting of ChE testing results but these types of problems continue to exist.

Step 2: Data clean-up

OEHHA obtained the ChE data from DPR through a secure access website (SAW). Over 110,000 records were downloaded for the period of 2011-2014. To assure data consistency within each laboratory, OEHHA used SAS (Statistical Analysis System, software that manages data and performs statistical analyses) to further clean the data (e.g., reformatting the data, flagging missing information, removing duplicates, and correcting typographical errors).

After cleaning, data processing included selection of test results and application of exclusion criteria for analysis, estimation of baseline values, calculation of variation from baseline, and implementation of a screening tool to quickly identify cases of interest. The processed data were exported back into Excel format to be analyzed using both Excel and GIS (geographic information system) software.

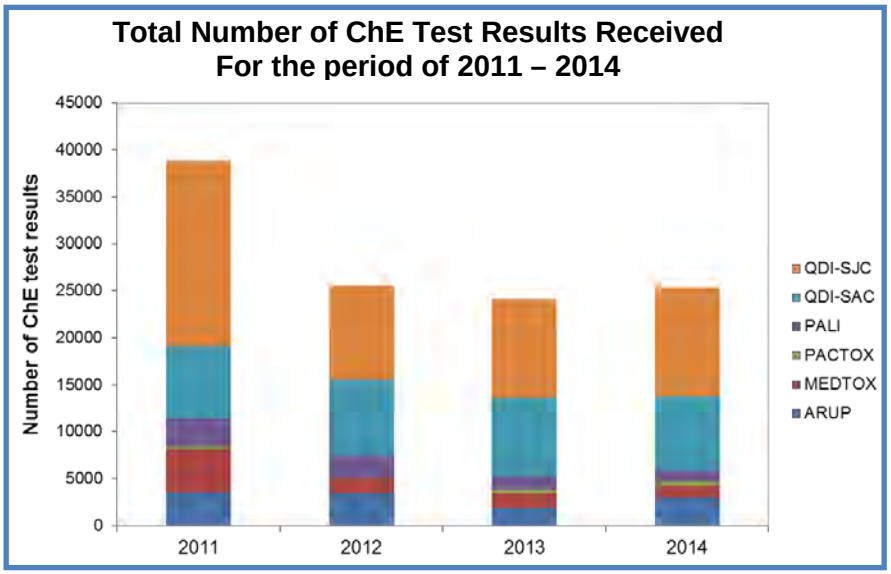


Figure C2: *Yearly number of ChE test results received by DPR from the 6 laboratories between 2011 and 2014. See Appendix B2 for complete laboratory names and locations.*

<u>Step 3: Application of exclusion criteria</u>

The laboratories reported all ChE test results to DPR, not just those related to the Program. As a result, OEHHA developed criteria for excluding irrelevant records from further analysis. Test results that fit any one of the following criteria were excluded from further analysis:

- Contained only RBC or plasma ChE activity levels, but not both
- Indicated employers that do not apply pesticides (e.g., California Department of Toxic Substance Control, San Francisco General Hospital)
- Showed that the age of the test subject was less than 16 or over 75 years old
- Showed that the physician who ordered the test was located outside California

In order to focus on records that were more likely to be related to the Program, tests were excluded from further analysis if they were ordered by a physician who did not order ChE tests for any other individuals from 2011-2013.

After application of our exclusion criteria there were 58,064 paired sample tests (RBC and plasma) for 11,735 apparent pesticide handlers. It should be noted that this process might have erroneously eliminated some data that were actually relevant to the Program or included some data that were not relevant to the Program.

In order to investigate the patterns of ChE activity level and the frequency of ChE depressions, we divided the dataset into two groups depending on whether individuals had more or less than two paired RBC and plasma ChE test results within any given year (Figure C3):

1. Individuals for whom a baseline was taken regularly (annually or every two years) but did not receive other periodic testing (follow-up).
2. Individuals for whom a baseline was taken regularly and received other routine periodic testing.

The second group was used for the analysis of individual ChE activity patterns, and frequency and type of depressions.

The histogram below shows a significant reduction in the number of ChE test results (Figure C4a) and number of individuals (Figure C4b) following the application of the exclusion criteria. The reduction is even more substantial when considering only individuals with periodic testing. This suggests that (1) a large number of ChE test results reported by the laboratories were not related to the Program, and (2) most individuals apparently in the Program (89%) did not have routine periodic testing. The apparent lack of longitudinal monitoring of individuals could be because (1) these individuals do not participate in the Program and the exclusion criteria failed to exclude them, (2) these individuals did not need to be tested more frequently because they did not handle pesticides more than six days per 30-day period, or (3) medical supervisors and/or employers failed to comply with the Program's requirements.

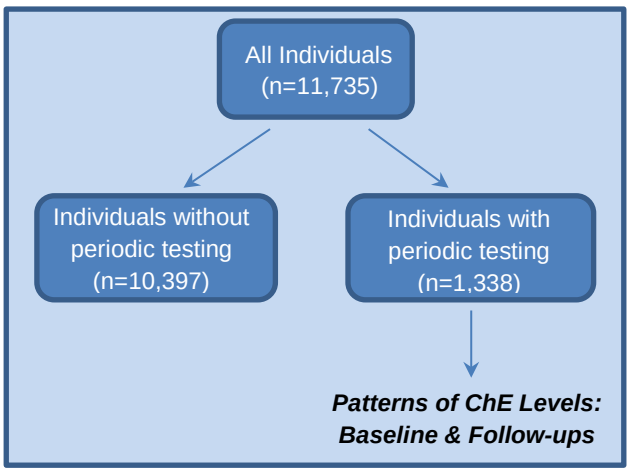


Figure C3: *Diagram of the dataset split in two groups for analysis. "Periodic testing" was defined as a record with more than two paired RBC and plasma ChE test results within any given year.*

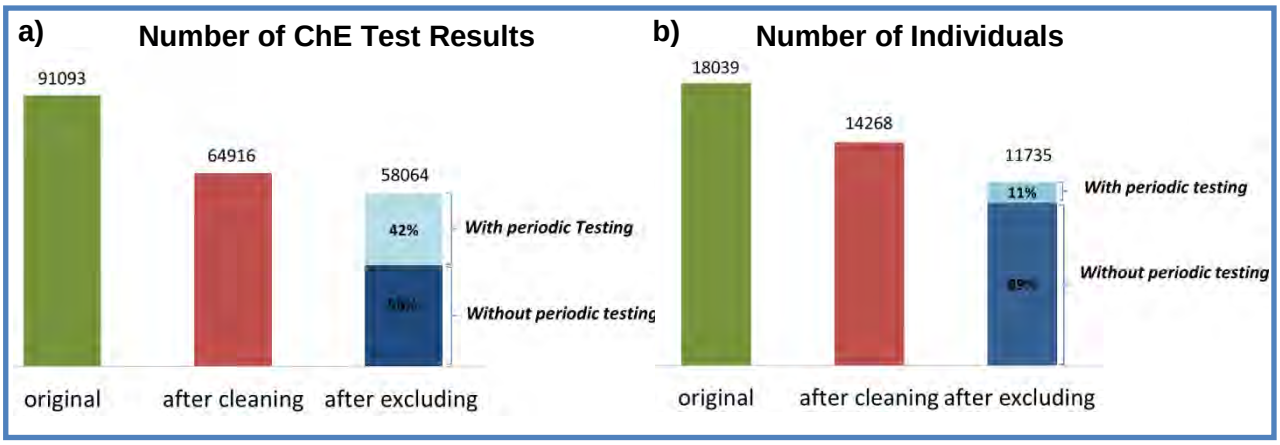
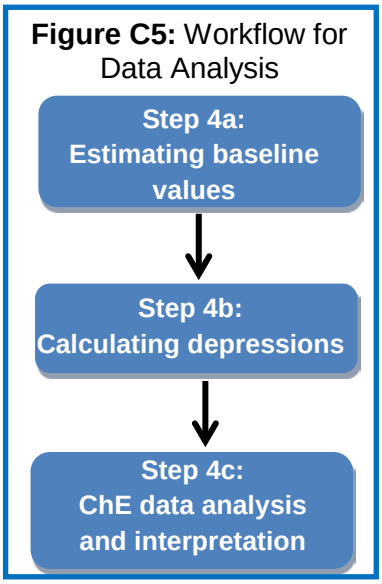


Figure C4: a) *Total number of ChE test results (RBC and Serum) before data clean-up (green), after additional data clean-up (red) and application of exclusion criteria (blue).* ***b)*** *Total number of individuals with ChE test results before data clean-up (green), after data clean-up (red) and application of exclusion criteria (blue).*

Figure C5: Workflow for Data Analysis



Step 4: Data Analysis

OEHHA analyzed the ChE data following the steps outlined in Figure C5.

Step 4a: Estimating baseline values

Since the purpose of the test for nearly all the records was not indicated or reliable, we explored alternative methods to analyze the data. We based our decision on the recommendations in the *Guidelines for Physicians,* which state that: 1) ideally, the baseline value should be the average of two or more tests taken

at least 72 hours but not more than 14 days apart following a 30-day exposure-free period[1] and 2) one baseline test is permissible if two were not obtained. Approximately half the population of presumed pesticide handlers appeared to have 14-day baselines. Their baseline values were calculated by averaging the two test results, and this method of baseline estimation was referred to as Approach 1. However, 14-day baseline estimates were not available for the rest of the population. In order to include these individuals in our analysis of depression frequencies, a different approach ("Approach 2") was adopted using the highest ChE test result obtained over the 2011-2013 period as an estimated baseline. Figure C6 illustrates how records from individuals with follow-up testing were divided into two groups to estimate the baseline.

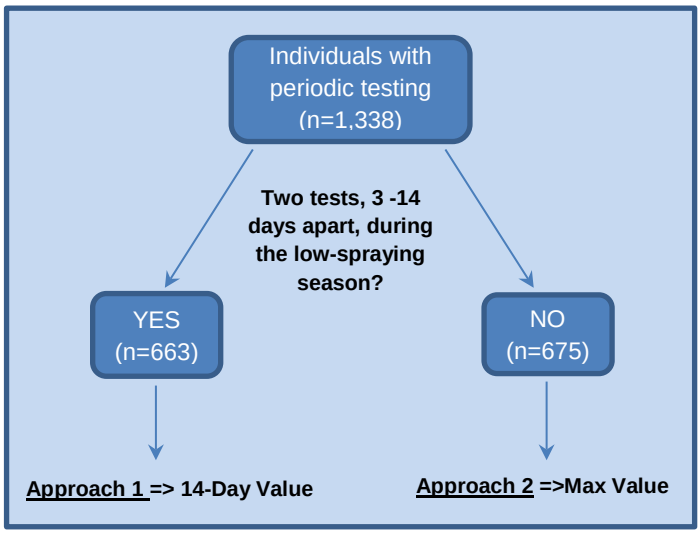


Figure C6: *Diagram of the two different approaches to determine baseline values for analysis.*

Approach 1. Baseline ChE activity level was determined by averaging results from two tests taken 3 to14 days apart during the low-spraying season[2] since pesticide handlers were most likely to be free of exposure during that period of the year. As recommended in the *Guidelines for Physicians*, if the first two baseline tests differed by more than 15% and a third test was performed within 14 days, the baseline was calculated as the average of the two closest results (Figure C7).

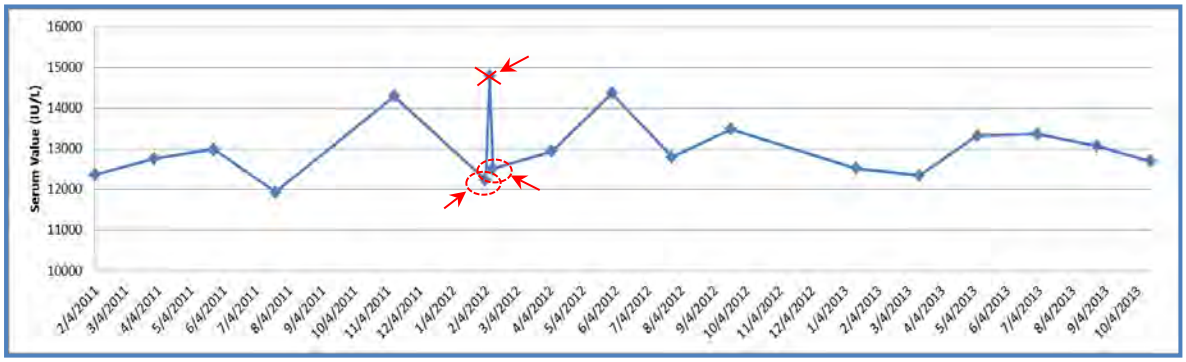


Figure C7: *ChE test results from an individual with three tests taken within 4 weeks. Since the three test results indicated by red arrows were taken over a short time period during the non-spraying season, we inferred that they were baseline test results. Furthermore, because the first two values differed by more than 15%, the baseline was estimated by averaging the first and third test result (i.e., the two circled data points).*

According to the *Guidelines for Physicians*, if a patient is recovering from ChE depression that required removal from OP/CB handling activities, the medical supervisor should promptly verify that ChE activities are returning to baseline. This situation also might lead to two samples being

[1] If two baseline tests differ by more than 15%, a third test should be performed, and the average of the two closest results should be used as an estimate of baseline ChE level.
[2] Five months with the lowest OP/CB pesticide use in California are November through March.

collected within a 14-day period. To avoid misidentifying "recovering" values as a baseline, we restricted the "baseline" period to the low-spraying season. Using statewide Pesticide Use Report data, we defined the low-spraying season as the five months with the lowest OP/CB pesticide use in California: November through March. Figure C8 illustrates how this decision rule was applied. On initial inspection, the figure appears to include three sets of paired baseline values. The two sets of two closely-spaced ChE test results surrounded by blue ovals probably represent the "true" baseline test results because they were taken during the non-spraying season, and they were used as such in our analysis. The red arrow indicates two closely-spaced ChE test results taken during the spraying season. These were probably not baseline measurements because they were collected during the spraying season and after the ChE activity level dropped below the first action threshold (<80% of baseline). In fact, they were likely to be taken during a recovery period following exposure to a ChE-inhibiting pesticide. The blue line represents the variation in ChE activity relative to the false baseline using the two values indicated by the red arrow. The red dotted line represents the variation in ChE activity relative to the baseline measurements surrounded by blue ovals.

Approximately half of the data (n=663) were amenable to this approach and they were analyzed using the 14-day baseline as the reference value.

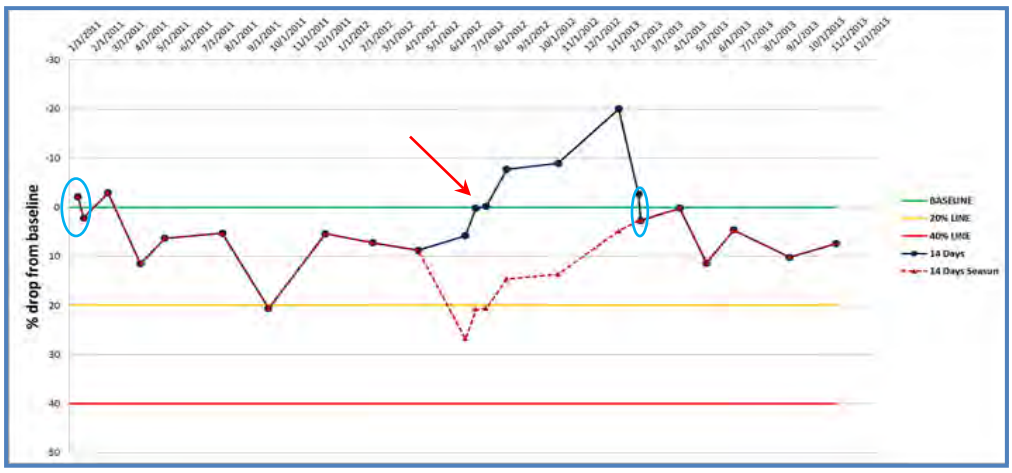


Figure C8: *Example of application of decision rules used to identify baseline estimates during non- or low-spraying season, and to reject samples collected within 14 days of one another during spraying season.*

Approach 2. For those data that were not amenable to Approach 1 (n=675), we assumed that the highest ChE test result obtained over the three-year period (2011-2013) was the baseline. We hypothesized that since baseline samples should be taken following a 30-day exposure-free period, the value of the baseline should be close or equal to the maximum ChE activity level observed.

In the main report, we compared frequency of depressions using Approach 1 on the population of individuals with a14-day baseline (n=663) to the frequency of depressions using Approach 2 on the entire population of individuals with periodic monitoring (n=1,338).

In this appendix, for the purpose of the discussion, the data set for subsequent analysis was comprised of records using ***either one or the other of the two approaches*** to define the baseline. Therefore if two successive sample results in an individual's chronological record did not appear to meet the first requirement (Approach 1), the alternative approach was used

(Approach 2). Since Approach 2 produced on average higher estimates of baseline activity than Approach 1, it led to a higher frequency of ChE depressions. Therefore the two approaches provided a range of estimates of the overall frequency and types of ChE depression. Use of both approaches also allowed us to include all suspected workers and doubled our sample size. To simplify the data analysis, all records were treated the same way once a baseline was determined. Results comparing both approaches are presented and discussed later in this Appendix.

We have compared frequency of individuals with depressions using Approach 2 on the total population of individuals (n=1,338) to the frequency of depressions obtained from the population of individuals without a 14-day value (n=675). As shown on Table C1, results from both populations are very similar.

Table C1: *Percentage of individuals with different levels of depressions using Approach 2 on all individuals (n=1,338) or just on individuals without a 14-day value (n=675).*

Action Level	RBC ChE		Plasma ChE	
	All Individuals	Individuals without 14-day values	All Individuals	Individuals without 14-day values
No action needed (80-100% of baseline)	87%	89%	62%	63%
Review of workplace practices (<80% of baseline)	9%	7%	33%	31%
Removal from further exposure (<60% of plasma baseline, or <70% of RBC baseline)	4%	4%	5%	6%

It is worth emphasizing that the need to use these two approaches to baseline estimation arose because the test purpose was seldom provided with the ChE test reports. Consequently, baseline ChE values were inferred solely from the data.

Step 4b: ChE data analysis and interpretation

We formulated five questions (A to E) to evaluate the Program and analyzed the ChE data to determine if it might provide insights and possible answers to these questions.

A. Can we infer from the reported ChE test results that workers who regularly handle category I and II OP and CB pesticides are participating in the Program?

Electronic reporting of ChE test results does not allow us to identify all the workers in California that handle OP/CB pesticides. It only provides a list of individuals who were tested for various reasons, and some of them might have been exposed to OPs/CBs. In an attempt to assess the degree of participation of workers in the Program, we analyzed the correlations between the temporal and spatial distribution of ChE test results and agricultural use of OP/CB pesticides in the state.

- **Geographic distribution of ChE test results and their association with pesticide use.**

We used geospatial analysis to determine if the overall number of ChE test results reported from each county was proportional to the amount of OP/CB used in that county. As shown in Figure C9, there is generally good correlation between geographic density of ChE test results and the areas of high pesticide use (Pearson's $r = 0.667$, $p < 0.0001$). In other words, the larger the quantity of OPs/CBs used in a county, the higher the number of ChE test results.

However, geographic analysis also revealed that there were very few ChE test results from several California counties that had relatively high OP/CB use (indicated with red arrow). Indeed, after applying the exclusion criteria, some counties with relatively high pesticide use (e.g., Butte, Glenn, Sutter, Yuba and Colusa counties in the northern Sacramento Valley) did not show any ChE test results. A lack of test results from these counties might be due to: 1) missing location information on the ChE test reports (16.1 % of total ChE test results), 2) uncertainty in identifying the employee's worksite (see explanations in the following paragraph), 3) seasonal migration of workers from one county to another, 4) small farms in these areas may have hired Pest Control Operators located in other counties to apply pesticides, and/or 5) employers failed to follow the Program requirements.

Geographic analysis also revealed that some counties with no or very low pesticides use (e.g., San Francisco) had disproportionally high number of tests. Further analysis revealed that these tests were from individuals not receiving periodic testing and most likely not participating in the Program (e.g., pre-operative testing, Alzheimer's drug monitoring, liver disease screening, and aging research studies).

Ideally, one would use employee's worksite data to generate the county-specific ChE test results and correlate the information with county-specific pesticide use data. However, employee's worksite data was not provided in the electronic ChE test reports. To overcome this data gap, we used the physician's location[3] to generate the county-specific ChE test results. This method may assign an employee to a wrong county if, for example, the employee was seen by a medical supervisor located in one county but was exposed in another county.

[3] When physician's location was missing, we used the location of the patient, the drawing lab or the employer instead.

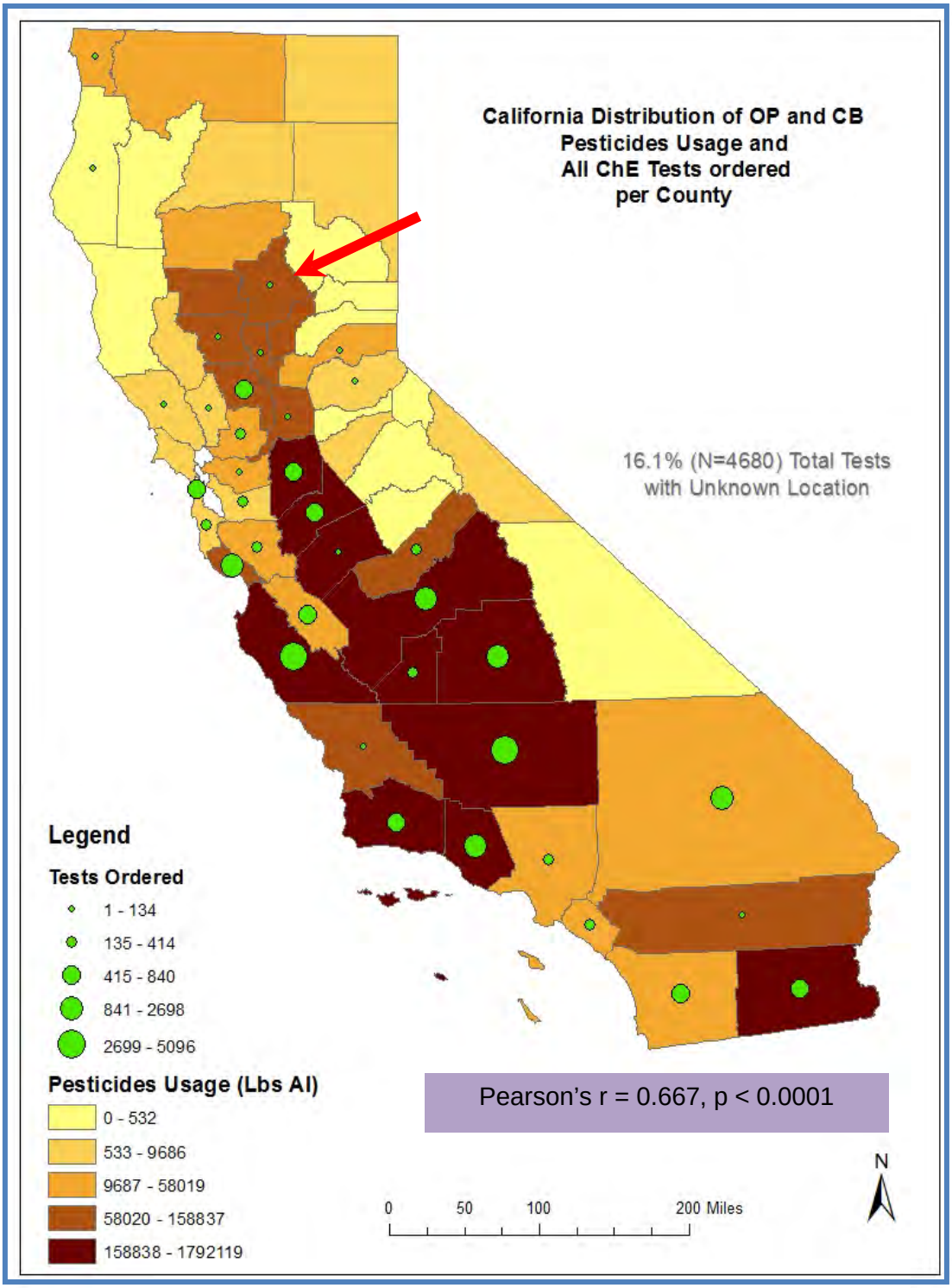


Figure C9: *Geographic distribution of OPs/CBs types I and II used (2011 – 2013) and number of ChE test results by county.*

- **Temporal distribution of tests ordered and their association with pesticide use.**

We used temporal analysis to determine if the monthly number of ChE test results reported was proportional to the monthly volume of OP/CB use. Figure C10 shows statewide monthly pesticides use with number of estimated baselines (a), and with number of estimated follow-up ChE tests (b) between 2011 and 2013 from dataset with periodic (follow-up) testing. As expected, the number of follow-up ChE tests (defined as total ChE tests minus baseline ChE

tests) showed a strong correlation with the volume of pesticide use (Pearson's r = 0.775, p < 0.0001) suggesting that an increase in the volume of OP/CB pesticide use leads to an increase in the number of follow-up tests being ordered (Figure C10b). Conversely, the number of estimated baseline ChE tests was inversely correlated (Pearson's r = -0.287, p < 0.1) with pesticide use (red line), reaching a peak between January and March of each year, just before the beginning of the spraying season (Figure C10a).

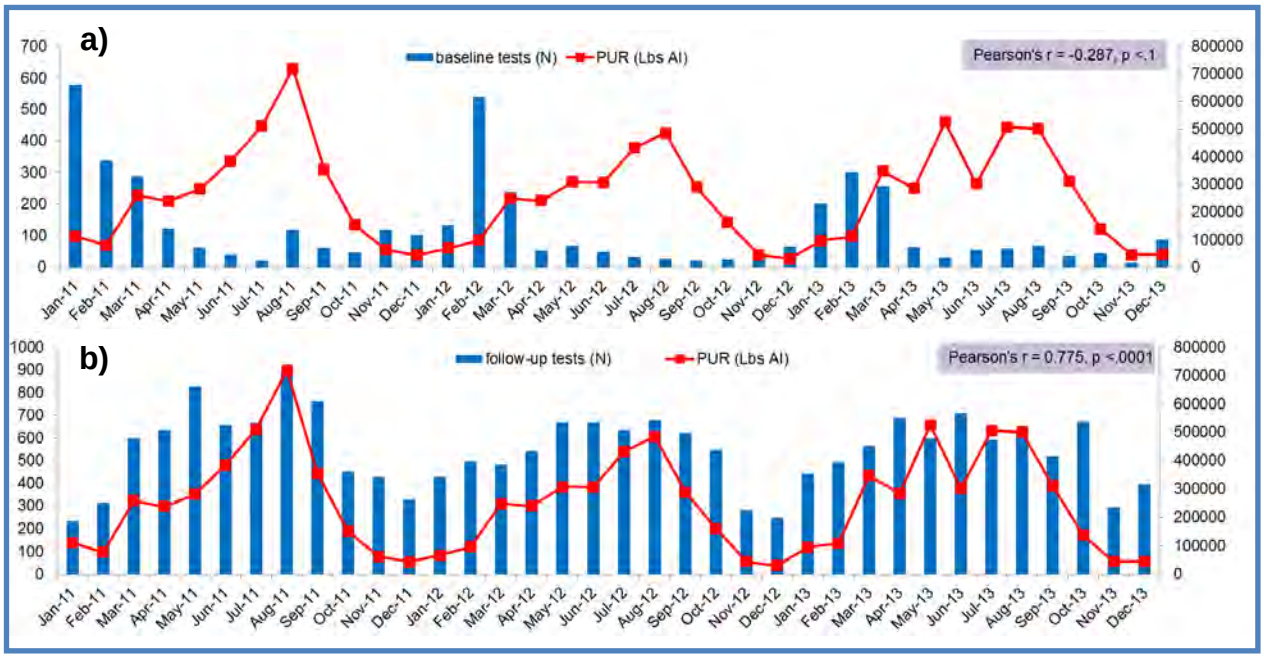


Figure C10: *Monthly OP/CB use (PUR) and* ***a)*** *number of estimated baseline ChE test results, and* ***b)*** *number of estimated follow-up ChE test results from dataset with periodic testing between 2011 and 2013. Red lines are pesticide use data (lbs AI/month, right y-axis) for all toxicity category I and II OPs and CBs. Bars are estimated number of monthly ChE test results.*

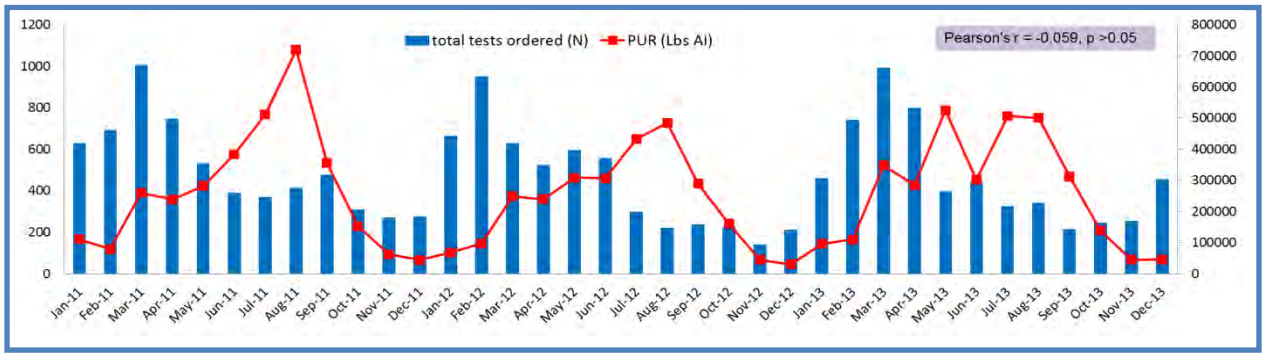


Figure C11: *Monthly OP/CB use (Pesticide Use Record) and number of ChE test results from dataset of individuals without periodic testing. Red lines are pesticide use data (lbs AI/month, right axis) for all toxicity category I and II OPs and CBs. Bars are estimated number of monthly ChE test results.*

Figure C11 shows statewide monthly pesticides use and number of tests ordered from the group of individuals that only had baselines taken and no other periodic testing (e.g., follow-ups). As expected, the number of tests from this dataset is similar to the one showing the baseline from the group with periodic testing (Figure C10a). Test results reached a peak between January and May of each year, just at the beginning of the spraying season (Figure C11). This suggests that a large number of these individuals participate in the Program even

though they did not have any follow-up testing. However some months with high pesticides use (e.g., June-August 2011) had significant number of tests. We presume that these test results are most likely from individuals not under the Program.

B. Can we infer from the reported ChE test results that depressions that exceed one or more of the action levels are occurring?

To investigate the frequency of ChE depressions (2011-2013), we used the dataset with routine periodic testing to look at the distribution of ChE test results that were 20, 30 or 40% below baseline (Figure C12). The proportion of ChE test results that appears to warrant action is relatively small. Three to twenty four percent of plasma ChE test results and 1-5% of RBC ChE test results appear to have required an evaluation of workplace practices, while only 1-5% of plasma ChE test results and <1-2% of RBC test results appear to have required removal of the worker from OP/CB handling activities. Nevertheless, from analysis of the ChE data alone, we cannot determine if any of these actions were actually taken.

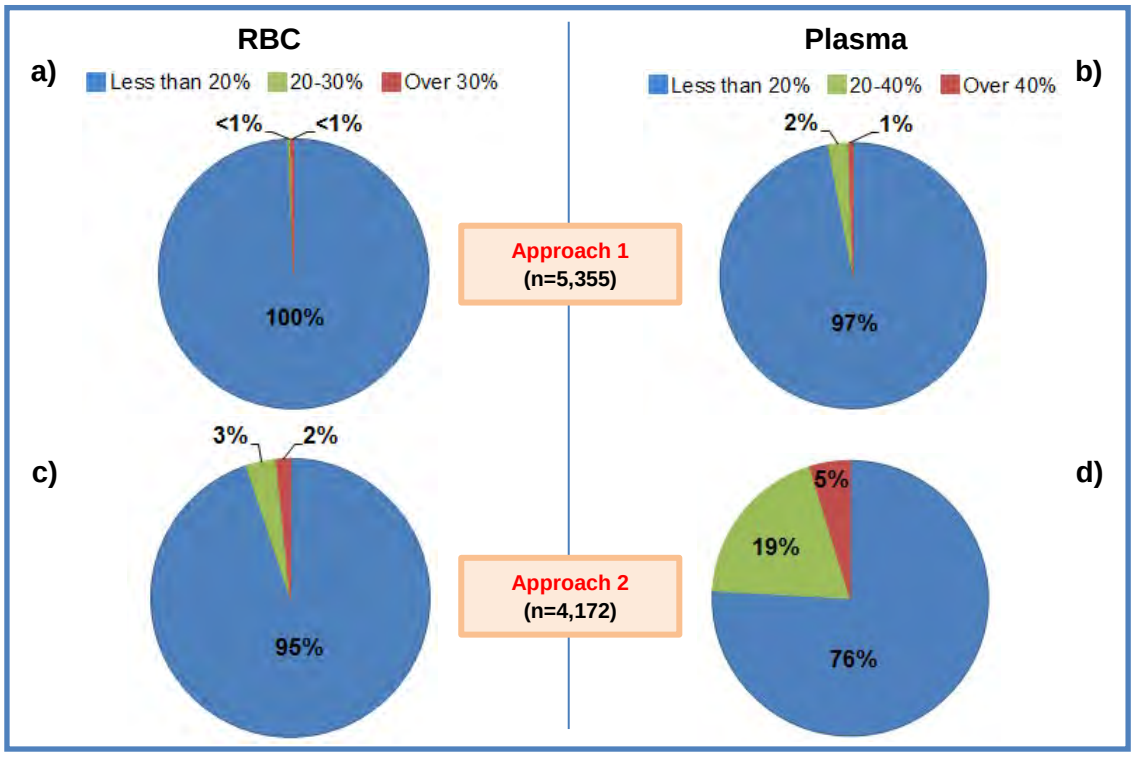


Figure C12: *Distribution of* ***test results from routine monitoring*** *with level of depressions requiring different level of action: no action needed (0-20%), review of workplace practices (20-30% for RBC and 20-40% for plasma), and removal from further handling of OP/CB (over 30% for RBC and over 40% for plasma) with Approach 1 (**a, b**) and Approach 2 (**c, d**).*

- **Geographic distribution of depressions and its association with the amount of pesticide use.**

We investigated the associations between the geographic distributions of apparent ChE depressions and county by county pesticide use to determine if depressions occurred more often in areas of high OP/CB use (Figure C13). The total number of depressions per county (represented on the map by the size of the circles) is significantly correlated with pesticide use

(Pearson's r = 0.315, p <0.05). The lack of ChE test results previously noted (Figure C9) in some counties with moderately high OP/CB use (e.g. northern Sacramento Valley), reduced the strength of correlation. In contrast, three high-use counties (Monterey, Ventura and Kern) had proportionally high number of depressions, and one county (San Benito) had a disproportionally large number of ChE depressions compared to the amount of OP/CB use.

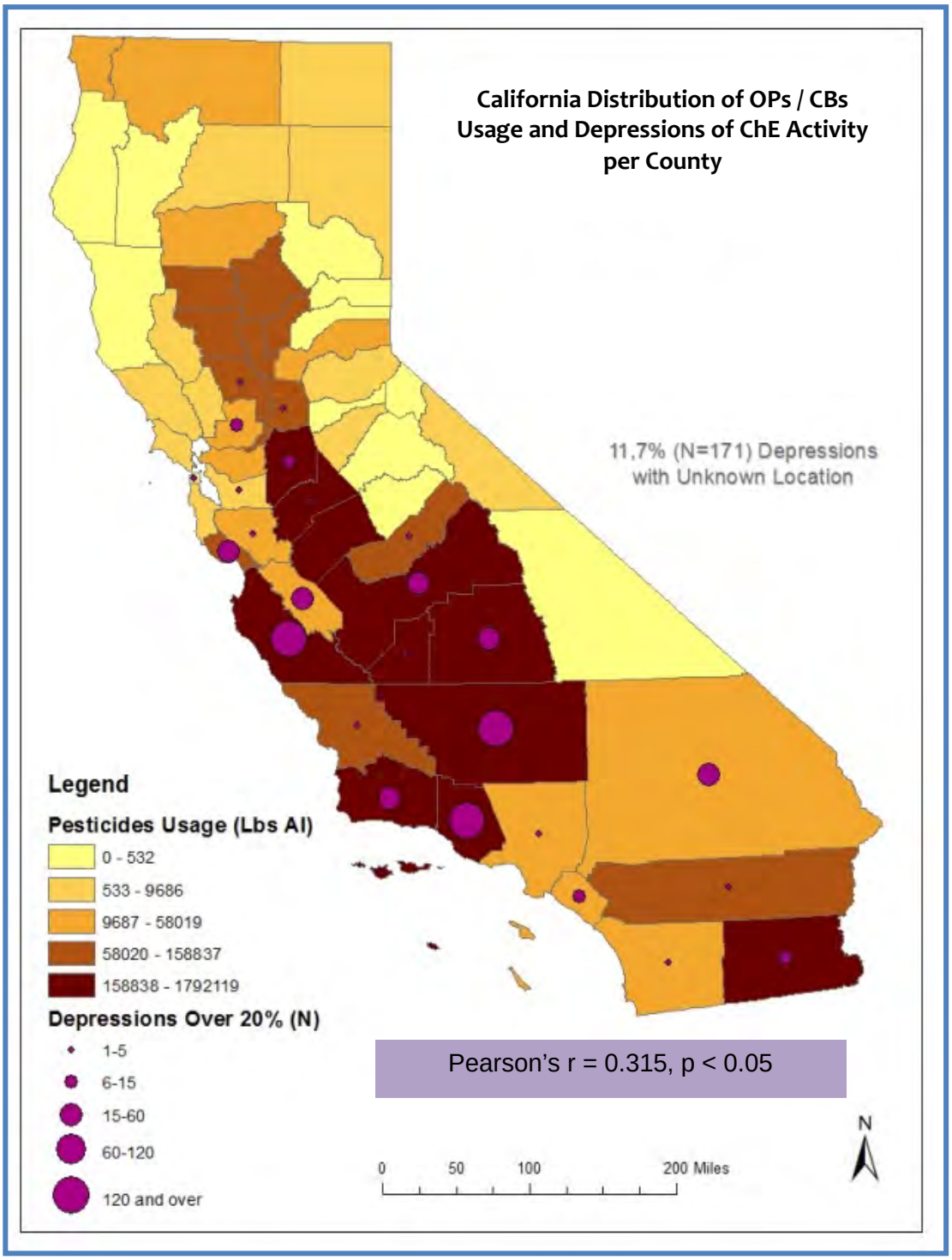


Figure C13: *Geographic distribution of OPs/CBs types I and II use and number of depressions by county across California (2011-2013).*

- **Temporal distribution of ChE depressions and its association with the amount of pesticide use.**

We investigated the associations between the temporal distributions of depressions and monthly pesticide use to determine whether depressions occurred more often during the months of high OP/CB use (Figure C14). Both monthly number (Pearson's r = 0.80, p < 0.0001) (Figure C14b) and monthly frequency (number of tests with significant depressions / total number of tests) (Pearson's r = 0.71, p < 0.0001) (Figure C14a) of depressions were strongly correlated with pesticide use.

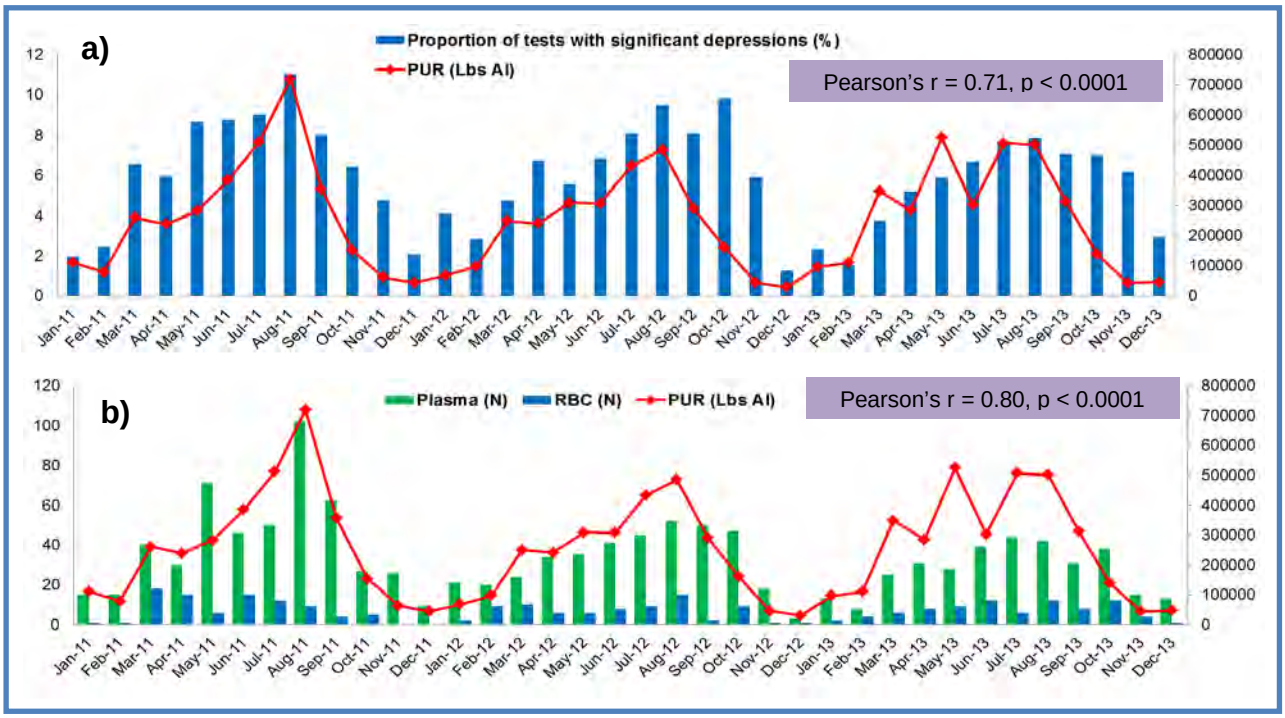


Figure C14: *Monthly pesticide use and ChE test results from 2011 to 2013. Red lines are pesticide use data (lbs AI/month, right axis) for all Toxicity I and II OPs and CBs.* ***a)*** *Bars are monthly percentage of ChE test results with depressions that met the minimum action level (>20%).* ***b)*** *Bars are number of Plasma ChE (green) and RBC (blue) depressions over 20%.*

C. Can we infer from the reported ChE test results that actions are being taken in the workplace in response to ChE testing?

There were 1,338 individuals who were tested numerous times over the three-year period, allowing a time course evaluation of ChE activity levels. Figure C15 represents five different patterns of individual ChE activity levels. These results illustrate variations in the frequency, magnitude and duration of ChE depression that meet or exceed the various action levels.

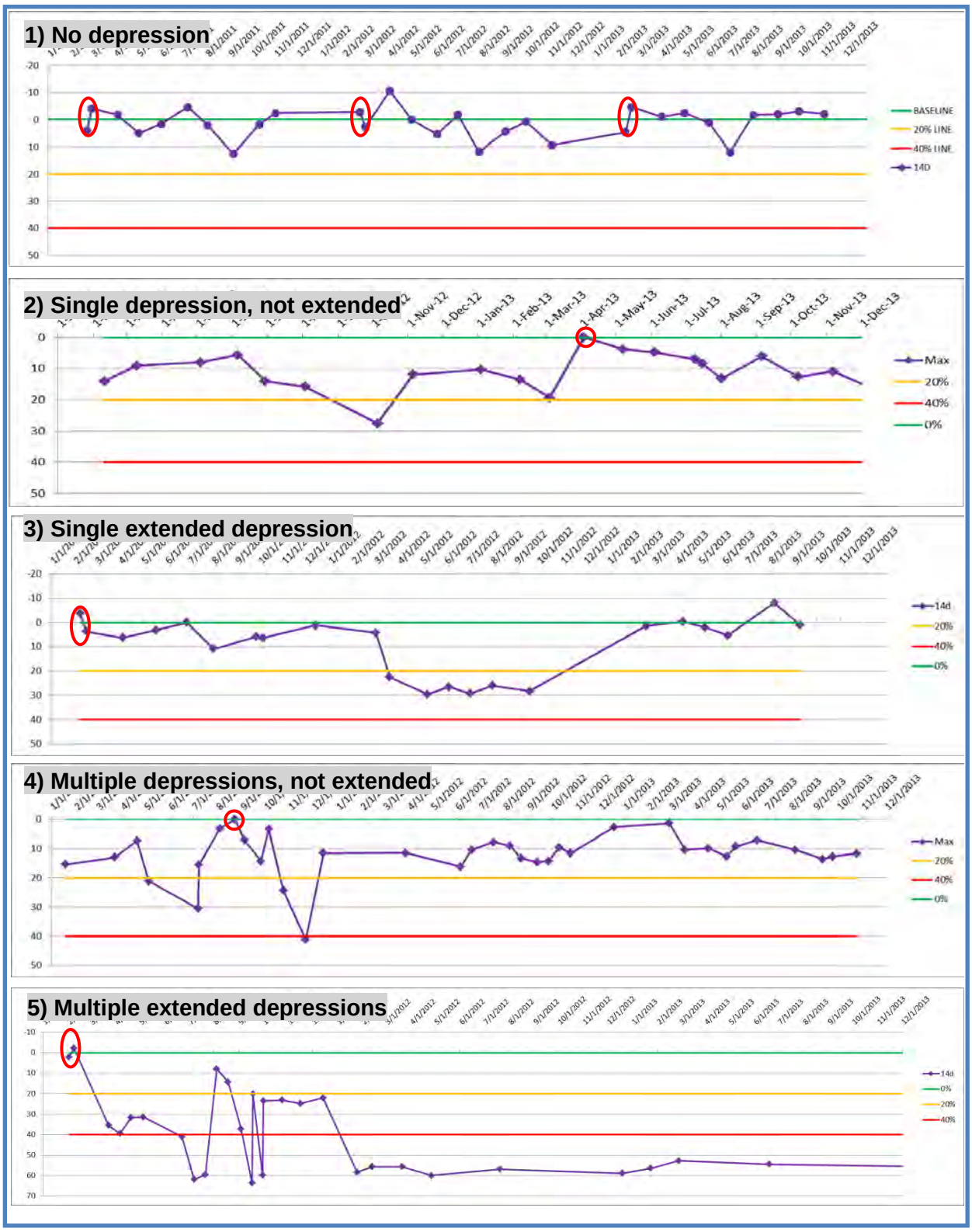


Figure 6: *Individual test results that represent different plasma ChE level patterns.* ***1)*** *no depression that exceeded action levels,* ***2)*** *single depression with prompt return to >80% of baseline level,* ***3)*** *single depression with slow return,* ***4)*** *multiple depressions with prompt return,* ***5)*** *multiple depressions with slow or no return. Y axis is percent depression from baseline. Green line represents the baseline of the individual. Red circled values are baselines.*

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A primary objective of the data analysis was to identify ChE test results that exceeded one or more action levels. For this purpose, we plotted the variation in ChE activity level of individual pesticide handlers over time. Often, the number of ChE tests over time is sufficient to provide some indication that a worker's activities were being managed in accordance with the recommendations of the *Guidelines for Physicians*, as reflected by their ChE test results. In some cases, insufficient records for individual workers prevented us from assessing whether corrective actions had been taken, or follow-up monitoring had been initiated, following ChE depression. Figures C16a and C16b are examples of longitudinal data with too few test results to indicate whether corrective action was taken (Figure C16a, which shows a 40% ChE depression with no subsequent test results), or to determine if a ChE depression was completely resolved (Figure C16b, which illustrates a 10-month gap between successive samples). Figure C16c is an example of time course data with sufficient test results to evaluate the pattern of ChE depression over the 3-year analysis period.

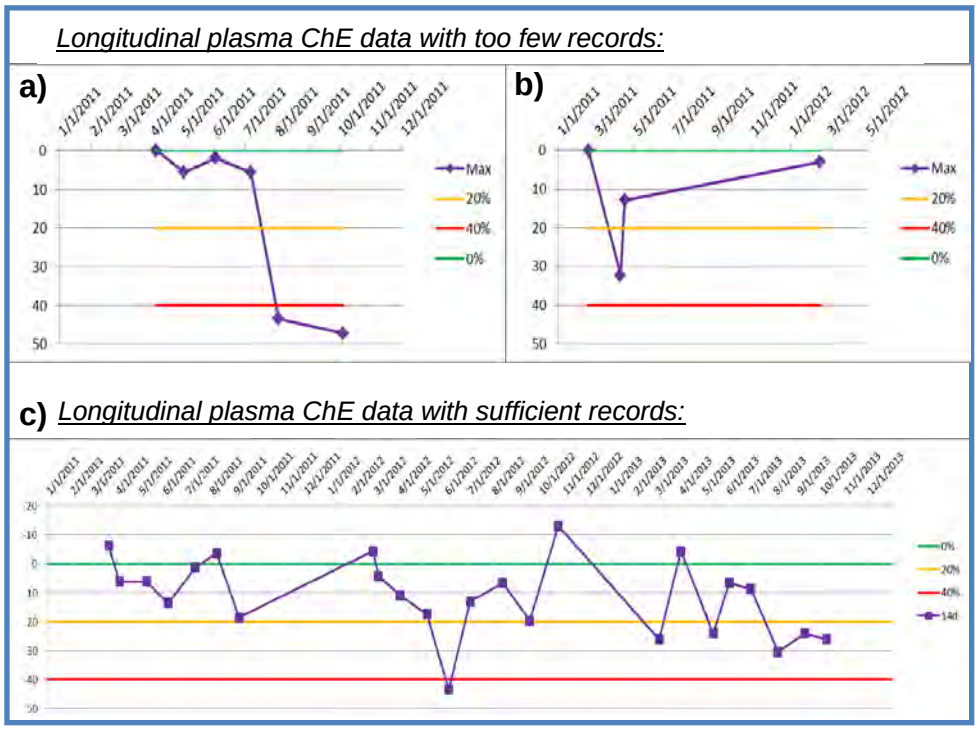

Figure C16: *Summary of visual examination of individual longitudinal variations extracted from the screening tool results. Examples show two individuals with too few records to interpret patterns of depression (**a** and **b**). Also shown is an example of an individual with sufficient records to identify ChE depression patterns (**c**). The Y axis is the percent depression from baseline.*

To examine if actions were being taken in the workplace in response to ChE test results, we investigated the number of individuals with ChE depression exceeding one or more action levels, the duration of time the ChE activity levels remained depressed (slow vs. rapid return to >80% of the baseline), and how often (single vs. multiple times) an individual experienced depressions of his/her ChE activity levels. For this purpose, we used SAS to develop a screening tool that allowed us to identify patterns of either RBC or plasma ChE variation over time.

Using the screening tool, we defined the five different time-courses of ChE activity over the 2011-2013 data analysis period (Figure C17):

1. **No** depression exceeding action levels: no depression below the minimum action level (<80% of baseline) occurred.
2. **Single** depression with **rapid** return to acceptable level: one or two consecutive depressions below the minimum action level occurred within a three month period, with rapid return to an acceptable level (>80% of baseline).
3. **Single** depression with **slow or no** return to acceptable level: three or more consecutive depressions below the minimum action level occurred within a three month period, with slow return or no return to an acceptable level.
4. **Multiple** depressions with **rapid** return to acceptable level: more than two discrete depressions below the minimum action level occurred, with rapid return to an acceptable level.
5. **Multiple** depressions with **slow or no** return to acceptable level: more than two discrete depressions below the minimum action level occurred, with at least one of these depressions returning slowly or not returning to an acceptable level.

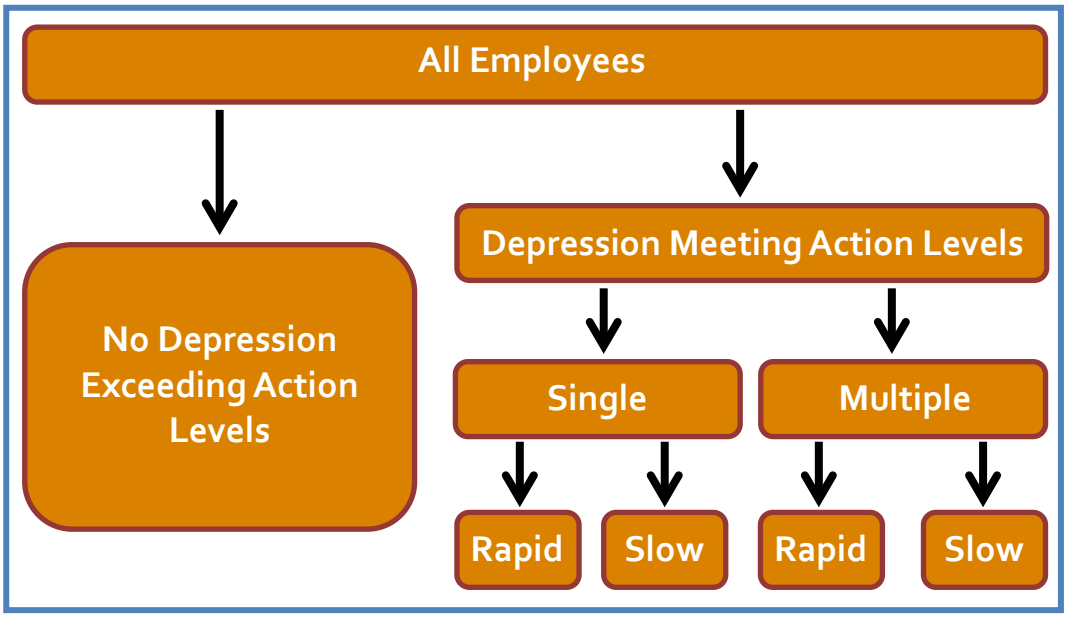

Figure C17: *Patterns of depressions of ChE activity*

We first investigated the number of individuals with ChE depression that exceeded any of the action levels, that is, those that required evaluation of workplace practices or immediate removal from work (Figure C18). Overall, 12-37 % individuals had at least one plasma ChE depression (> 20%) and 2-11% had at least one RBC ChE depression (>20%). However, only 1-6% of the individuals had at least one depression requiring removal from work based on plasma ChE depression (>40%) and only 1-4% of the individuals had at least one depression requiring removal from work based on RBC ChE depression (> 30%).

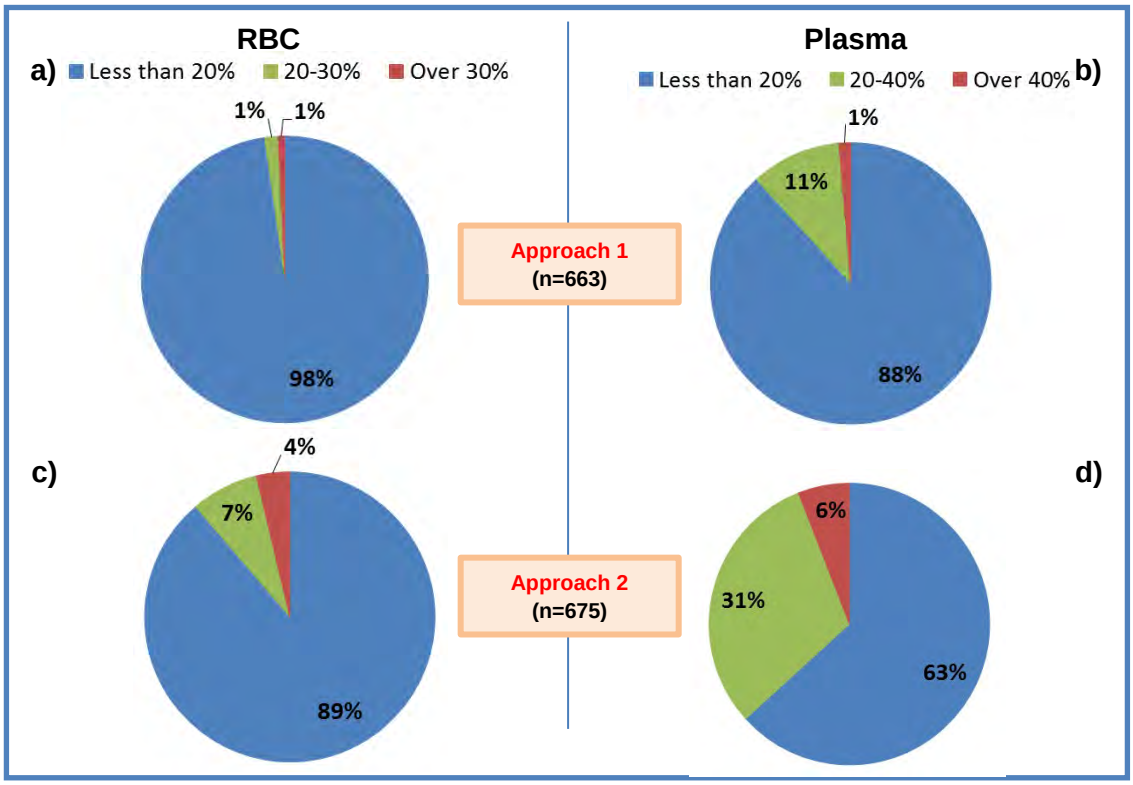

Figure C18: *Distribution of* ***individuals*** *with levels of ChE depression requiring different levels of action: no action needed (0-20%), review of workplace practices (20-40% for plasma ChE and 20-30% for RBC ChE) and removal (over 40% for plasma ChE and over 30% for RBC ChE) with* ***Approach 1*** *(**a, b**) and* ***Approach 2*** *(**c, d**).*

We used the screening tool to investigate the percentage of individuals who experienced repeated depressions of ChE and those whose ChE activity level remained depressed for an extended period of time. These results are shown in Figure C19. Sixty-three to eighty-eight percent of the individuals had no plasma ChE depression that exceeded an action level and 89-98% had no RBC depression. For individuals with plasma ChE depressions > 20%, 8-23% experienced multiple depressions and 4-14% had a single depression. With regard to RBC ChE activity levels, 1-5% of individuals had single depressions while 1-6% had multiple depressions. However, multiple depressions were generally short in duration and promptly returned to a level that would allow a worker to return to pesticide handling activities (i.e., >80% of the baseline). Two to eight percent of the individuals experienced multiple extended plasma ChE depressions while 1% or less experienced multiple extended RBC ChE depressions. These results suggest that in most cases, immediate action was taken following a depression of >20%, resulting in a prompt return to an acceptable ChE activity level. This analysis also suggests that, in some cases, long-term remedies may not have been implemented to prevent further excess pesticide exposure and consequent reoccurrence of ChE depression.

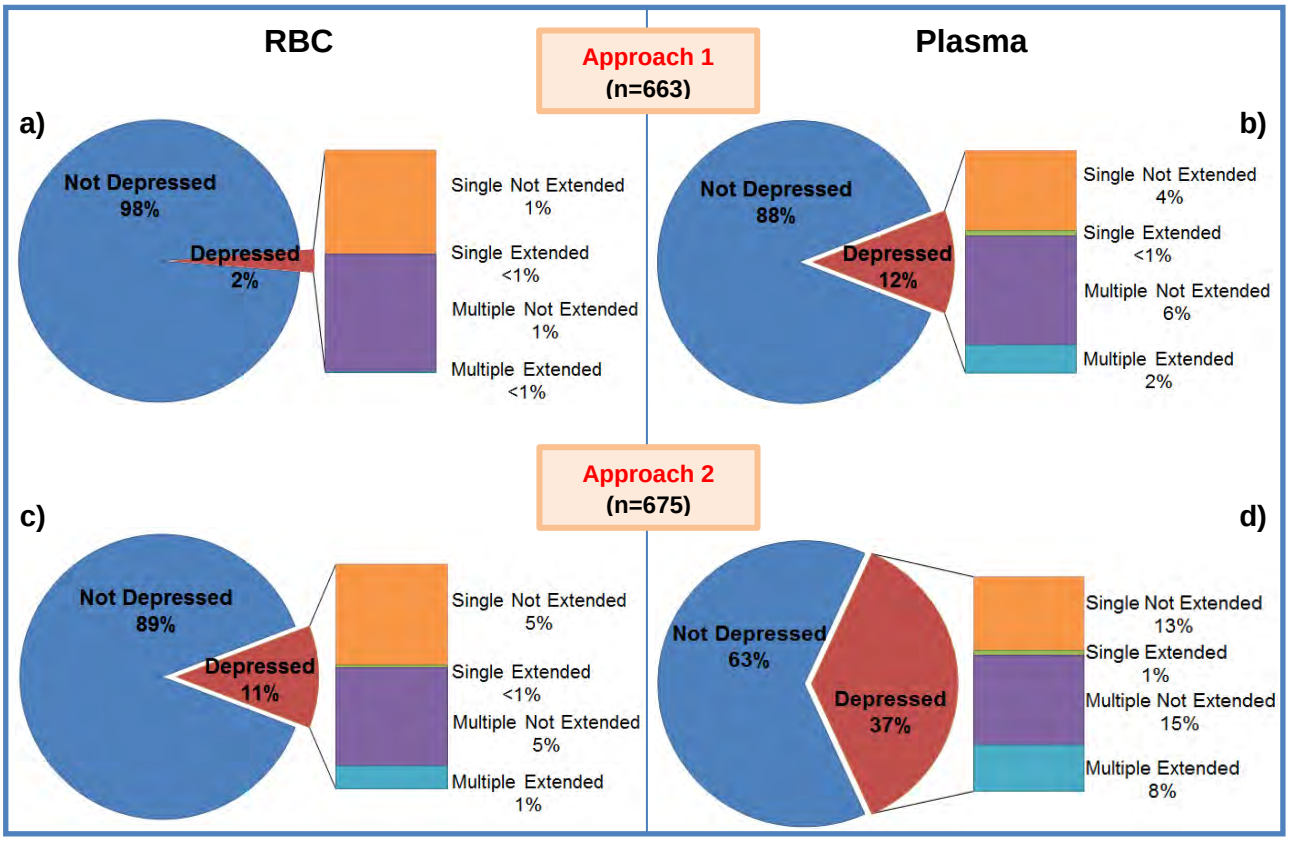

Figure C19: *Overall distribution of* ***individuals*** *by type of depressions (single, multiple, extended or not extended): RBC (left) and Plasma (right) with Approach 1 (**a, b**) and Approach 2 (**c, d**).*

- **Challenges resulting from not having the purpose of the test reported with the ChE data**

As mentioned earlier, it was not always possible to identify baseline test results in the dataset. To overcome this shortcoming, we used the two approaches described in Step 4a of this appendix ("Estimating baseline values"). However, both approaches are based on inferences and have limitations:

Limitations of Approach 1:

<u>Reduce the sample size</u>
Only approximately 50% of the ChE data were amenable to this approach (that is, had one or more 14-day baselines). The other 50% of the data had to be either evaluated using an alternative approach or discarded.

<u>May potentially bias the findings</u>:
It is possible that the individuals whose baselines were determined using Approach 1 were monitored more closely by both their employer, who was willing to cover the additional cost of a second baseline test, and their medical supervisor, who followed the *Guidelines for Physicians* recommendations more strictly. This may provide a biased picture on the overall effectiveness of the Program.

Error in defining the exposure-free period using the season:
Since we were unable to verify that an individual was exposure free for 30 days prior to collection of the first baseline sample, we limited the period for baseline samples to the statewide "low spraying" season. However, the spraying season varies within California. Some counties, especially those in southern California and along the central coast, do not have an "off-season" for agricultural production. This makes it very difficult to distinguish between baseline and post-exposure testing periods based solely on the traditional spring/summer/fall/winter seasons.

Limitations of Approach 2:

Overestimation of baseline value:
By definition, the baseline cannot be higher than the maximum ChE value. Therefore, using the maximum value as the baseline could lead to an over-estimation of the extent and frequency of ChE depressions. However, it provides a health-protective reference point for evaluating the blood ChE data when a 14-day baseline estimate cannot be determined.

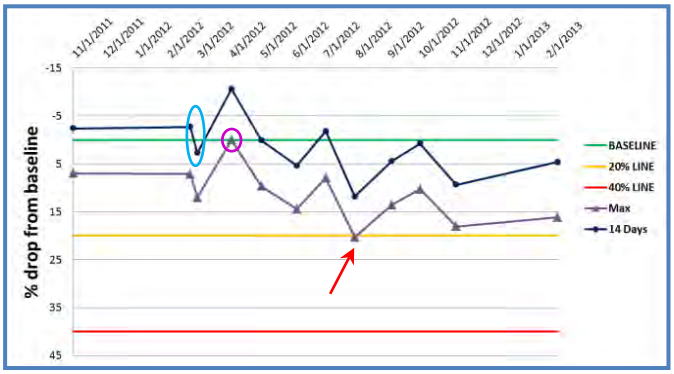

Figure C20: *An example of the error that might be introduced using Approach 2.*

An example of the error that might be introduced by Approach 2 is illustrated in Figure C20. The blue line represents the variations in ChE activity observed using the 14-day estimate of baseline (shown on the left side as the average of the two circled values). The purple line was obtained using the maximum value as the baseline (shown as the circled value from a sample collected in April, 2012). The red arrow represents the point where the 20% action level (yellow line) was exceeded using the maximum value baseline estimate but not the 14-day baseline estimate.

We estimated the degree of over-estimation of baseline activity introduced using Approach 2. The 14-day baseline estimate derived using Approach 1 was compared with the maximum value estimate derived using Approach 2 for those workers who had both values available. On average, the Approach 2 estimate of baseline was 12% higher than the estimate derived using Approach 1. Assuming that Approach 1 produces the "true" baseline (and there are uncertainties this regard, as noted above), Approach 2 may overestimate the number of depressions that exceed one or more of the action levels.

Earlier in this appendix, we presented results using Approach 1 for individuals who had 14-day baselines and Approach 2 for individuals who did not have 14-day baselines. We also compared results using each of the two approaches for individuals who had 14-day baselines (n=663) and obtained the following results:

1. Frequencies of *ChE test results* with depressions that met an action level (at least 20% below baseline) were much lower with Approach 1 than with Approach 2: <1 vs. 7 % for RBC ChE and 3 vs. 27 % for plasma ChE.

2. Frequencies of *individuals* with depressions that met an action level (at least 20% below baseline) were much lower using Approach 1 than Approach 2: 3 vs. 15 % for RBC ChE and 12 vs. 38 % for plasma ChE.

3. Regardless of which approach was used to establish a baseline ChE level, the relative proportions of single vs. multiple and extended vs. not-extended depressions were similar.

As expected, the evaluation using data generated by Approach 2 identified more depressions and more workers with at least one depression than Approach 1. But, as noted earlier, neither approach provides a definitive baseline; both approaches are based on inferences.

D. Does electronic reporting of ChE test results have an impact on the medical supervision program?

- **Annual number of tests reported**

In order to assess whether electronic laboratory reporting improved as a result of DPR's work with the analytical laboratories on their reporting practices, we evaluated the number of tests reported from 2011 to 2013. Figure C21 shows the number of test results before and after applying the exclusion criteria. The number of test results reported in 2012 and 2013 dropped by 40.5% compared to 2011, but the number of tests that we suspect were related to the Program declined by just 13.5%. This suggests that over the three year period, the laboratories improved their ability to eliminate irrelevant records from their reports. Therefore, DPR's efforts to improve the laboratory reporting process appeared to be effective and should be continued.

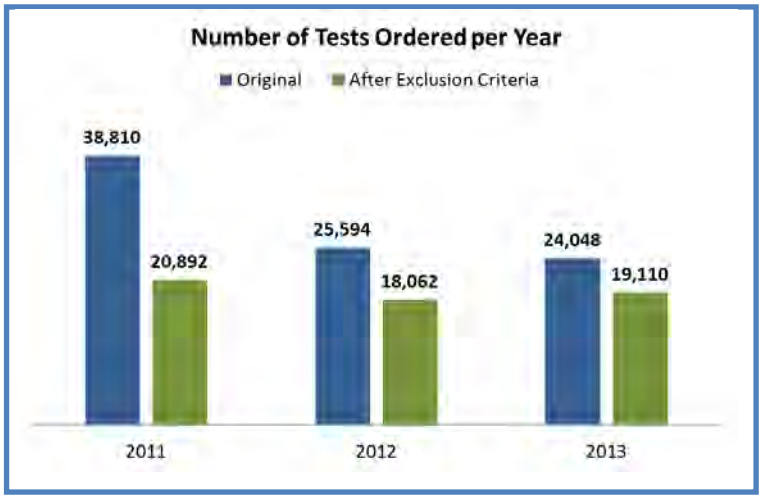

Figure C21: *Yearly number of tests before (blue) and after (green) applying all exclusion criteria*

- **Annual number of depressions**

Figure C22 shows the annual number of depressions observed, and the use of category I and II OP/CB pesticides from 2011 through 2013. There was a 30.4% decline in the number of depressions, but this decline did not coincide with a corresponding trend in OP/CB use. Improved work practices may have been responsible for the decline in depressions. Differences in the handling and/or processing of blood samples, or changes in the reporting process may also be contributing factors. Regardless of the cause, there was a general decline in ChE depressions over the three years.

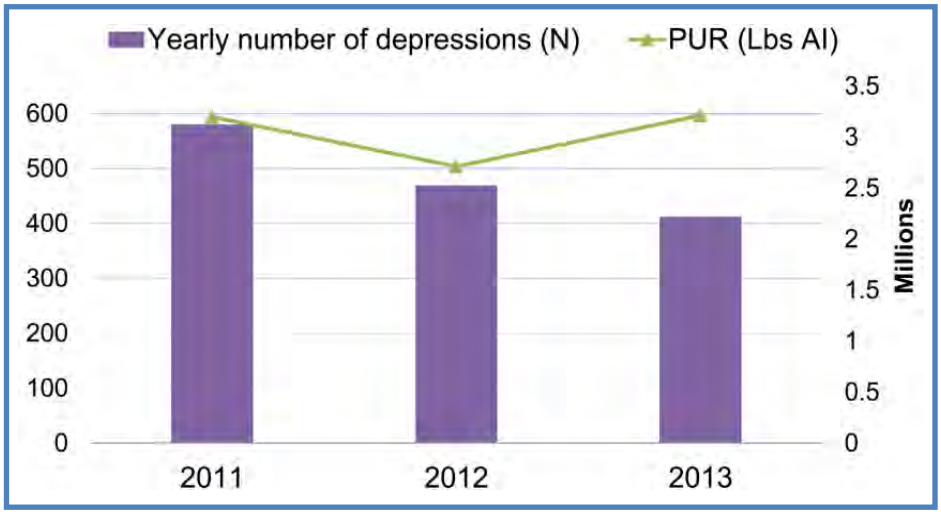

Figure C22: *Yearly number of depressions (purple bars, left axis) and yearly pesticide use (green line, right axis in millions of pounds AI, as reported in the PUR).*

E. Does intra- and inter-laboratory variability affect the reliability of monitoring of the workers?

- **Inconsistency of ChE test results reported from individual analytical laboratories**

Ninety-three percent of the Program-related test results were reported by three of the six laboratories [MEDTOX, Quest Diagnostics-Sacramento (QDI-SAC), and Quest Diagnostics-San Juan Capistrano (QDI-SJC)].

There was also a large difference in the frequency of ChE depressions detected by each laboratory. The percentage of depressions relative to the number of Program-related ChE tests (both RBC and plasma) reported by Physicians Automated Lab, Inc. (PALI) and MEDTOX were much higher than the other four labs (Figure C23). QDI-SJC had the overall highest number of depressions (n=562) from 2011-2013.

To explain the overall three-year decline in depressions (Figure C22) and the differences in the percentage of ChE depressions from the various laboratories (Figure C23), we analyzed the time course data from individual workers whose ChE records had been reported by each of the laboratories. We found that the high number of depressions observed with QDI-SJC appeared to be due primarily to frequent and large variations in ChE activity level that only occurred during 2011 (Figure C24). These cases of depression were the same as the ones we had previously identified from the geographic analysis in Ventura County. We are unable to determine the cause of this abrupt change in the variability of ChE test results but possible explanations may include improvements in pesticide handling practices or changes in blood sample handling procedures. Another possible explanation, based on information obtained from the focused growers' headquarters inspections, is that some workers continued to be tested even though they stopped handling OPs/CBs after 2011.

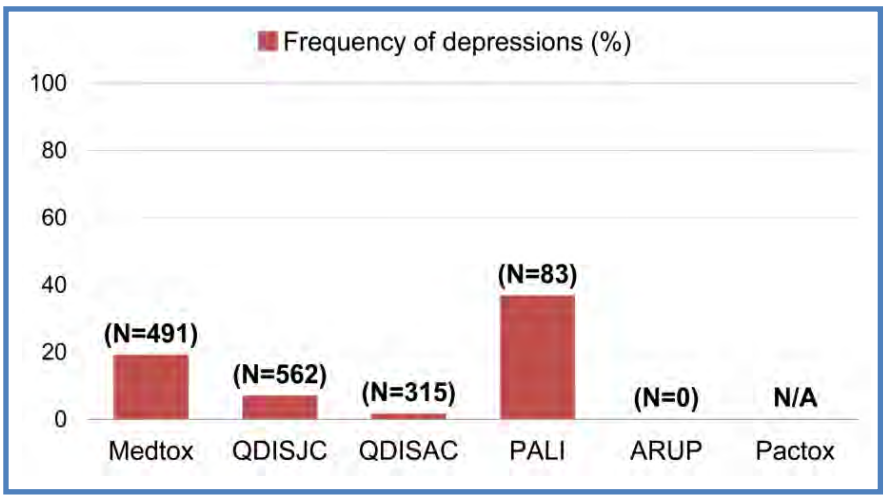

Figure C23: *Frequency of depressions (number of depressions divided by the total number of Program-related ChE test results), per laboratory. Three years of data were analyzed (2011-2013).*

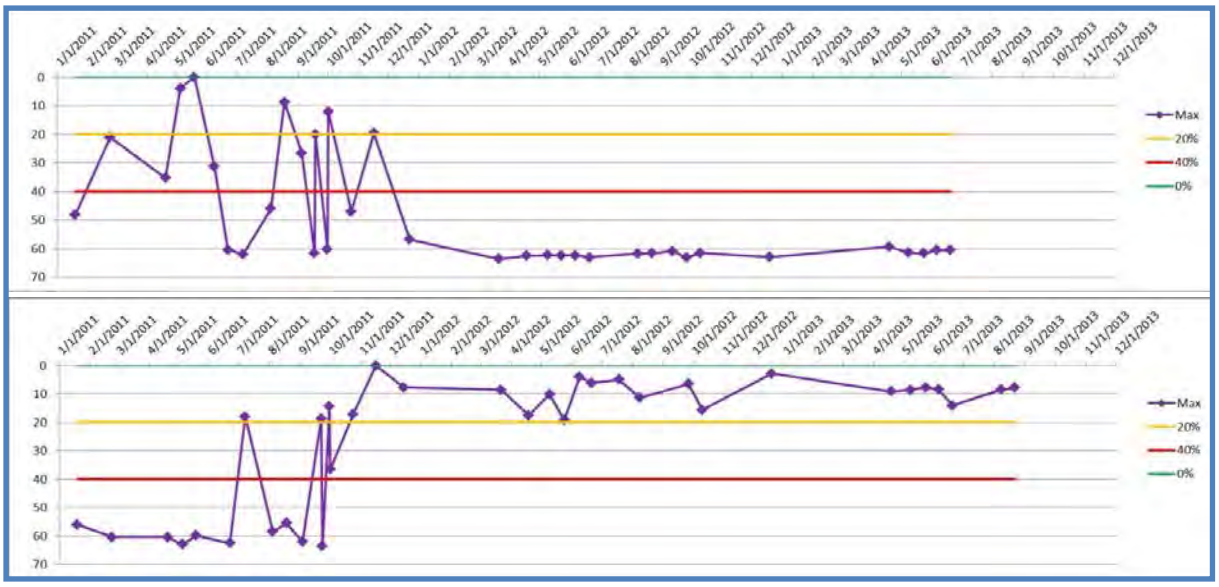

Figure C24: *Individual longitudinal variations from max values of Plasma ChE from QDISJC. Y axis is percent depression from baseline.*

- **Inconsistencies in the "normal reference range" of blood ChE activity levels reported by the reference laboratories.**

While CCR Title 3, Section 6728 specifies that baseline and subsequent follow-up ChE assays should be conducted by the *same laboratory method*, the *Guidelines for Physicians* recommend using the *same laboratory* for baseline and follow-up testing. All six laboratories are approved by CDPH and use either the Ellman or Modified Ellman method for ChE analysis. Nevertheless, there is considerable variation in the normal ChE range that the six laboratories provided to us (Table C2) clearly indicating that it is important to follow the *Guidelines for Physicians* recommendation. Of the ChE test results from the 1,338 suspected workers (2011-2013), 91% of the blood samples were analyzed by the same reference laboratories. Eight percent of the samples were analyzed by two different laboratories during the 3 year period, but at each spraying season both baseline and follow-up tests were analyzed by the same laboratory. Only 1% of the tests results were analyzed by different laboratories over a spraying season Figure C25).

Table C2: *Normal Reference ranges for ChE test results (IU*

Laboratory	RBC		Plasma	
	Low	High	Low	High
Pactox	6.6	15.5	1.8	5.2
Pali	11.19	16.7	3.17	6.33
ARUP	7.9	17.1	2.9	7.1
Medtox	6.3	13	1.9	5.5
QDISAC	9.57	15.03	2.5	7.03
QDISJC (women)	9.57	15.03	2.5	6.2
QDISJC (men)	9.57	15.03	3.33	7.03

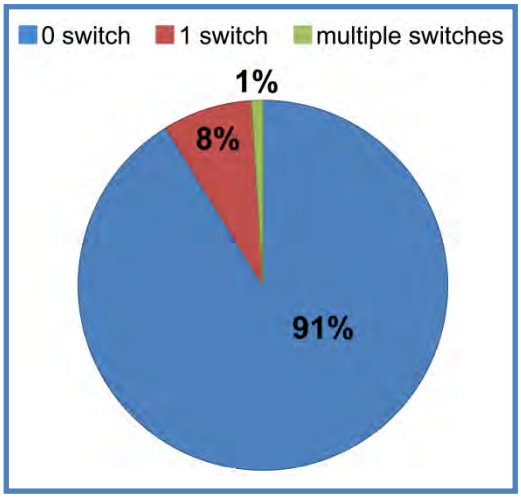

Figure C25: *Percentage of individuals whose blood specimens were sent to one, two or multiple labs for analysis over the 3 years.*

If blood samples from a single individual were analyzed by different reference laboratories, it would be difficult to interpret the results over time since changes in the ChE activity level may reflect inter-laboratory variation, not exposure to OPs/CBs. For this reason, all blood samples from an individual should be analyzed for ChE by the same reference laboratory.

Summary of Findings from Analysis of Electronically-Reported ChE Data

Laboratory-based reporting is a valuable tool for evaluating the Program. ChE test reports can be used to evaluate the implementation of the Program and assess its effectiveness on a statewide basis.

Analysis of electronically-reported ChE data allowed us to draw the following conclusions:

1. The number of workers who participate in the Program was proportional to OP/CB pesticide use.
   - The temporal distribution of the *number of estimated baselines* was inversely correlated with pesticide use. Conversely, the number of estimated follow-up tests and the number of ChE depressions were directly correlated with pesticide use.
   - When the data were analyzed on a county-by-county basis, there was good concordance between the geographic density of the *number of ChE test results* and the relative amount of OP/CB pesticide use, although some exceptions were also observed (for example, counties in the northern Sacramento valley). This suggests that in areas with heavy pesticide use, there is a high degree of worker participation in the Program.
   - Similarly, there was a good concordance between the geographic density (on a county-wide basis) of the *number of ChE depressions* and the relative amount of OP/CB pesticide use.
2. There were large differences in the frequency and magnitude of depressions using the two approaches used to identify baseline ChE activity levels. Regardless of the approach used, the relative proportions of single, multiple, short-term or extended depressions were similar.
   - Most of the workers did not have a ChE depression that reached a level requiring any action to be taken by the medical supervisor or the employer.
   - Some cases of ChE depression reached a level requiring an assessment of workplace practices. Even fewer cases required immediate removal from work.
   - There were also cases where 1) ChE activity levels remained depressed for an extended period of time (several months) and 2) ChE activity levels were depressed repeatedly.
   - Workers who experienced depression of their plasma ChE activity level had repeated depressions more often than single depressions. Most of these depressions were followed by a rapid return to an acceptable ChE activity level. This suggests that, in most cases, prompt actions were taken based on the recommendation from the medical supervisor, but long-term remedies were not implemented to prevent subsequent OP/CB exposure.

Our analysis of ChE test results and laboratory-based reporting also helped us identify program elements that can be improved. For example, the distribution of ChE test results that exceeded action levels could be interpreted as an indicator of the effectiveness of the Program. Ideally, we would hope to see minimal number of cases of ChE depression, or if there is a single ChE depression, the level does not exceed 30% below RBC ChE baseline or 40% below plasma

ChE baseline. This would indicate that the employer took action to prevent additional exposure. These patterns of depression and recovery could be identified from our analysis of the ChE test results.

However, analysis of ChE data was hampered because critical information was not provided in the submitted test reports. We encountered numerous obstacles in effectively analyzing the ChE test results, primarily due to not having the purpose of the ChE test indicated in the reports. Lacking the information on the purpose, we have to use certain assumptions in evaluating the ChE data, and that could affect our findings. In addition, the large number of extraneous ChE test results (not related to the Program) compromised our ability to focus our analysis on the population of interest (i.e., OP/CB pesticide handlers). As a result, we applied broad inclusion criteria to increase our confidence that the data reflected the work activities of all workers in the Program. Limiting analysis of ChE test results to agricultural workers will greatly improve the ability of DPR and OEHHA to use these test results to evaluate the medical supervision program. Better quality data would not only improve our ability to evaluate the Program and make recommendations for improvement but also help us meet our mandates to protect California's agricultural workers.